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J Orthop Sci (2006) 11:359–364
DOI 10.1007/s00776-006-1036-7

JOURNAL OF
ORTHOPAEDIC SCIENCE

Original article

Preparing a version of the Nottingham Adjustment Scale (for psychological adjustment) tailored to osteoarthritis of the hip

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Abstract

Background. The Nottingham Adjustment Scale–Japanese version (hereinafter referred to as NAS-J) was developed to measure psychological adaptation to visually impairment. Several disease-specific modified versions have been developed in Japan. The purpose of this study was to develop and test the reliability, validity, and responsiveness of the NAS-J for hip osteoarthritis patients.

Methods. Patients with osteoarthritis of the hip managed as outpatients at the Department of Orthopaedic Surgery of one university hospital gave informed written consent to be enrolled in this study. Subjects were asked to complete a questionnaire consisting of the NAS-J — Hip edition (hereinafter referred to as NAS-J-HIP), health-related QOL (Short Form 36). Subjects' medical and treatment histories, and the Japanese Hip Society's Evaluation Chart of Hip Joint Functions (hereinafter referred to as the JOA score) were also collected from their medical records. Psychometric analyses were conducted to test reliability, validity, and responsiveness.

Results. A total of 231 patients agreed to participate in the survey, and responses were obtained from 168 (72.7%). Their mean ± SD age was 52.5 ± 12.4 years, and the mean JOA score was 80.9 points. By factor analysis using the principal factor method, seven factors were extracted: (1) anxiety/depression, (2) self-esteem, (3) attitude, (4) locus of control, (5) acceptance, (6) self-efficacy, and (7) attributional style. Concurrent validity was the result according to the near hypothesis. Cronbach's α-coefficient ranged from 0.68 to 0.83, indicating high internal consistency.

Conclusions. For hip osteoarthritis patients, construct validity was confirmed for NAS-J-HIP. Furthermore, seven factors comprising 27 items with high internal consistency were incorporated into NAS-J-HIP. This scale can be used to assess the psychological adaptation of hip osteoarthritis patients.

Offprint request to: Y. Koyama
Received: February 2, 2006 / Accepted: April 25, 2006

Introduction

In patients with osteoarthritis of the hip, daily living is disturbed progressively due to pain and restriction of the range of motion (ROM).[1] It is a chronic, progressive disease involving regressive degeneration of bone. In some foreign countries, total hip replacement is performed at relatively early stages of this disease to alleviate pain and the restricted ROM. In Japan, total hip replacement is often performed for patients with end-stage osteoarthritis of the hip aged over 60 years.[1] In Japan, arthritis of the hip secondary to acetabular dysplasia has a high prevalence. If this condition disturbs the activities of daily living (ADL) due to pain, beginning at relatively young ages, arthroplasty is often performed as a means of symptomatic treatment, followed by conservative treatment (primarily self-control by the patient) until total hip replacement is indicated. These patients often have restrictions in their daily lives due to pain and restricted ROM and are probably exposed to mental stress. To date, however, few reports have been published concerning the psychological aspects of patients with osteoarthritis of the hip,[2] and no psychometric scale for patients with this condition has been developed.

In the field of rehabilitation, it has long been discussed that psychological factors greatly affect the resumption of physical function or social activity of patients. Also in Japan, the view that the acceptance of disabilities is related to the progression of rehabilitation has been widely prevalent, and hypotheses about the psychological process to acceptance have been used extensively. However, the term "acceptance" tends to be used without clear definition, and reports published concerning this topic are confined to theoretical descriptions. Thus, few studies based on clinical data or evidence have been published.

An attempt to explain the structure of psychological adjustment to disabilities using several psychological

factors (including acceptance of disabilities) was made by Dodds et al.[3,4] Dodds and colleagues developed the Nottingham Adjustment Scale (NAS) for multifaceted measurement of the psychological adjustment of patients to visual disorders. They thus created a structural model of psychological adjustment.[5] The NAS, which is used internationally, is composed of seven subcategories (made up of 55 items): (1) anxiety/depression; (2) self-esteem; (3) attitude toward visually impaired people; (4) locus of control; (5) acceptance; (6) self-efficacy; and (7) attributional style. In Japan, Suzukamo et al. prepared a Japanese version of this scale (NAS-J) and its reliability and validity were tested.[6] Furthermore, psychometric evaluation of visually impaired individuals has been made using the NAS-J, and consistency of the evaluation results with the results of the study in British patients conducted by Dodds has been reported.

Dodds et al. noted that NAS is potentially applicable to individuals with disabilities other than visual impairment. In Japan, versions of NAS-J tailored to Parkinson's disease, stroke, and laryngeal cancer[7] have been prepared. These versions of NAS-J involve an architecture of factors resembling that of the original NAS-J for visually impaired people.

Subjects and methods

Subjects

Of the patients with osteoarthritis of the hip managed as outpatients at the Department of Orthopaedic Surgery of one university hospital, those who satisfied the following requirements and gave informed consent in writing were enrolled in this study: (1) age over 20 and less than 80 at the time of enrollment; (2) communication in Japanese was possible; (3) free of dementia and able to complete the survey form by himself/herself; and (4) judged by the attending physician as appropriate for the study.

Methods

With the cooperation of six physicians, patients were asked to participate in the study, and the examiner gave information about the study in writing to each patient in a separate room. Written consent was obtained.

After acquisition of informed consent, the survey form was delivered to each patient. It was filled in by the patient on the spot and immediately recollected after completion. Disease history, details of treatment provided, and other information about individual patients were collected from medical records. The survey lasted from May to July 2004.

Questionnaire items

The term "visually impaired people" or "eye problem" used in the questions contained in NAS-J version 1.0 was replaced with "osteoarthritis of the hip" to yield NAS-J-HIP version 1.0. The NAS-J has seven subcategories: (1) anxiety/depression (6 items to measure slightness of anxiety and depressive state); (2) self-esteem (5 items to measure one's feeling of self-respect); (3) attitude to visually impaired people (5 items to measure a positive attitude for visually impaired people); (4) locus of control (3 items to measure the degree of conviction in which success or failure of rehabilitation is determined by one's actions); (5) acceptance (9 items to measure the degree in which the current state of disability is accepted); (6) self-efficacy (4 items to measure the degree of confidence in having the ability to practice the necessary steps for rehabilitation); and (7) attributional style (6 items to measure the degree in which success is assumed to depend not on external determinants but on one's own competence). The subscale score is calculated as: Subscale score = [(low score – minimum score)/(maximum score – minimum score)] × 100. High scores for each subcategory are associated with more advanced psychological adjustment. For concurrent validity evaluation of NAS-J-HIP version 1.0, the Medical Outcome Study Short Form 36 version 1.2[8–10] (hereinafter referred to as SF-36) was used. On the basis of the medical records for individual patients, background variables (age and sex), the Evaluation Chart of Hip Joint Functioning prepared by the Japanese Orthopaedic Association (JOA score),[11] affected side (bilateral or unilateral), duration of sickness, frequency of past operations, operative procedure, and length of time (years) after operation were investigated.

