Cytotechnology **36:** 49–53, 2001.

Adhesion, growth and detachment of cells on modified polystyrene surface

V. Hendrick[1], E. Muniz[2,3], G. Geuskens[2] & J. Wérenne[1*]
[1] *Animal Cell Biotechnology (C.P. 160/17), Université Libre de Bruxelles, 1050 Brussels, Belgium;* [2] *Polymer Chemistry (C.P. 206/1), Université Libre de Bruxelles, 1050 Brussels, Belgium;* [3] *Present address: Departamento de Química, Universidade Estadual de Maringá, 87020–900, Maringá, Brazil*
(Author for correspondence)*

Received 4 January 2001; accepted 30 July 2001

Key words: adsorption of poly(N-isopropylarcrylamide), cell adhesion, surface modification of polystyrene, temperature dependent detachment

Abstract

By adsorbing poly(N-isopropylacrylamide) (PNIPAAm) from an aqueous solution onto oxidised polystyrene without the need for grafting the polymer to the surface, we showed here that cells (CHO-K1) adhere and grow well at 37 °C and are detached by lowering the temperature to 10 °C without any other deleterious treatment. Both bacterial culture grade polystyrene Petri dishes and polystyrene beads (120 to 250 μm diamaters) commercially available used in static conditions of growth were tested with similar results. The contact angle of modified Petri dishes with a water droplet increases from 36 to 58° when the temperature is raised from 25 to 37 °C indicating change in hydrophilicity of the surface as a function of temperature.

Introduction

The incorporation of functional groups in polymers to modify their surface without changing the bulk properties of the material has been widely studied (Kubota and Ujita, 1995; Kubota and Shiobara 1998; Yakushiji and Sakai, 1998; Liang et al; Okahata et al., 1986). This is especially important in biomaterials where interactions between the surface and cells are requested (Makino et al., 1999; Ong et al., 1999; Seto et al., 1998). Nowadays a large number of surface-modification technologies are available. For instance, irradation with ultraviolet-visible light or gamma rays (Onyiriuka et al., 1993), plasma treatment (Idage and Bamdrinarayanan, 1998), and ion-beams irradiation (Zhao et al., 1999).

Grafting is also a technique used to modify polymer surfaces (Fujishige et al., 1989; Geuskens and Tiriaux, 1993; Grierson et al., 1994; von Recum et al., 1998; Geuskens et al., 2000). Polymers such as poly(N-isoproylacrylamide) (PNIPAAm) that exhibit a lower critical solution temperature (LCST) have been grafted (Fujishige et al., 1989). The resulting material is hydrophilic below 32 °C (the LCST of PNIPAAm) and hydrophobic above that temperature. Several works have shown that this effect may be used in biotechnology allowing the detachment of cells without introduction of chemicals like EDTA or proteolytic enzymes that could, sometimes, damage the cells (Grierson et al., 1994; von Recum et al., 1998).

However though this low temperature detachment presents interesting practical potentialities, it has not been commercially applied so far. Reproducibility of this observation has been indeed disputed (Blümi, 1999) and therefore further studies were needed. Further support for the feasibility of this interesting idea is provided in this work in which we modified the surface of polystyrene beads by adsorbing PNIPAAm from aqueous solution. The resulting material behaves as classical PNIPAAm-grafted-polystyrene surfaces: the cells adhere, spread and grow at incubation temperature (37 °C) but can be totally detached by lowering the temperature from 37 to 10 °C. The process of adsorption will be described in this paper as well as the results from the surface characterization and cell culture and detachment.

Materials and methods

Synthesis of PNIPAAm

The PNIPAAm was synthesized by redox polymerization as published by Muniz and Geuskens (2000). Briefly, 200 ml of aqueous solution of N-isopropylacrylamide (NIPAAm, 5 wt %) and sodium persulfate (4 wt %) were prepared in distilled water.

The solution was deoxygenated by nitrogen bubbling for 30 min. Afterwards, 0.8 ml of aqueous solution of tetramethylethylenediamine (TEMED) (6 ml l^{-1}) was added and the polymerisation was carried out at ambient temperature.

The resulting polymer was precipitated by pouring the aqueous solution in hot water (80 °C). The white precipitation was dried in vacuum at 50 °C for two days.

The PNIPAAm obtained has $[\eta]$ = 80.0 ml g^{-1}, measured in water at 25 °C. The M_v is ca. 5.3 × 10^5 g mol^{-1}, determined using the Mark-Houwink-Sakurada equation. based in the values of **a** = 0.97 and **K** = 2.26 × 10^{-4} g^{-1}, published by Chiantore et al. (1979).

