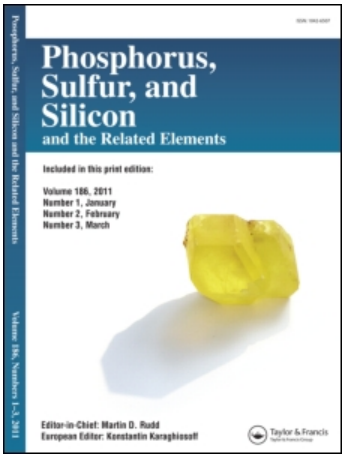

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The Study of the Cultivation of Chinese Hamster Ovary and Bows Cell Lines

Meisetsu Kajiwara[a]
[a] Department of Applied Chemistry, Faculty of Engineering, Nagoya University, Nagoya, Japan

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THE STUDY OF THE CULTIVATION OF CHINESE HAMSTER OVARY AND BOWS CELL LINES

MEISETSU KAJIWARA
Department of Applied Chemistry, Faculty of Engineering,
Nagoya University, Furo-cho, Chkikusa-ku, Nagoya, 464-01 Japan.

Abstract Sixteen poly(organophosphazenes) were prepared by reaction between polydichlorophosphazene and nucleophilic reagents such as phenoxides and amino compounds.
Adhesive and colony formation percent were investigated using V-79 Chinese hamster cells on poly(organophosphazene) films. It was found that $[NP(OC_6H_5)_{2-x}(NHBu\text{-}n)_x]_n$ (x=0.2, 1.8) gave the best percent adhesiveness. This value was similar to that of Falcon^R. On the other hand, $[NP(OC_6H_5)(NHBu\text{-}n)]_n$ film showed best colony percent formation. This was of a higher value than that of Falcon. The $[NP(OC_6H_5)_2]_n$ properties were poor for cultivation of useful Bowes and chinese hamster ovary cell lines in comparison with Cytodex III.

INTRODUCTION

Poly(organophosphazenes) were prepared by reaction between polydichlorophosphazene $(NPCl_2)_n$ and various nucleophlic reagents such as alkoxy, phenoxy, and amino compounds. Application of poly(organophosphazenes) have been considered by many companies. However, Firestone Rubber and Tire Co. has been the first to succeed in producing a commercial elastomer known as PNF (1). Ethyl Co. and Atochem Co. have also developed Aypel F or A (2) and Orgaflex type F and A (3), respectively. Furthermore, Goedemond (4) reported that some polyphosphazenes were suitable for development of implantable antitumor devices. However, there are no avaliable report regarding the study of bioreactor materials using poly(organophosphazenes). This report describes the study of adhesive properties and the degree of colony formation of chinese hamster ovary (V-79) cells and cultivation of (V-79) and Bowes cell lines or poly(organophosphazenes).

EXPERIMENTAL

Preparation of $(NPCl_2)_3$, $(NPCl_2)_n$, $(NPR_2)_n$ and $(NPR^1R^2)_n$

The $(NPCl_2)_3$ was prepared by the method of Saito (5). Also, $(NPCl_2)_n$ was prepared by the method of Kajiwara (6). That is, after the poly-

merization was complete, the product, treated with tetrahydrofuran (THF) was repricipitated by addition of n-heptane. In so far as the precipitated and purified $(NPCl_2)_n$ was unstable in water and moisture, chlorine in $(NPCl_2)_n$ was substituted for the nucleophlic reagent in THF for 24 h. The following typical substitution reaction was described.

Poly(bisphenoxyphosphazene) $[NP(OC_6H_5)_2]_n$; A solution of $(NPCl_2)_n$ in THF was added slowly to a stirred solution of sodium phenoxide prepared from sodium and phenol in THF. After the mixture was refluxed at 74°C for 24 h, THF solvent was then added to n-heptane. The precipites were filtered off, washed with acetone, and dried under the vacuum conditions for one night.

Poly(diethylaminobutylaminophosphazene) $[NP(NEt_2)(NHBu\text{-}n)]_n$; A solution of $(NPCl_2)_n$ dissolved in THF was added dropwised over 2 h to a stirred solution of diethylamine in 100 mL of THF. The reaction was allowed to proceed at 25°C for 24 h. Then n-butylamine and triethylamine dissolved on 50 mL of THF were added dropwised over 2 h. The reaction mixture wwas allowed to remain at 25°C for 24 h.The mixture was then filtered to remove amine salts, and the polymer was precipitated from the filtrate by addition of n-heptane. Purification was effected by precipitation of the polymer three times from solution in dilute aqueous sulfuric acid in ethanol into methanol that contained a trace of triethylamine. The polymer was then dried under vacuum over P_2O_5.