Method of analyses

Face validity

The questions contained in the NAS-J-HIP version 1.0 were checked for inappropriate expressions that could offend the respondents. This check was done by five patients with osteoarthritis of the hip, three physicians specializing in osteoarthritis, and five nurses with clinical careers of more than 3 years in the field of orthopedic surgery.

Factor validity

Factor analysis (principal factor method, promax rotation) was conducted to examine the structure of the factors. The criterion factor load was set at 0.4. Items with factor loads smaller than 0.4 or items with loads on multiple factors larger than the criterion level were eliminated one after another from the analysis. Factor

analysis was thus performed repeatedly until the domain became interpretable.

Concurrent validity

The following hypotheses were examined using Pearson's correlation coefficient.

1. "Anxiety/depression" and "self-esteem" would be highly correlated with "mental health" and "vitality" on the QOL measure of the SF-36.
2. "Acceptance" would be highly correlated with "physical functioning," "bodily pain," "vitality," and "mental health" on the QOL measure of the SF-36.
3. "Attitudes," "locus of control," "acceptance," "self-efficacy," and "attributional style" would not be correlated with any of the SF-36 subscales.
4. No correlation exists among each subscale of NAS-J-HIP except for "acceptance," which would be correlated with the lower measure of the JOA score.

Internal consistency

As a measure of internal consistency, Cronbach's α-coefficient was calculated for each subscale. In this research, two-sided testing was conducted, with the level of significance established at the $P < 0.05$ level. All statistical analyses were done with SAS software, S version 9.1 for Windows (SAS Institute, Cary, NC, USA).

Ethical considerations

The protocol of the present research was approved by the Tokyo University Medical Research Ethics Committee at the University of Tokyo. Furthermore, to ensure subjects' voluntary participation, the authors explained to all patients that nonparticipation would not have any effect on their treatment, that personal information collected concerning this research would be kept strictly secret, that all questionnaire responses and interview data would never be conveyed to the patients' doctors, and that all surveys would be conducted in person in a private room. Patients' agreement to participate in the present research was confirmed in writing.

Results

Subjects' characteristics

A total of 231 patients were asked to participate, and 168 patients (72.7%) agreed and responded to the questionnaire. The reason of those who did not agree were the following explanations: "I do not have enough time" (60 patients), and "I cannot understand the necessity for the research" (3 patients). Five patients were excluded from the analysis because they had not responded to more than 20% of the questionnaire items. Thus, 163 patients were included in the final analysis.

Subjects' backgrounds

Patient characteristics are shown in Table 1. The mean age of subjects was 52.5 years (SD ± 12.4), and the mean JOA score was 80.9 points (SD ± 16.8).

Table 1. Characteristics of the sample ($n = 163$)

Parameter	No.	
Sex		
Male	10 (6.1%)	
Female	153 (93.9%)	
Age (years)		52.5 ± 12.4 (23.0–77.0)
Duration of osteoarthritis (years)		9.3 ± 8.1 (0.1–36.0)
JOA score (points)		80.9 ± 16.8 (7.0–100.0)
Weight (kg)		52.9 ± 8.9 (35.0–86.0)
BMI (kg/m^2)		22.2 ± 3.5 (15.6–33.3)
Surgical treatment		
Rotational acetabular osteotomy	49 (30.1%)	11.2 ± 8.7[a]
Chiari pelvic osteotomy	1 (0.6%)	17.0[a]
Varus osteotomy	4 (2.5%)	9.0 ± 6.1[a]
Valgus osteotomy	12 (7.4%)	10.4 ± 8.0[a]
Total hip arthroplasty	22 (13.5%)	5.1 ± 5.8[a]
Revision	9 (5.5%)	6.2 ± 5.5[a]
Second revision	1 (0.6%)	2.0[a]
Asthrodesis	1 (0.6%)	24.0[a]
Conservative treatment	64 (39.3%)	7.8[b] ± 7.6

BMI, body mass index
[a] Duration after the operation
[b] Duration of osteoarthritis with conservative treatment

Table 2. Results of factor analysis of the NAS-J-H: factor loadings after principal factor method (promax rotation) and Cronbach's alpha coefficients (n = 163)