Adsorption of PNIPAAm on oxidised polystyrene Petri dishes

25 ml of aqueous sodium persulfate (30 wt %) was added to 125 ml of PNIPAAm solution (8 wt %) and the mixture deoxygenated by bubbling nitrogen for 30 min. Afterwards, 0.4 ml of an aqueous solution of TEMED (6 mol l^{-1}) was added and nitrogen bubbling was performed for another 10 min before pouring 5 ml of final solution in cleaned untreated bacterial culture grade polystyrene (PS) Petri dishes. These were kept overnight at room temperature before being copiously washed with distilled water. Their surface was characterized by i) contact angle with a water droplet and ii) adhesion, growth and detachment of cells.

Contact angle measurements

Small pieces from the modified Petri dishes washed copiously with water were wiped dry with a soft piece of tissue. Static contact angles were measured with a specially assembled apparatus involving an optical projection technique to magnify a droplet of water placed on the polymer. The image of the droplet is projected on a screen and the contact angle measured directly. To measure the contact angle at temperatures higher than room temperature the polymer was put on a hot plate with temperature control.

Adsorption of PNIPAAm on oxidised polystyrene beads

Beads of PS kindly supplied by Petrofina (Belgium) were sieved and the portion between 180 and 250 μm was used. The sieved beads were washed with methanol an dried in vacuum at 50 °C. 10 g of beads were suspended in 125 ml of aqueous solution of PNIPAAm (8 wt %) and 25 ml of aqueous sodium persulfate (30 wt %) were added to the flask. Nitrogen was bubbled for 30 min before addition of 0.4 ml of aqueous solution of TEMED (6 mol l^{-1}). The system was further degassed for 10 min, closed and stirred overnight at room temperature. Afterwards the beads were filtered, washed copiously with distilled water and dried in vacuum at 50 °C. The surface of modified beads was characterized by their ability to permit adhesion, growth and detachment of cells.

Sterilisation

The Petri dishes and PS beads, treated as described above, were packed in polypropylene bags that were properly sealed. The bags were sterilised by exposure to a dose of 1 Mrad of γ-radiation from a ^{60}Co in a Gammacell 220 (tomic Energy of Canada Ltd.).

Adhesion, growth and detachment of cells

Beads (0.05 g ml^{-1}) were suspended in 10 ml of phosphate buffer solution (PBS) then centrifuged at 3000 rpm for 10 min and resuspended in 10 ml of culture medium for 2 days.

The cell line used was CHO-K1 (Chinese Hamster Ovary, type K1). The basal medium consisted in a DMEM/HAM F 12 mixture (1/1 v/v), buffered with potassium bicarbonate at 3.7 g ml^{-1}, supplemented with 5% (v/v) of Foetal Calf Serum (Gibco, Life Technologies), 20 mmol l^{-1} glucose, 4.5 mmol l^{-1} glutamine and 2% (v/v) MEM essential amino acids and 1% (v/v) non essential amino acid from Gibco stock solution 50× and 100× respectively.

The cells were cultivated on modified PS Petri dishes and modified beads. For comparison, untreated Petri dishes and PS beads were also used. The experiments for the evaluation of cell growth were performed at 37 °C in an incubator under 5% CO_2 atmosphere. The total amount inoculated is 7.5 × 10^5 cells in 5 ml. After 24 hr the medium was removed to

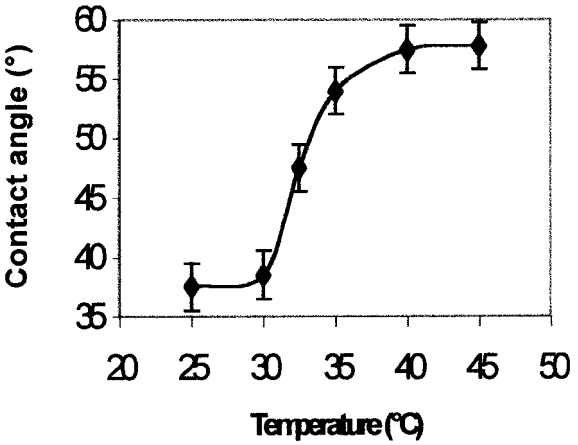

Figure 1. Contact angle of a modified PS surface with a water droplet as a function of temperature (surface modification contact angle measurement as described in material and methods).

eliminate cells that have not adhered on the different surfaces.

Cell concentration was determined with an haemacytometer and viability by trypan blue exclusion. Every day, the modified Petri dishes or beads were placed at 10 °C and after one hour the cells were resuspended readily in cold medium and counted.

