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The Study of the Cultivation of Chinese Hamster Ovary and Bowes Cell Lines with Poly(organophosphazene) Membranes

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ABSTRACT

Sixteen poly(organophosphazenes) were prepared by reaction between polydichlorophosphazene $(NPCl_2)_n$ 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® On the other hand, $[NP(OC_6H_5)\text{-}(NHBu\text{-}n)]_n$ 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.

Index Entries: Cultivation, chinese hamster ovary; poly(organophosphazene); membranes.

INTRODUCTION

Poly(organophosphazenes) were prepared by reaction between polydichlorophosphazene $(NPCl_2)_n$ and various nucleophilic reagents such as alkoxy, phenoxy, and amine compounds. Applications 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 available reports 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 Dichlorocyclotriphosphazene $(NPCl_2)_3$ and Polydichlorophosphazene $(NPCl_2)_n$

The $(NPCl_2)_3$ was prepared by the method of Saito (5). Five hundred of phosphorus pentachloride(PCl_5) was reacted with 200 g of ammonium chloride dried (NH_4Cl) in 1.5 L of terachloroethane or chlorobenzene for 24 h at 125°C. After the reaction was complete, separated $(NPCl_2)_3$ was purified by several vacuum distillations. The melting point of purified $(NPCl_2)_3$ was 112°C. Also, $(NPCl_2)_3$ was prepared by the method of Kajiwara (6). A mixture of 20-30 g of $(NPCl_2)_3$ and a small amount of sulfur recrystallized from benzene was placed in a Pyrex tube having the dimension of 200 mm × 40 mm. They were evacuated to 10 mm Hg for 1 h, sealed, and heated in an oven just below 300°C. When the polymerization was complete, the product, treated with tetrahydrofuran (THF) was precipitated by addition of n-heptane.

Preparation of Poly(organophosphazenes) $(NPR_2)_n$ and $(NPR^1R^2)_n$

Insofar as the precipitated and purified $(NPCl_2)_n$ was unstable in water and moisture, chlorine in $(NPCl_2)_n$ was substituted for the nucleophilic 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(bistrifluoroethoxyphosphazene) $[NP(OCH_2CF_3)_2]_n$

A solution of sodium trifluoroethoxide was prepared by addition of sodium 2,2,2-trifluoroethanol in dry THF. The reaction was exothermic and the mixture was heated to keep the solvent boiling for 20 h. At the conclusion of the reaction, the mixture was cooled and acidified with dilute hydrochloride acid, and benzene was added to coagulate the polymer. After isolation of the polymer by filtration, it was washed with an excess of water to remove salts and then precipitated from THF into benzene to remove oligomer and low-mol-wt polymers. The polymer was then exhaustively dried under vacuum.

Poly(diethylaminobutylaminophosphazene)
$[NP(NEt_2)(NHBu\text{-}n)]_n$

A solution of $(NPCl_2)_n$ dissolved in THF was added dropwise 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 dropwise over 2 h. The reaction mixture was 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 into dilute ammonium hydrochloride, and once from dilute acetic acid in ethanol into methanol that contained a trace of triethylamine. The polymer was then dried under vacuum over P_2O_5.

Preparation of a Culture Fluid and a Bed of the Cells 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 0.01 poise and 1.01g/cc, respectively. A bed for cell cultivation was prepared with poly(organophosphazenes). After the solution dissolved 1 g of poly(organophosphazenes) in THF, the film was formed by casting, then, 5 mL of culture liquid having 4×10^4 of V-79 cells was added to the film, where they remained at 37°C for 4 h or 72 h. In addition, Falcon® Petri dishes 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 (Pharmacia) 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

A typical colony formation is shown in Fig. 1 and the results of percent colony formation adhesiveness using V-79 for 13 poly(organophosphazene) films are summarized in Table 1.