Analysis	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Communality estimates
Anxiety/depression: 77.5 ± 20.5 (mean ± SD), range 0–100[a] α = 0.80								
Have you recently found everything getting on top of you?	0.68	−0.07	0.01	−0.07	0.20	−0.05	0.05	0.50
Have you recently been feeling nervous or strung out all the time?	0.65	0.28	−0.06	−0.04	0.08	−0.01	0.01	0.64
Have you recently felt constantly under strain?	0.57	0.27	0.00	−0.04	−0.03	−0.08	−0.04	0.49
Have you recently found at times you couldn't do anything because your nerves were so bad?	0.33	0.40	−0.06	0.22	0.14	−0.13	−0.02	0.59
Self-esteem: 60.8 ± 20.9 (mean ± SD), range 0–100[a] α = 0.81								
I feel that I do not have much to be proud of.	0.05	0.76	−0.05	0.11	−0.08	0.07	−0.05	0.65
I certainly feel useless at times.	−0.02	0.73	0.04	−0.01	0.10	−0.01	0.00	0.62
At times I think I am no good at all.	0.13	0.64	0.06	−0.12	−0.02	0.11	−0.06	0.51
I wish I coud have more respect for myself.	0.15	0.49	0.31	−0.11	0.00	0.10	0.14	0.52
Attitudes: 53.3 ± 20.9 (mean ± SD), range 0–100[a] α = 0.68								
Most osteoarthritis of the hip people believe that disability of the hip is the worst thing that could happen to them.	−0.13	0.23	0.52	0.12	0.11	−0.08	0.06	0.46
Most osteoarthritis of the hip people keep a lot of things to themselves.	0.17	−0.10	0.45	0.11	0.17	−0.08	−0.11	0.43
Osteoarthritis of the hip people are generally more easily upset than healthy people.	−0.07	0.09	0.41	0.17	0.28	−0.06	−0.06	0.49
Locus of control: 67.2 ± 23.1 (mean ± SD), range 0–100[a] α = 0.76								
I have little or no control over my progress from now on.	−0.05	−0.02	0.07	0.76	0.04	−0.02	0.06	0.60
My own contribution to my rehabilitation doesn't amount to much.	−0.07	0.00	0.09	0.68	0.00	0.15	−0.05	0.59
Acceptance 65.2 ± 23.1 (mean ± SD), range 0–100[a] α = 0.86								0.32
My osteoarthritis of the hip problem prevents me from doing just about everything I really want to do and from being the kind of person I really want to be.	−0.01	−0.02	−0.01	−0.05	0.87	−0.02	−0.04	0.69
Because of my osteoarthritis of the hip problem, other people's lives have more meaning than my own.	0.09	0.06	0.03	0.23	0.61	0.02	0.04	0.68
I feel satisfied with my abilities, and my osteoarthritis of the hip problem doesn't bother me too much.	0.18	0.11	0.06	0.01	0.55	0.10	0.04	0.57
It makes me feel very bad to see all the things healthy people can do that I cannot.	0.18	−0.04	0.04	−0.06	0.50	0.04	−0.04	
In just about everything, my osteoarthritis of the hip problem is so annoying that I can't enjoy anything.	0.01	0.00	0.05	0.00	0.75	0.07	0.01	0.65
Self-efficacy 61.4 ± 21.2 (mean ± SD), range 12.5–100.0[a] α = 0.83								0.65
I give up easily.	0.01	0.06	0.00	−0.08	−0.03	0.84	−0.01	0.69
When trying to learn something new, I soon give up if I am not initially successful.	−0.04	0.11	0.08	0.06	−0.10	0.75	0.03	
I avoid trying to learn new things when they look too difficult for me.	−0.04	0.10	−0.10	0.14	0.05	0.70	−0.03	0.63
Failure just makes me try harder.	−0.10	−0.15	−0.15	−0.01	0.30	0.55	0.04	0.39
Attributional style 56.5 ± 17.6 (mean ± SD), range 5–100[a] α = 0.79								0.57
If things go well it's just good luck.	0.24	−0.06	−0.05	0.12	−0.08	0.12	0.77	0.63
Any successes I have had have been due to good fortune.	−0.03	−0.16	0.08	−0.07	−0.02	0.01	0.73	
Any successes I've had have been due to the fact that circumstances have happened to be right.	−0.06	0.02	0.09	−0.07	0.08	0.07	0.73	0.53
If things go well it's because the system helped me.	0.05	−0.04	−0.13	0.13	−0.21	−0.16	0.57	0.49
Any successes I've had have been due to outside influences.	−0.32	0.33	−0.12	−0.08	0.18	−0.15	0.48	0.46

Total contribution 55.7%

[a] All measures are scored from 0 to 100

Table 3. Correlation matrix (Pearson's correlation coefficient) between the seven subscales of the NAS-J-H and the eight subscales of the SF-36 (n = 163)

	SF-36							
NAS-J	Physical functioning	Role physical	Bodily pain	General health	Vitality	Social functioning	Role emotional	Mental health
Anxiety/depression	0.29***	0.22**	0.32***	0.42***	0.54***	0.37***	0.22**	0.64***
Self-esteem	0.28***	0.18*	0.24**	0.36***	0.43***	0.36***	0.28***	0.54***
Attitudes	0.32***	0.22**	0.25**	0.36***	0.34***	0.25**	0.27***	0.37***
Locus of control	0.26**	0.20**	0.26**	0.31***	0.21**	0.23**	0.23**	0.29***
Acceptance	0.47***	0.27***	0.43***	0.49***	0.45***	0.38***	0.32***	0.50***
Self-efficacy	0.08	0.01	0.10	0.17*	0.09	0.00	0.03	0.15
Attributional style	0.10	0.08	−0.02	−0.20*	−0.16*	−0.02	0.09	−0.14

Numbers that are underlined indicate Pearson's correlation coefficient >0.40
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 4. Correlation matrix (Pearson's correlation coefficient) between the seven subscales of the NAS-J-H and the four subscales of the JOA score (n = 163)

	JOA score				
NAS-J	Total	Pain	Range of motion	Walking	ADL
Anxiety/depression	0.21**	0.15	0.07	0.11	0.16*
Self-esteem	0.25**	0.14	0.15	0.17*	0.20*
Attitudes	0.20*	0.22**	0.08	0.14	0.21**
Locus of control	0.23**	0.22**	0.22**	0.20*	0.22**
Acceptance	0.43***	0.40***	0.27***	0.39***	0.35***
Self-efficacy	0.09	0.08	0.14	0.03	0.07
Attributional style	0.11	0.07	0.07	0.14	0.08

ADL, activities of daily living
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Factor validity

The distribution of responses for each item of the NAS-J-HIP was examined. More than 80% of responses were concentrated on one choice in one item of "anxiety/depression"; thus, this item was excluded. A factor analysis was conducted on the remaining 27 items. The purpose of the factor analysis was to identify seven factors of NAS-J. "Have you recently found at times you couldn't do anything because your nerves were so bad?" in "anxiety/depression" took it for "self-esteem" in the factor load quantity 0.40. But it thought about semantic unity, we decided to keep it in "anxiety/depression" (Table 2).

Factor analysis extracted the following seven factors and their component items: "anxiety/depression" (4 items), "self-esteem" (4 items), "attitudes" (3 items), "locus of control" (2 items), "acceptance" (5 items), "self-efficacy" (4 items), and "attributional style" (5 items).

Internal consistency

Cronbach's α-coefficient was 0.80 for "anxiety/depression," 0.81 for "self-esteem," 0.68 for "attitudes," 0.76 for "locus of control," 0.86 for "acceptance," 0.83 for "self-efficacy," and 0.79 for "attributional style." These results indicate high internal consistency.

Concurrent validity

The relation between NAS-J-HIP and scores on the eight subscales of the SF-36 was examined with correlation analysis (Table 3). "Anxiety/depression" had moderate correlations for "general health" (r = 0.42), "vitality" (r = 0.54), and "mental health" (r = 0.64) of the SF-36. For all subscales except "role-physical" of the SF-36, moderate correlations were observed with "acceptance" (r = 0.32–0.50). "Locus of control," "self-esteem," "attributional style," and "attitudes" showed only mild correlations with the SF-36 subscales. Regarding JOA scores, no correlation was observed for any subscale except "acceptance" of the NAS-J-HIP.