For beads treated with PBIPAAm, a special procedure was used since they do not sediment easily: beads were centrifuged at 1000 rpm for 5 min, the supernatant was removed and the beads were collected in PBS. The system was further centrifuged twice at 400 rpm for 5 min to eliminate dead cells. After one hour, cells are detached by cooling at 10 °C and 500 μl of cell suspension was added to 500 μl of trypan blue for the counting.

Results and discussion

Contact angle measurements

The contact angle of modified Petri dishes with a water droplet increases when the temperature is raised from 25 to 37 °C. This is clear evidence that the surface is more hydrophilic below the LCST of PNIPAAm (32 °C) than abobe (Figure 1).

Cell culture

Adhesion and detachment of cells on modified PS Petri dishes and beads

The modified polystyrene beads exhibit a very interesting behaviour since the cells adhere very well on them at 37 °C. Once they adhere they grow and spread somewhat when incubated and cultured at 37 °C. Moreover, the cells detach easily when the beads are cooled down from 37 to 10 °C after 24 and 48 hr of cell culture (Table I).

Table 1. Percentage of cells detached from confluent Petri dishes or microcarrier beads after 24 and 48 hr of cell culture (cells were counted in homogenous supernatant 1 hr after the shift of temperature at 10 °C; non-detached cells were counted after enzymatic treatment to evaluate the total cell number in order to calculate the percentage of detached sell). 12 independent experiments were used and the standard deviation is ± 4%

Material	% (24 hr)	% (48 hr)
Untreated Polystyrene Dishes	30	18
Commercial T.C. Petri dishes	2	18
PNIPAAm treated Polystyrene dishes	100	100
Untreated Polysyyrene beads	3	3
Commercial T.C. beads (Cytodex-3)	18	15
PNIPAAm treated Polystyrene beads	100	100

The same experiments were done in Petri dishes. We observed a total detachment at 10 °C on modified PS Petri dishes and beads while this was very low with commercial tissue culture grade dishes. In dynamic conditions (spenner) the cells do not adhere to the treated beads.

With untreated bacterial grade PS Petri dishes and untreated beads neither spreading nor growth of cells was observed. This shows that without firm attachment, the growth is inefficient. Therefore the attachment of cells onto modified PS beads still has to be improved in order to allow the use in PNIPAAm treated beads for cell culture ion dynamic conditions.

Kinetics of cell growth

Figure 2 presents the growth behaviour of CHO-K1 cells performed on PNIPAAm modified PS Petri dishes. For comparison, tissue culture grade Petri dishes (positive control) and bacterial culture grade Petri dishes (negative control) were used. The kinetic curves show that the factor of multiplication is about 4 for tissue culture grade (▲ : T.C.) and PNIPAAm treated Petri (○ : Wi PNIPAAm). The plateau, in fact, is reached when the surface of the polymer is completely covered by cells resulting in a monolayer of cells that spread however only partially. Adsorption of PNIPAAm on oxidised PS surface results in a growth kinetics of CHO-K1 similar to that observed

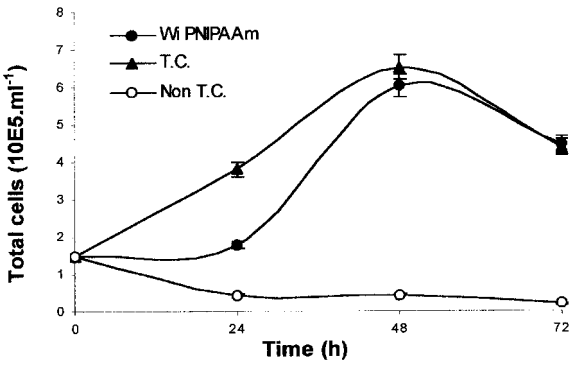

Figure 2. Growth kinetics of CHO-K1 on different type of PS Petri dishes in function of time culture (♦ Wi PNIPAAm = PS Petri dishes modified by oxidation and adsorption of KNIPAAm – ○ TC = commercial Tissue Culture grade (TC) Petri dishes – ● non TC = Commecial bacterial culture grade Petri dishes) (1×10^5 cells were inoculated and adhering cells were counted every 24 hr as described in the text).

with commercial tissue culture Petri dishes. However, in those commercial tissue culture dishes cells do not detach spontaneously when the temperature is reduced and cells must be detached by enzymatic treatment (trypsine) for the counting.

Cell growth on untreated Petri dishes (○ : Non T.C.) is inefficient because cells do not adhere on unmodified PS.

The re-use of the beads after the detachment of the cells was investigated. After detachment of the cells by shifting the temperature from 37 to 10 °C, the beads were copiously washed with distilled water and sterilised. The re-used beads modified by adsorption of PNIPAAm show the same capability as presented in the first run. This shows that the beads can be re-used after cell detachment for further cell growth (data not shown).