Preparation of Culture Fluid and a Bed of the cell cultivation: A culture fluid was prepared as follows; Minimum essential medium(MEM) was added to 10 wt% of fetal calf serum. The viscosity and density of this culture fluid had 0.01g/cc, respectively. A bed for cell cultivation was prepared with poly(organophsphazenes). After the solution dissolved 1 g of poly(organophosphazenes) in THF, the film was formed by casting, then, 5 mL of culture liquid having $4x10^4$ of V-79 cells was added to the film, where they remained at 37°C for 4 h or 72 h. In adddition, Falcon Petri dishs were used as standard samples. Bowes cells(myeloma cell) and Chinese hamster ovary cells, which produced tissue plasminogen activator, were cultured using the cultivation on polystylene. Furthermore, Cytodex III(Pharmarcia) was used as the

standard sample.

RESULTS AND DISCUSSION

Adhesiveness and percent colony formation of lung tissue of chinese hamster(V-79) on poly(organophosphazene) films

The results of percent colony formation adhesiveness using V-79 for 13 poly(organophosphazdne) films are summarized in Table 1.

TABLE 1 Adhesive (A) and percent colony formation (B) of poly(organophosphazenes) using V-79 cells

$(NPR^1R^2)_n$		(%)		$10^{-11} cm^2 s^{-1} mmHg^{-1}$
R^1	R^2	(A)	(B)	D_k
OCH_2CF_3	OCH_2CF_3			11.7
OC_6H_4F-p	OC_6H_4F-p			
$OC_6H_4CH_3$-p	$OC_6H_4CH_3$-p	58	50	5.5
OC_6H_4Cl-p	OC_6H_4Cl-p			4.5
OC_6H_4Et-p	OC_6H_4Et-p	62	50	19.7
$OC_6H_4CH_3$-m	$OC_6H_4CH_3$-m	70	46	0.2
OC_6H_5	OC_6H_5	77	50	6.4
NHPr-n	NHPr-n	83	19	15.5
NHBu-n	NHBu-n	61		44.2
NHC_5H_{11}	NHC_5H_{11}			27.7
NHPr-n	NEt_2			55.2
NHBu-n	NEt_2	33		55.2
NHC_5H_{11}	NEt_2	2		16.0
Falcon		88	65	

It was found(Table 1) that poly(bisphenoxyphosphazne), poly(bis-p-ethylphenoxyphosphazne), and poly(bisbutylaminophosphazene) have good percent adhesiveness in comparison to other poly(organophosphazenes). On the other hand, percent colony formation on poly(bis-p-methylphenoxyphosphazene), poly(bis-p-ethylphenoxyphosphazene), and poly(bisphenoxyphosphazene) are about 50% less as shown in Table 1. It was also observed that adhesiveness and percent colony formation of poly-(organophosphazene) films using V-79 cells are lower than that of

Falcon Petri dishes. It was also found that halogen-containg polymer are poor for adhesiveness or colony formation since V-79 cells did respond positively to the halogens. Furthermore, as colony formation of V-79 cells is related to oxygen permeability of poly(organophosphazene) films in water, oxygen permeability (D_k) of these polymers was determined by the method of Kajiwara (7). The results obtained are summarized in Table 1.

It seems that percent colony formation is dependent on oxygen permeability of the films, expect for films having no halogen atoms or NC_5H_{11} and NEt_2 groups. The films having D_k=5 - 20 give percent good colony formation. To prepare the films having the highest adhesiveness and percent colony formation, some modified poly(organophosphazenes) such as $[NP(OC_6H_5)_{2-x}(NHBu\text{-}n)_x]_n$ were synthesized since poly(bisphenoxyphosphazene) and poly(bisbutylaminophosphazene) had higher percent adhesiveness. In this study, compositions of the best films having the highest percent colony formation are $[NP(OC_6H_5)_{1.8}(NHBu\text{-}n)_{0.8}]_n$ and the percent colony formation of these is a little lower than that of Falcon Petri dishes.

The cultivation of Bowes and Chinese hamster ovary cell lines using polystylene beads coated with poly(bisphenoxyphosphazene)

In the case of Bowes cell line, after 4 h of cultivation, the cell number decresed and closely approached the steady state. Cytodex III, used as the standard sample, showed increased cell numbers with increasing cultivation time. In the case of Chinese hamster ovary cells, after 4 h of the cultivation, the cells were decreased and the degree of the percent cultivation reached the steady state after 2 d, or compared with the results of Cetodyx III used as the standard sample. However, the cells disappeared completley after 3 d.

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