Discussion

Validity of the NAS-J-HIP

The results of the investigation among hip OA patients indicate that the NAS-J-HIP has a factor structure similar to that of the personal edition of the NAS-J for visually impaired people, thus indicating construct validity. In addition, seven factors consisting of 27 items with high internal consistency were incorporated into the NAS-J-HIP. Regarding concurrent validity, results of a comparison between the SF-36 subscales and JOA scores supported our hypotheses.

The fact that the correlation between the SF-36 sub-scales of "general health," "vitality," and "mental health" and the "anxiety/depression" subscale of the NAS-J-HIP was high is probably because "anxiety/depression" is a sympathy movement factor, whereas other factors are recognition-like ones in the NAS-J and because hip OA is a progressive disease.

A moderate correlation was observed between scores of "pain," "walking," and "ADL" of the JOA score and the "acceptance" score in the NAS-J-HIP (Table 4). This may reflect the fact that major symptoms of hip OA are pain and disability of ADL. As has been indicated in previous research on psychological adaptation,[12] the subscale of "acceptance" refers to the patient's willingness to control his or her own symptoms and to "live his/her own life within the limitation."[12] Thus, it is possible for hip OA patients to "accept their disabilities" if they are able to control their pain. Furthermore, because "acceptance" was correlated with health-related QOL, it is also possible to improve health-related QOL by controlling the pain and by promoting "acceptance." However, further research is required to clarify this point.

As for reliability, the Cronbach's α-coefficient was high (>0.76) for most factors; however, the value for "attitudes" was low (0.68). This may be related to the fact that many hip OA patients tend to regard themselves as healthy despite the pain and limited ROM. Therefore, it can be concluded that it is possible to measure psychological adaptation using the NAS-J-HIP.

In this research, we established the first scale for assessing the psychological adaptation of hip OA patients in Japan.

Limitations of the present research and future research topics

The sample population in the present study was limited to individuals from one hospital; therefore, the results should be interpreted carefully. It is possible that the psychological adaptation of hip OA patients differs among those who have undergone surgery and those who are still receiving conservative treatment. This should be taken into consideration when the NAS-J-HIP is applied.

Using the scale developed in the present research, the relation between the psychological adaptation of hip OA patients and the care provided by medical staff can be elucidated. It is also expected that the NAS-J can be used to examine the differences in psychological adaptation between hip OA patients and patients with other diseases, such as visually impaired people.

Acknowledgments. We express our sincere gratitude to those individuals who aided in the creation of the questionnaire and the completion of this survey; Dr. Mabuchi, Dr. Fukatani, and Dr. Miura (Tokyo University Hospital), who not only solicited their patients to participate in this research but provided valuable expert advice; and our colleagues at the Adult Nursing/Palliative Care Nursing, School of Medicine, Tokyo University, who all provided beneficial advice at every phase of this research. NAS-J-HIP and the Scoring Manual can be downloaded from http://www.i-hope.jp.

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Stress response of adherent cells on a polymer blend surface composed of a segmented polyurethane and MPC copolymers

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Received 2 December 2005; revised 22 February 2006; accepted 7 March 2006
Published online 6 June 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.30820

Abstract: To better understand the effect of 2-methacryloyloxyethyl phosphorylcholine (MPC) copolymer in improving the biocompatibility of segmented polyurethane (SPU), the expression of heat shock protein (HSP) mRNA in HeLa S3 cells adhered on SPU blended with MPC copolymers was measured. Conventionally, MPC copolymers (PMEH) were synthesized by changing the feed ratios of MPC and 2-ethylhexyl methacrylate. X-ray photoelectron spectroscopic analysis of the SPU/PMEH film indicated that the surface concentration of MPC units on the SPU/PMEH film increased with an increase in PMEH composition. HeLa S3 cells were cultured on SPU/PMEH films. The number of adherent cells on the SPU/PMEH films decreased with an increase in the concentration of PMEH. When the PMEH composition was greater than 0.5 wt %, cell adhesion and proliferation decreased markedly. Expressions of HSP27 and HSP47 mRNA were detected using the reverse transcription-polymerase chain reaction (RT-PCR). After incubation for 24 h, both the HSP mRNA expressions in the HeLa S3 cells showed no significant differences among all samples. In HeLa S3 cells that adhered to the SPU film for 48 h, the expressions of HSP27 and HSP47 mRNA increased significantly when compared with those incubated for 24 h. In contrast, the two kinds of mRNA expressions decreased in the HeLa S3 cells that adhered to the SPU/PMEH films for 48 h. From these results, we concluded that PMEH was quite important in suppressing the stress response of adherent HeLa S3 cells. Therefore, SPU/PMEH blend polymers are useful as implantable biomedical materials. © 2006 Wiley Periodicals, Inc. J Biomed Mater Res 79A: 476–484, 2006

Key words: mRNA expression; heat shock protein (HSP); MPC polymer; biocompatibility; segmented polyurethane

INTRODUCTION

Segmented polyurethanes (SPUs) have soft segments that mostly consist of polyether chains and hard segments that are primarily composed of polyurethane blocks.[1] The phase separation of the hard and soft segments dominates the complicated and desirable characteristics of the SPUs. The good stability, excellent mechanical and physical properties, and thermoplasticity of the SPUs should enable long-term applications as biomedical devices with various shapes, such as catheters, diaphragms for artificial hearts, coating materials for pacemaker leads, etc.[1] However, the biocompatibility and chemical stability of SPUs under biological conditions are not satisfactory for long-term implantation. We have been studying a method of improving SPU biocompatibility with 2-methacryloyloxyethyl phosphorylcholine (MPC) copolymers.[2–5] In particular, a polymer blend composed of SPU and poly(MPC-*co*-2-ethylhexyl methacrylate (EHMA)) (PMEH) was successfully used in the fabrication of blood-compatible, small-diameter ($\wp$ 2 mm) vascular prostheses.[6,7] The blood compatibility of the polymer blends was quite stable and was not even affected by heat processing.[8]

Detailed elucidation of the interactions between cells and biomaterials is important in the development of implantable biomaterials. When a cell is exposed to such materials, the cell–material interaction consists of various biological processes.[9] A great amount of research has been done using different kinds of cells and various techniques, such as counting adherent cells, morphological observations, and the evaluation of bioactive substances secreted from adherent cells.[10–12]

