7.14 Adhesion of Yeast Cells on Surface of Polymers Produced by Radiation Polymerization

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ABSTRACT

The adhesion of yeast (Sacharomyces formesences) cells on plymers was studied thermodynamically. The polymers were laminally prepared by means of radiation polymerization.

By measuring contact angles, we calculated dispersion component and polar component of surface free energy of the polymers and the cells, and interfacial free energy between the polymer and the cells. Then interfacial free energy change of the cell adhesion to surface of the polymer was evaluated.

The adhesion behavior of yeast cells on the polymers was observed by optical microscope.

From above results, we conclude that the initial adhesion of the cells is related to the surface free energy of the polymer, but the irreversible adhesion may be close to the polar component in surface free energy. The high polar component is favourable the irreversible adhesion of yeast cells.

KEYWORDS: Adhesion; Yeast cell; Radiation Polymerization; Surface free energy.

INTRODUCTION

A microbial suspension may be interpreted as a living colloidal system and the microorganism adhesion to a carrier is often regared as solid-solid interaction. The physical chemistry of interfaces provides two theoretical frames for describing adhesion of a partical to a surface. The initial step of adhesion can in first approxiation be described by colloid chemical theories such as the DLVO theory, accounting for long-range interactions, point out the importance of electronstatic forces (Rutter and Vincent, 1980; Tadros 1980). The thermodynamic decripation of adhesion process emphasized the role of surface energy and hydrophobicity and accounts thus for short-range force (Busscher et al 1980; Absolom et al 1983).

In our previous work, the filamentous fungi, Trichoderma reesei were immobilized by adhesion onto ionic polymer carrier prepared by radiation polymerization. It was found that the ionic properties of polymer affected markedly adhesion of filamentous cells and the activity of adhered cells, and that the cationic prcperty of polymer promoted the adhesion of the cells, indicated the importance of electrostatic interactions (Lu et al 1994). In present work, we prepared a series of acrylic and methacrylic esters using radiation polymerization technique in order to investigate on adhesion behavior of yeast cells on the polymer surface and to discuss adhesion of the cells, thermodynamiclly.

Materials and Methods

Microorganism and culture   Saccharomyces formesensis was used in this work. The yeast cells were precultured under aerobic condition for 48 h at 28℃ in a medium consisting of 1% glucose, 0.1% molasses, 0.5% pepton, 0.3% yeast extract and 0.3% malt extract (pH 4.8). And Yeast cells after 48h of culture were gathered, washed by distilled water and centrifuged three times with 4000 rpm.

Carrier preparation   Hydroxyethyl acrylate(HEA), hydroxypropyl methacrylate (HPMA), hydroxyethyl methacrylate(HEMA), and diethyleneglycol dimethacrylate (2G), tetraethyleneglycol dimethacrylate(4G), and nonaethyleneglycol dimethacrylate(9G) were obtained from Shin Nakamura chemicals Co. Ltd. Acrylamide (AM) was puchared from Beijing Dongfeng chemical plant, respectively.

The preparation of polymer piece was performed as following precedure: the monomer adding 10%(v/v) of methanol was poured between the two slide glasses, it was bound, then it was irradiated with 20kGy by γ-rays from Co-60 source at room temperature.

Contact angle measurement   The contact angle measurement was carried out using sessile drop technique at 25℃ with bidistilled water and CH_2I_2 (DIM) as wetting agent. The contact angle was read in 3 sec. and measured three times. A series of contact angle (mean value) data on a given surface yields the solid surface energy by a least squre fitting procedure (Busscher et al 1983).

The centrifuged cells were coated on the slide glass as homogenuous and smooth as possible, dried in the room temperature and used for the measurement of contact angle of the cells. The measurement of the contact angel of the cells and the calculation of suface free energy of yeast cells were carried out by the same method as has been described above.

Adhesion test   The polymer piece (1×1 cm) was placed horizontally into a flask (100ml), in which a cell- water suspension was poured and settle during 8, 24h. The carrier was then rinsed and lightly washed in the distilled water three times. Immediately, the adhesion state was examined by optical microscope (Olympus, Japan). The adhesion was characterized by a rough quatitative evalution of cell adhesion on the carrier. Four grades was used as grade 0, no adhesion; grade 1, low adhesion; grade 2, medium adhesion; grade 3, good adhesion.