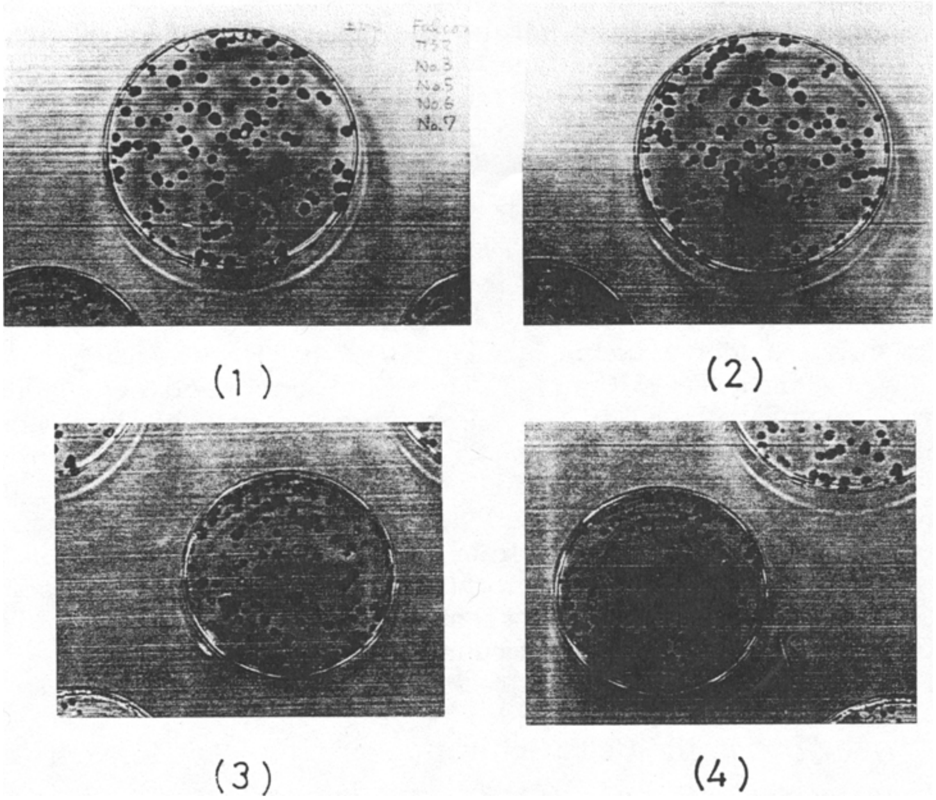

Fig. 1. The typical formation of colony using V-79 (1) Falcon, (2) $[NP(OC_6H_4CH_3\text{-}p)_2]_n$; (3) $[NP(OC_6H_4Et\text{-}p)_2]_n$; and (4) $[NP(OC_6H_4CH_3\text{-}p)_2]_n$.

It was found (Table 1) that poly(bisphenoxyphosphazene) $[NP(OC_6H_5)_2]_n$, poly(bis-p-ethylphenoxyphosphazene) $[NP(OC_6H_4Et\text{-}p\text{-})_2]_n$, and poly(bis-butylaminophosphazene) $[NP(NHBu\text{-}n)_2]_n$ have good percent adhesiveness in comparison to other poly(organophosphazenes). On the other hand, percent colony formation on poly(bis-p-methylphenoxyphosphazene) $[NP(OC_6H_4CH_3\text{-}p)_2]_n$, poly(bis-p-ethylphenoxyphosphazene) $[NP(OC_6H_4Et\text{-}p\text{-})_2]_n$, and poly(bisphenoxyphosphazene) $[NP(OC_6H_5)_2]_n$ are about 50% less as shown in Table 1. It was also observed (Table 1) 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 (Table 1) that halogen-containing polymers such as $[NP(OCH_2CF_3)_2]_n$, $[NP(OC_6H_4F\text{-}p)_2]_n$ and $[NP(C_6H_4Cl\text{-}p)_2]_n$ are poor for adhesiveness or colony formation since V-79 cells did respond positively to the halogens. In the case of the polymers having NC_5H_{11} or NEt_2 groups, adhesiveness and percent colony formation are lower than that of amino phosphazene polymers such as $[NP(NHPr\text{-}n)_2]_n$ or $[NP(NHBu\text{-}n)_2]_n$, owing to basicity. The V-79 cells may be grown in harmony with basicity. Furthermore, as colony formation of V-79 cells is related to oxygen permeability of poly-

Table 1
Adhesive (A) and Percent Colony Formation (B)
of Poly(organophosphazenes) Using PV-79 Cells

$(NPR^1R^2)_n$ R^1	R^2	(%) (A)	(B)	10^{-11} cm^2S^{-1} $mmHg^{-1}$ 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	-	-	4.7
OC_6H_4Et-p	OC_6H_4Et-p	62	50	19.0
$OC_6H_4CH_3$-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}	-	-	
NHPr-n	NEt_2	-	-	27.7
NHBu-n	NEt_2	33	-	55.2
NHC_5H_{11}	NEt_2	2	-	16.0
Falcon		88	65	

(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 (Table 1) that percent colony formation is dependent on oxygen permeability of the films, except 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) $[NP(OC_6H_5)_2]_n$ and poly(bisbutylaminophosphazene) $[NP(NHBu\text{-}n)_2]_n$ had higher percent adhesiveness. The results obtained are presented in Fig. 2 and Table 2.