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Contract grant sponsor: New Energy and Industrial Technology Development Organization (NEDO), Japan; contract grant number: 04A02025

Novel tools and techniques for studying cell function have recently been developed, and cell responses to materials have come to be evaluated in more detail. The reverse transcription-polymerase chain reaction (RT-PCR) is one of the useful techniques for evaluating gene expression in cells. Gene expression in a small number of cells can be analyzed by the RT-PCR because it is the most sensitive among available methods such as Northern blotting.[13]

In 2003, the expression of interleukin-1β (IL-1β) mRNA as a probe gene in the differentiated promyelocytic leukemia HL-60 cells that adhered to surfaces coated with poly(MPC-*co*-*n*-butyl methacrylate) was published.[14] The IL-1β mRNA expressions on the MPC polymers were significantly decreased in comparison with widely used reference biomaterials. IL-1β is one of the proinflammatory cytokines that mediates the activation and proliferation of lymphocytes, fibroblasts, and endothelial cells.[15] It was concluded that MPC copolymers minimize inflammatory response to adherent HL-60 cells. However, the study of inflammatory cells alone is not sufficient in understanding the body's reaction to foreign materials. It would also be wise to study other types of stress proteins that mRNAs accumulated in other cells.

Heat shock protein (HSP) creation is an important molecular bioresponse when cells are subjected to physical or chemical stress.[16] The creation of HSPs is induced in cells by a variety of stress stimuli including heat shock, oxidative free radicals, toxic metal ions, and ultraviolet irradiation.[17–19] A few studies of HSP gene expressions in cells exposed to biomaterials have been reported.[13,20,21] Kishida and coworkers reported on the expression of HSP (HSP70, HSP90, and HSP47) mRNA in cells on various polymers.[22] In this study, we measured the generation of HSP27[23] and HSP47[24] to determine cell responses. HSP27 is one of the small HSPs and is overexpressed in response to thermal stress, oxidative stress, inflammatory cytokines, and retinoic acid.[23,25] HSP27 is thought to be involved in various cellular processes such as displaying the chaperone function, contributing to the organization of the cytoskeleton, and protecting the cell against apoptosis under oxidative stress. HSP47 was found to be a collagen-binding glycoprotein in collagen-producing cells, and this protein was heat inducible and sensitive to malignant transformation.[24]

The purpose of this study was to show the expressions of HSP mRNAs in cells cultured on MPC polymer surfaces. HSP27 and 47 are typical proteins of the main inducible HSPs. HSP27 levels in unstressed cells are generally low; the protein rapidly increases during a stress event.[26] In addition, Kishida and coworkers evaluated the biocompatibility of biomaterials, focusing on the expression of HSP47 mRNA as a stress marker.[27] We then determined the expression of HSP27 and 47 mRNA from cells in contact with biomaterials. The HeLa S3 cell, which has been known as a typical HSP-inducible cell,[28] was selected. In this evaluation, the SPU/MPC polymer surface was used to alter the surface concentration of MPC units. The results could be very helpful in understanding the efficiency of MPC copolymer-additives in improving SPU biocompatibility.

MATERIALS AND METHODS

Materials

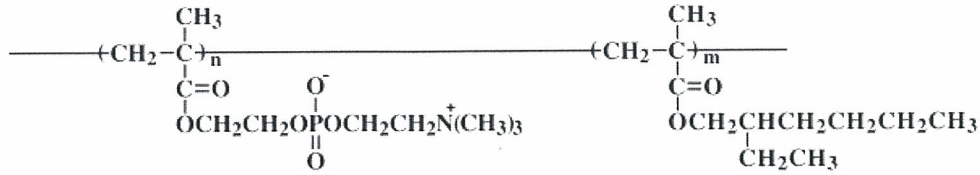
MPC was synthesized by a method reported previously.[29] The EHMA was purified by distillation under reduced pressure in an argon atmosphere, and a bp 56.0°C/1.0 mmHg fraction was used. 2,2′-Azobisisobutyronitrile (AIBN) was recrystallized from methanol. As an SPU, Tecoflex® EG-60D was obtained from Thermedics (MA, USA) and purified by reprecipitation so as to remove all additives. Ethanol (EtOH) and methylene dichloride (CH_2Cl_2) were purified by distillation. The other reagents were of extra pure reagent grade and used without further purification. As typical culture material, tissue culture polystyrene (TCPS; NUNC Co., Denmark) was used.

Preparation of poly(MPC-*co*-EHMA) (PMEH)

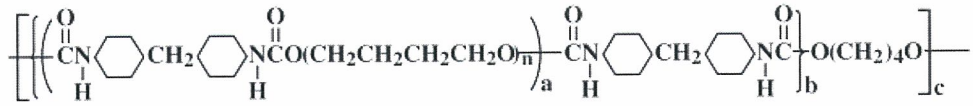
The PMEH was synthesized by conventional radical copolymerization with corresponding monomers using AIBN as the initiator in EtOH. The PMEH was purified using a reprecipitation technique. The structure of the PMEH was confirmed by ^{1}H NMR and FT-IR, and the MPC unit composition in the PMEH was determined by phosphorous analysis. The molecular weight (M_w) of the PMEH was evaluated by gel-permeation chromatography (GPC, Jasco, Tokyo, Japan) in a methanol/water mixture (7/3 by volume) at room temperature. Calibration was based on the elution time of the GPC curve using poly(ethylene oxide) standards. The chemical structures of PMEH and SPU are shown in Figure 1.

Preparation of SPU/MPC polymer blend film

Solutions containing 5.0 wt % SPU and 5.0 wt % PMEH in an EtOH/CH_2Cl_2 mixture (3/7 by volume) were separately prepared. The PMEH solution was mixed into the SPU solution in specific polymer compositions of 0.125, 0.25, 0.5, 1.0, 2.5, and 5.0 wt %. The mixed solutions were then stirred for 30 min and sonicated for another 30 min at room temperature. The solutions (20 mL) were cast on 20-cm^2 glass dishes. To evaporate the solvents, each dish was kept for 5 h at room temperature, and glass plates for covering the dishes were used to control the rate of solvent evaporation. The dishes were kept at 60°C in air overnight. To completely



PMEH



SPU

Figure 1. Chemical structure of PMEH and SPU.

evaporate the remaining solvents, the dishes were then dried in a vacuum at 60°C for another overnight period. The SPU/PMEH films that formed in the dishes were carefully peeled off. The same procedure was used to prepare the SPU films without the PMEH. The films were 200 μm thick. In subsequent experiments, the side of the film contacting the glass was used.