Conclusions

Treatment of PS Petri dishes or beads by a KNIPAAm solution in the presence of sodium persulfate and TEMED results in simultaneous oxidation of the PS surface and adsorption of the water soluble polymer. The resulting material exhibits hydrophilic behaviour at low temperature and hydrophobic properties at temperatures above 37 °C, the LCST of PNIPAAm in wtaer. We have shown that adsorption of PNIPAAm without grafting permits adhesion and growth of CHO-K1 cells on PS and detachment of cells at 10 °C in very mild conditions without deleterious treatment. These properties are of considerable interest for the adhesion and growth of cells in connection with interactions between the polymer surface and cells.

References

Chiantore O, Guaita M & Trossarelli L (1979) Solution properties of poly(N-isopropylacrylamide). Macromol Chem 190: 969–973.

Fujishige S, Kubota K & Ando I (1989) Phase transition of aqueous solution of poly(N-isopropylacrylamide) and poly(N-isopropylmethacrylamide). J Phys 92: 3311–3312.

Geuskens G & Thiriaux Ph (1993) Surface modification of polymers II. Photo-oxidation of SBS containing anthracene and grafting initiated by photo-generated hydroperoxides. Eur Polym J 29: 351–353.

Geuskens G, Etoc A & Di Michelle P (2000) Surface modification of polymers – VII. Photochemical grafting of acrylamide and N-isopropylacrylamide onto phlyethylene initiated by anthraquinon-2-sulfonate adsorbed at the surface of the polymer. Eur Polym J 36: 265–271.

Muniz EC & Geuskens G (2000) Influence of temperature on the permeability of polyacrylamide hydrogels and semi-IPNs with poly(N-isopropylacrylamide). J Membrane Sci 172: 287–293.

Grierson I, Hiscott P, Hogg P, Robey H, Mazure A & Lakin G (1994) Development, repair and regeneration of the retinal-pigment epithelium. Eys 8: 255–262.

Idage SB & Bandrinarayanan S (1998) Surface modification of polystyrene using nitrogen-plasma. An X-ray photoelectron spectroscopy study. Langmuir 14: 2780–2785.

Kubota H & Shiobara N (1998) Photografting of poly(N-isopopylacrylamide) on cellulose and temperature-response character of the resulting grafte cellulose. Reactive and Functional Polymers 37: 218–224.

Kubota H & Ujita S (1995) Reactivity of glycidyl methacrylate grafted cellulose prepared by means of photografting. J Appl Polym Sci 56: 25–31.

Liang L, Feng XD, Liu J, Rieke PC & Fryxell GE (1998) Reversible surface properties of glass plate and capillary tube grafted by photopolymerization of N-isopropylacrylamide. Macromolecules 31: 7845–7850.

Makino K, Umersu M, Goto Y, Nakayama A, Suhara T, Tsujii J, Kikuchi A, Ohshima H, Sakurai Y & Okano T (1999) Interaction between harged soft microcapsules and red blood cells: effects of PEGylation of microcapsule membranes upon their surface properties. Colloids and Surfaces B 13: 287–297.

Okahata Y, Noguchi H & Seki T (1986) Functional capsule membranes .23. thermoselective permeation from a polymer-grafted capsule membrane. Macromolecules 19: 493–494.

Ong Y-L, Razatos A, Georgiu G & Sharma MM (1999) Adhesion forces between *E. coli* bacteria and biomaterial surfaces. Langmuir 15: 2719–2725.

Onyiriuka EC (1993) The effects of high-energy radiation on the surface chemistry of polystyrene: a mechanistic study. J Appl Polym Sci 47: 2187–2194.

Seto F, Fukuyama K, Muraoka Y, Kishida A & Askashi M (1998) Thermosensitive surfac properties of polyethylene film with poly(N-isopropylacrylamide) chains prepared by corona discharge induced grafting. J Appl Polym Sci 68: 1773–779.

von Recum H, Kikuchi A, Okuhara M, Sakurai Y, Okano T & Kim SW (1998) Retinal pigmented epithelium cultures on thermally responsive polymer porous substrates. J Biomater Sci Polymer Edn 9: 1241–1253.

Yakushiji T, Sakai K, Kikuchi A, Aoyagi T, Sakurai Y & Okano T (1998) Graft architectural effects on thermo-responsive wettability changes of pol(N-isopropylacrylamide)-modified surfaces. Langmuir 14: 4657–4664.

Zhao Q, Zhai G-J, Ng DHL, Zang Z-Z & Chen Z-Q (1999) Surface modification of Al_2O_3 bioceramic by NH_2^+ ion implantation. Biomaterials 20: 595–599.