It was found (Fig. 2) that colony formation is increased with increasing x value. It was also found (Table 2) that in this study, compositions of the best films having the highest percent colony formation are $[NP(OC_6H_5)_{1.8}(NHBU\text{-}n)_{0.2}]_n$ and the percent colony formation of these is a little lower than that of Falcon* Petri dishes. It is difficult to explain why these polymers have the highest adhesiveness and percent colony formation in comparison with other films. There is no relation between the x values of $[NP(OC_6H_5)_{2-x}(NHBu\text{-}n)_x]_n$ and percent adhesiveness or colony formation. Furthermore, the new behavior is observed which occurs after colonies are grown and removed from the film's surface. It is difficult to judge if removing cells represents a good or bad influence of the bioreactor materi-

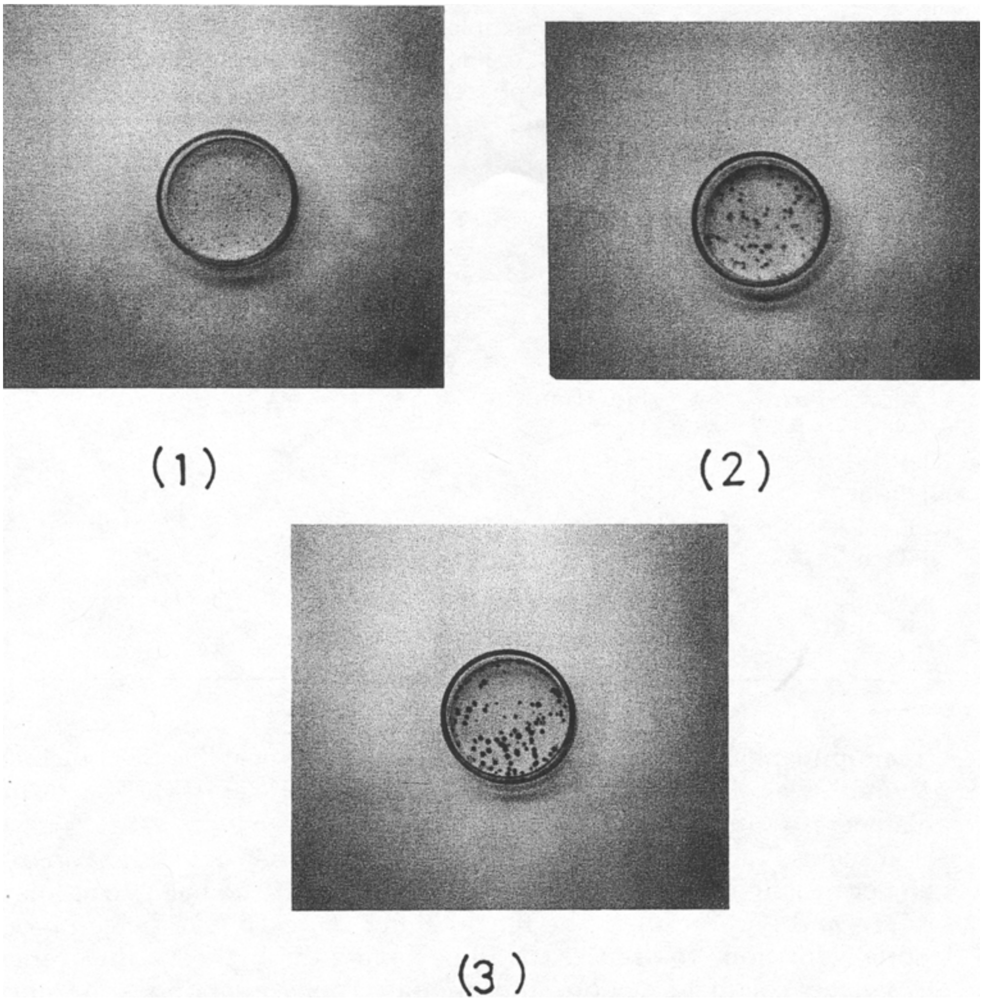

Fig. 2. Formation of colony with $[NP(OC_6H_5)_{2-x}(NHBu\text{-}n)_x]_n$. (1) x = 0.2; (2) x = 1.0; (3) x = 1.8.

Table 2
Adhesive (A) and Percent Colony Formation (B) of Poly(organophosphazene) $[NP(OC_6H_5)_{2-x}(NHBu\text{-}n)_x]_n$ Using PV-79 Cells

$[NP(OC_6H_5)_{2-x}(NHBu\text{-}n)_x]_n$	(%)	
x	(A)	(B)
0	77	50
0.2	84	54
1.0	62	77
1.8	84	53
2.0	61	-