Results and Discussion

Table 1 presented the surface free energy of polymers, dispersion compotnent, polar component, interfacial free energy of the cell adhesion and the test result of the adhesion of yeast cells on surface of the polymers produced by radiation polymerization and photographs show the adhesion behavior of cells on the surface of polymers. The surface free energy of cells was 42.63 erg/cm^2. The surface free energy of the polymers were in 40-60 erg/cm^2, in which the dispersive component is about 40 erg/cm^2. The result is accordance with Van Pelt's result that γ^d is about 40 erg/cm^2 (an exception being polytetrafluorethylene) for most materials (Van Pelt et al 1984).

The surface free energy of polymers is following order of decrease: HEA> HEMA> AM> 9G> 4G> 2G> HPMA. The degree of the cells adhered was in the order of HEMA= 9G> AM= HEA= HPMA> 4G> 2G (Table and Figure 1). The result indicates that yeast cells easily adhere on the polymer having high surface free energy. However, according to the thermodynamic approach, the adhesion process is considered as creating a new interface, cell-solid(cs), by disrupture of two preexisting interfaces, cell-liquid(cl) and solid-liquid(sl). This involves thus a molecular contact between the two adhering surface. The tendency of the surfaces to associate is expressed by the free energy change of the process. Adhesion may be expected by

$$\Delta G_{adh} = \gamma_{cs} - \gamma_{cl} - \gamma_{sl}$$

there, γ_{cs}, γ_{cl} and γ_{sl} are the interfacial free energy between cell and polymer, cell and liquid, polymer and liquid, respectively. If $\Delta G_{adh} < 0$, the cell adhesion is favorable energetically.

From table 1, it can be seen that all ΔG_{adh}(interfacial free energy change of adhesion) is less than zero, indicating the cell adhesion on these polymers is energetically favorable. But the degree of cell adhesion was not expressed the tendency that the smaller the cell adhesion, the lower ΔG_{adh}. For example, ΔG_{adh} of poly(2G) was lowest, but adhesion of the cells on this surface was hardly observed over 48h. On surfaces of the polymer having higher ΔG_{adh}, such as poly(HEMA), poly(AM), more cells adhered.

Table 1:interface free energy and the adhesion of yeast cells

polymer	γ	γ^d	γ^p	γ^p/γ^d	Δ_{adh}	grade
HEA	66.52	41.46	25.06	0.60	-16.72	2
AM	58.29	35.35	22.94	0.65	-17.53	2
HEMA	51.54	32.58	18.96	0.58	-20.17	3
9G	57.21	38.58	18.63	0.48	-21.10	3
4G	55.37	39.28	16.09	0.41	-23.25	1
2G	46.46	35.35	11.11	0.31	-27.42	1^-
HPMA	40.27	29.79	10.48	0.35	-27.42	2
YEAST	42.63	28.69	13.94	0.49		

γ : surface free energy (erg/cm^2), γ^d: dispersion component , γ^p: polar component, Δ_{adh}: interface free energy of adhesion.

From above result, it was found that the thermodynamic approach may predict the initial adhesion of the cells, energetically, but it may be difficult to predict irreversible adhesion of the cells.

By further analysis, it was discovered that the larger γ^{p}/γ^{d} of polymer surface, the more the cells adhered on this surface, suggesting that the polar component in surface free energy of the polymer play an immpotant role in the irreversable adhesion of the cells on the surface. γ^{p} is related to polar group in molecule, which yield the dipole-dipole interaction and dipole-induced dipole interaction, so that strengthen the initial adhesion of the cells owing to the electrostatic interaction between the cells and the polymer. Another reason is assumed that the polymer having polar groups NH, OH, COOH may be recognized by the cells, whom surface has same polar groups, then induce the secretion of biopolymer to strengthen the initial adhesion and to promote subsequent multiplication. Suzuki has reported that the ion implanted polystyrene (PS) and segmented polyurethane(SPU) lead a dramatic increase in the extent of cell adhesion and spreading of endothelial cells comparing with unimplanted PS and SPU because the ion implantation broke the original chemical bond to form new radicals such as OH, >C=O, SiH and condensed rings (Y. Suzuki 1992).

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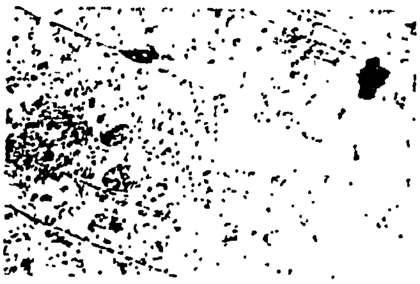
Figure 1: The adhesion of Yeast Cells on Surface of Polymers

HEA polymer

AM polymer



HEMA polymer

9G polymer

4G polymer

2G polymer

HPMA polymer