Surface analyses of SPU/PMEH films

Surface analyses of the SPU and SPU/PMEH films were performed using an X-ray photoelectron spectroscope (XPS; AXIS-HIS, SHIMADU, Kyoto, Japan). Measurements were performed at room temperature. The take-off angle of the photoelectrons was 90°.

Culture of cells on the SPU/PMEH film surfaces

The SPU/PMEH films were placed in a 24-well tissue culture plate (NUNC Co., Denmark). Human cervical carcinoma cells (HeLa S3; IFO50011, obtained from the Health Science Research Resources Bank (HSRRB), Osaka, Japan) were added to each well at a concentration of 2×10^4 cells/well in a cell culture medium (Eagle's MEM; Nissui Pharmaceutical, Tokyo, Japan) with 10% fetal bovine serum (FBS; GIBCO BRL, Rockville). The cells were incubated for a specific time at 37°C, 5% CO_2, and 95% humidity. After incubation, the surfaces were washed with phosphate buffered saline (PBS). The cells that adhered to the surface were fixed with 10% formaldehyde neutral buffer solution for 2 h at room temperature. The cells on the surface were then dehydrated in a graded series of EtOH (50, 60, 70, 80, 90, and 100%) for 10 min. Finally, they were vacuum-dried and coated with gold to enable examination of the morphology of the adherent cells using a scanning electron microscope (SEM) (JSM-5400; JEOL, Tokyo, Japan). The number of adherent cells was determined by lactate dehydrogenase (LDH) assay. After incubation, the films were rinsed with PBS and then placed in 0.5% Triton®X-100 to lyse the adherent cells. LDH activity in the lysed cell suspensions was measured using a Wako LDH-cytotoxic Test Kit (Wako Pure Chemical Industries, Osaka, Japan). The measurements were performed using triplicate samples for each film.

Measurement of adsorbed fibronectin on SPU/PMEH film surfaces

The bovine serum fibronectin adsorbed on the SPU/PMEH film surfaces from the cell culture medium was detected by a method based on the antigen–antibody reaction using enzyme-labeled immunoglobulin.[30] The SPU/PMEH films (14 mm diameter) were placed in a 24-well tissue culture plate and secured with a silicone rubber ring. They were allowed to contact PBS overnight at room temperature to equilibrate the surfaces. After the PBS was removed, the cell culture medium was added to each well and kept for 1 h at 37°C. As a negative control, each kind of films was soaked in medium that did not include FBS. The SPU/PMEH films were then rinsed with PBS. For the determination of fibronectin adsorption, the SPU/PMEH films were soaked in bovine serum albumin (BSA; Sigma, St. Louis, MO) solution (1 wt % BSA in PBS) to inhibit any undesirable reactions and nonspecific adsorption with the following antibody. The SPU/PMEH films were kept at 4°C overnight and then rinsed with PBS. They were incubated with the primary antibody (anti-bovine fibronectin rabbit polyclonal antiserum; Yagai Co., Yamagata, Japan) for specific proteins for 1 h at 25°C. The primary antibody reacted with the adsorbed fibronectin on the SPU/PMEH surfaces, and then the SPU/PMEH films were rinsed with PBS. A secondary antibody was then applied to the SPU/PMEH films. Horseradish peroxidase (HRP)-conjugated immunoglobulin (anti-rabbit IgG peroxidase conjugate, Sigma) was used as the secondary antibody. After sufficient rinsing with PBS for the enzyme-linked immunoassay, a solution containing the substrate for HRP, 3,3',5,5'-tetramethylbenzidine (TMBZ; Sumitomo Bakelite Co., Ltd., Tokyo, Japan), was added. Absorbance of the solution at 450 nm after 5 min was recorded. Measure-

TABLE I
Synthetic Results of the PMEH

Abbreviation	MPC Mole Fraction		M_w (10^4)[b]	M_w/M_n[b]	Yield (%)[c]
	In Feed	In Copolymer[a]			
PMEH	0.30	0.32	4.3	1.3	69

[a] Determined by phosphorus analysis.
[b] Determined by GPC with poly(ethylene oxide) standard, M_n and M_w represent the number- and the weight-average molecular weights, respectively.
[c] [Monomer] = 1.0 mol/L, [AIBN] = 5 mmol/L. Polymerization was carried out at 60°C for 2 h in ethanol.

ments were performed using triplicate samples for each polymer. A comparative analysis was done using an analysis of variance and Student's *t*-test.

RNA isolation and RT-PCR

Isolation of total RNA and RT-PCR were performed based on the method reported by Kishida and coworkers[20–22] HeLa S3 cells were cultured on polymer plates for 24 and 48 h. Total RNA was isolated from the cells that adhered to the SPU/PMEH films by the acid guanidine method using ISOGEN (Nippon Gene, Tokyo, Japan) and was dissolved in water treated with diethylpyrocarbonate (DEPC; Sigma Chemical). The RNA concentration was spectrophotometrically measured at 260 nm. The RNA purity was confirmed from the ratio of the absorbance at 260 nm to that at 280 nm. First-strand cDNA synthesis was performed using 1 μg of total RNA. The RNA samples were reversibly transcribed to cDNA using the SuperScript First-Strand Synthesis System (GIBCO BRL, Rockville). The PCR reaction was performed in a volume of 25 μl at a final concentration of 10 mM Tris–HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.2 mM of each dNTP, 0.5 μM of each primer, and contained 1.5 units of Taq DNA Polymerase (Roche Diagnostics, Mannheim, Germany). The HSP27 primer pair was purchased from StressGen, Victoria, BC, Canada. The primer pairs used for β-actin and HSP47 were obtained from published sequences[31] and were purchased from Takara Biochemicals, Kyoto, Japan. The PCR cycle consisted of 94°C for 30 s, 54°C for 1 min, and 72°C for 45 s with the primer sets (β-actin, HSP27, HSP47). The number of PCR cycles was determined to be within a linear range of amplification for the genes of β-actin, HSP27, and HSP47. The products of the PCR reaction were fractionated by electrophoresis on an agarose gel in Tris-acetate-EDTA buffer at 100 V and were visualized by ethidium bromide staining. The amplified products were confirmed to be those of the gene transcripts by the detection of an 821-bp band (β-actin mRNA), a 285-bp band (HSP27 mRNA), and a 492-bp band (HSP47 mRNA). The values of each band were measured by NIH image analysis (NIH, Bethesda, MD).[32] The expressions of HSP27 and HSP47 mRNA were determined as relative values to that obtained for β-actin. In general, amplified β-actin mRNA was used as an internal standard for semiquantification.[33,34] The experiments were performed three times. A comparative analysis was done using an analysis of variance and Student's *t*-test.