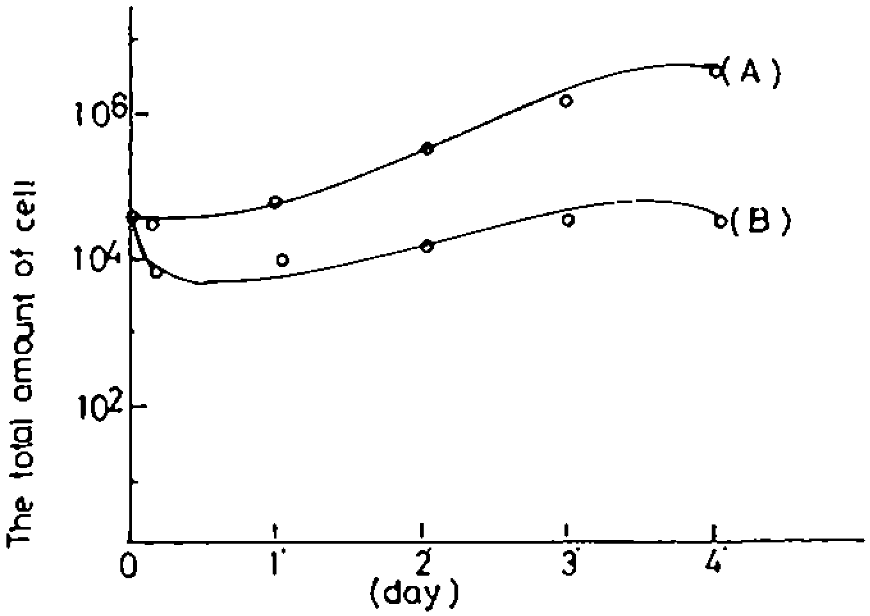

Fig. 3 The relation between the degree of cell cultivation and the reaction time, using Bowes cell line (A) Cytodex III; (B) $[NP(OC_6H_5)_2]_n$.

als using poly(organophosphazenes). This phenomenon may be suitable for artificial skins because the film can be removed from the skin with regeneration of the skin after some poly(organophosphazene) film would be coated on the broken skin and which would hold for several days.

The Cultivation of Bowes and Chinese Hamster Ovary Cell Lines Using Polystyrene Beads Coated with Poly(bisphenoxyphosphazene) $[NP(OC_6H_5)_2]_n$

It was found (Table 1) that the percent adhesiveness and cultivation of V-79 cells for $[NP(OC_6H_5)_2]_n$ film was similar to the values of Falcon® Petri dishes. The cultivation of Bowes and Chinese hamster ovary cell lines were carried out using polystyrene beads coated with the polymer such as $[NP(OC_6H_5)_2]_n$. First, 4×10^4 cells were cultivated on polystyrene beads. The total cell number was estimated by counting them for the period of cultivation. The results obtained are shown in Fig. 3 and Fig. 4.

In the case of Bowes cell line, after 4 h of cultivation, the cell number decreased and closely approached the steady state. Cytodex III, used as the standard sample, showed increased cell numbers with increasing cultivation time as shown in Fig. 3. 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 Cytodex III used as the standard sample. However, the cells disappeared completely after 3 d.

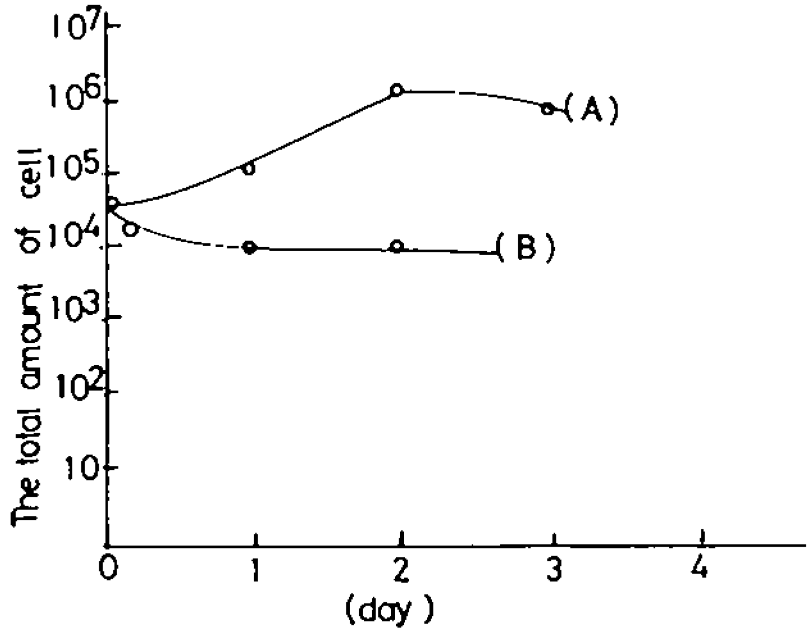

Fig. 4. The relation between the degree of cell cultivation and the reaction time, using Chinese hamster ovary cells. **(A)** Cytodex III; **(B)** $[NP(OC_6H_5)_2]_n$.

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