RESULTS

Synthesis of PMEH

Table I shows the results of PMEH synthesis. The MPC unit composition in PMEH was 0.32 as a mole fraction, which is almost as same as that in the feed (0.30). The molecular weight of PMEH was 4.3×10^4 and the M_w/M_n was 1.3 as determined by GPC with poly(ethylene oxide) standards.

Surface properties of SPU/PMEH films

The XPS charts of the SPU and SPU/PMEH films are given in Figure 2. In the case of the SPU film, the XPS signals attributed to the carbon in the CH_3— or —CH_2—, —COC—, and —C (=O)— groups, and the nitrogen in the —OCONH— bond were observed at 285.0, 286.6, 288.5, and 400.8 eV, respectively. The same signals were also observed on the surfaces of the SPU/PMEH films. In addition to these signals, new signals at 133 and 403.5 eV were observed; they became remarkable at a higher concentration of PMEH. These signals were attributed to phosphorus and nitrogen in the phosphorylcholine group in the MPC unit. Figure 3 indicates the relationship between the ratio of the peak area of phosphorus to that of carbon (P/C) detected by XPS analysis and the composition of PMEH in the SPU/PMEH films. The value of P/C increased with an increase in the amount of PMEH in the SPU/PMEH film. In particular, a considerable increase in the P/C value was found in the low composition range (0–1.0 wt %).

Cell adhesion on SPU/PMEH film surfaces

The number of HeLa S3 cells that adhered to the SPU/PMEH films for 6, 24, and 48 h is shown in Figure 4. On the SPU/PMEH films, the number of adherent cells decreased with an increase in the con-

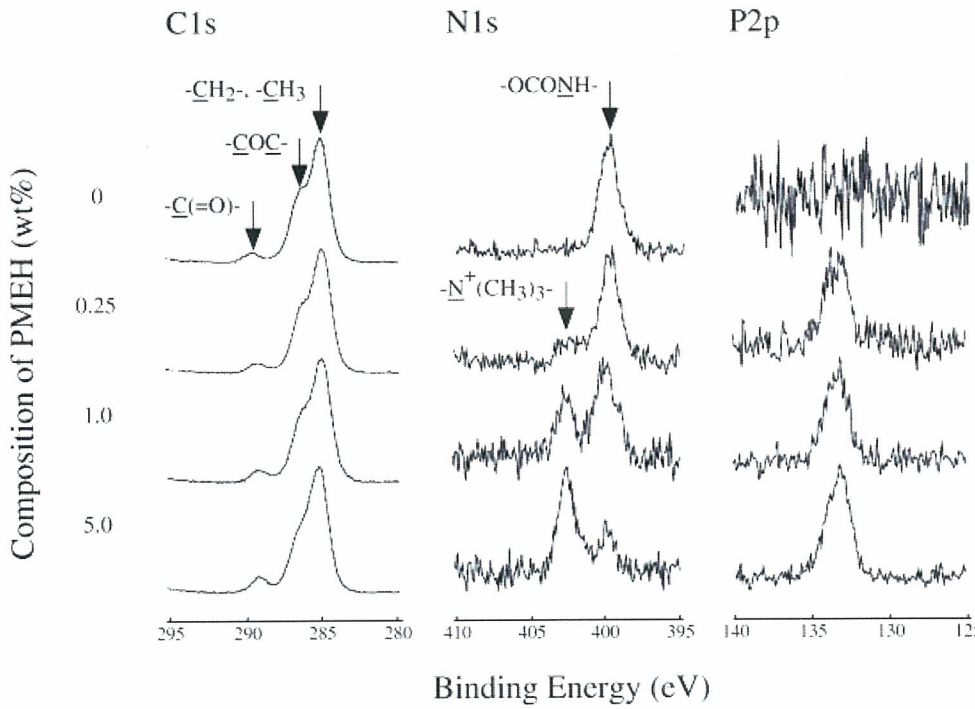


Figure 2. XPS charts of surfaces of SPU/PMEH films.

centration of PMEH. With a PMEH concentration of 0.5 wt % or above, cell adhesion and proliferation were reduced markedly.

Figure 5 shows SEM pictures of the HeLa S3 cells that adhered to the SPU/PMEH film after 24 h of culture. The shape of the cells that adhered to the SPU/PMEH films became round at higher concentrations of PMEH, but those on SPU and TCPS had a spreading morphology.

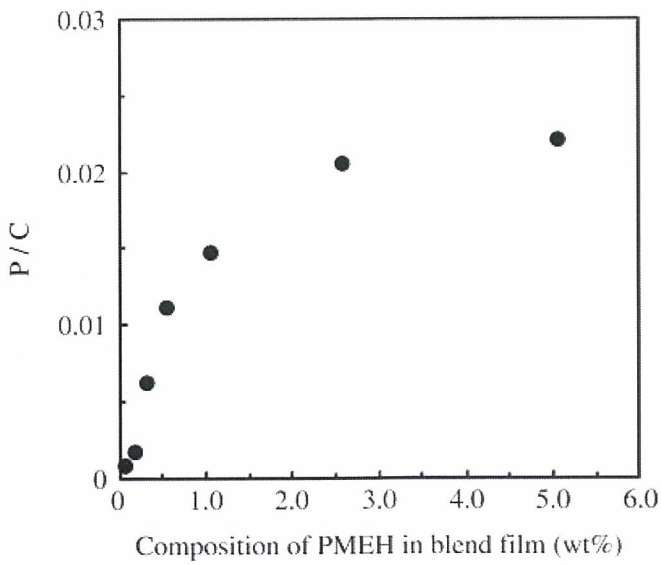


Figure 3. Relationship between phosphorus/carbon ratio determined with XPS and PMEH composition in SPU/PMEH films. The P/C value corresponded to the concentration of the phosphorylcholine group in PMEH near the surface.

Fibronectin adsorption on film surfaces

Figure 6 shows the results of the enzyme-linked immunoassay for fibronectin adsorbed on the SPU/

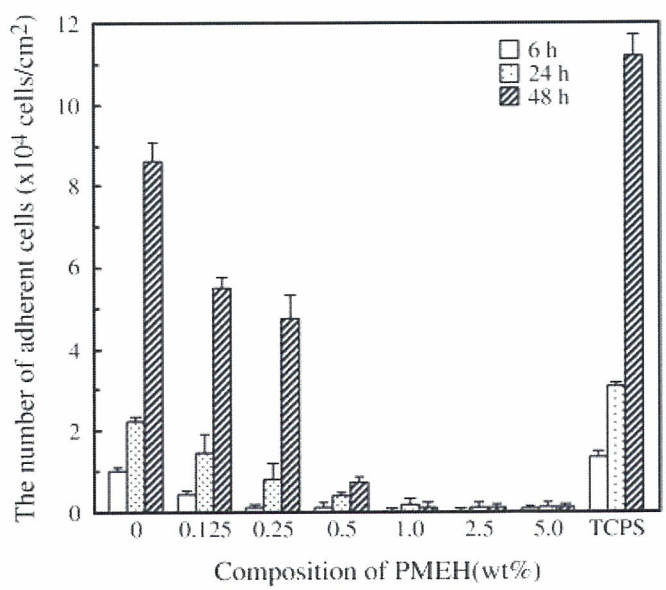


Figure 4. Number of HeLa S3 cells adherent to the SPU and the SPU/PMEH films after incubation for 6, 24, and 48 h. Mean values of three measurements and standard deviations are indicated.

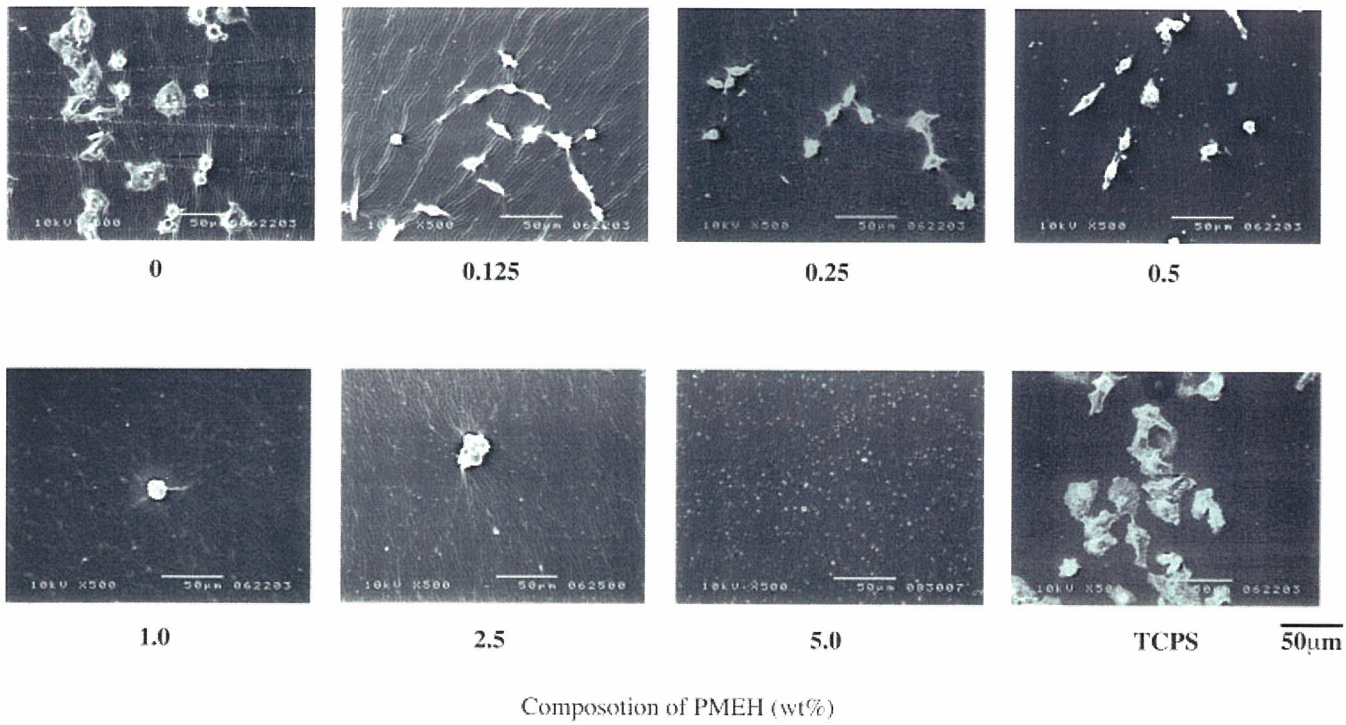


Figure 5. SEM pictures of adherent HeLa S3 cells on the SPU and the SPU/PMEH films after incubation for 24 h.

PMEH film surfaces. The vertical axis is the product absorbance in the reaction solution that is proportional to the amount of fibronectin. Although the amount of fibronectin adsorbed on the SPU/PMEH films was lower than that on the SPU film, the difference among all samples was not pronounced.

HSP27 and HSP47 mRNA expressions

The expressions of HSP27 and HSP47 mRNAs were determined by RT-PCR analysis. Figures 7 and 8 show the relative expressions of the HSP27 and HSP47 mRNAs in HeLa S3 cells that adhered to the SPU/PMEH

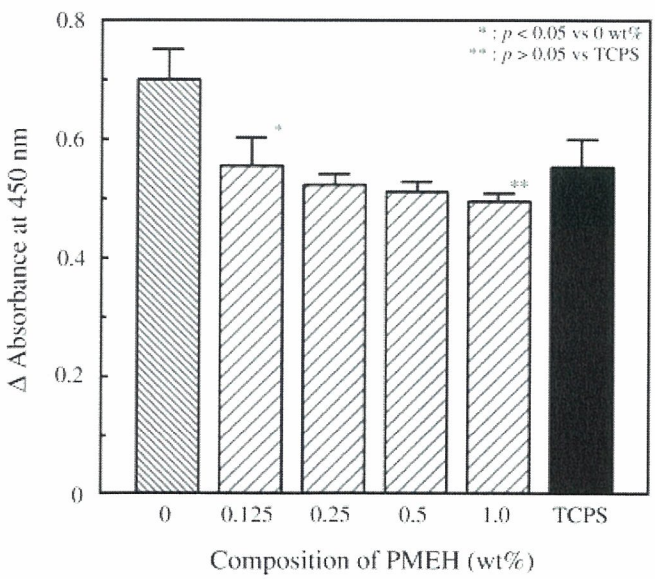


Figure 6. Amount of fibronectin adsorbed on the SPU and the SPU/PMEH films from cell culture medium determined by enzyme-linked immunoassay. Mean values of three measurements and standard deviations are indicated.

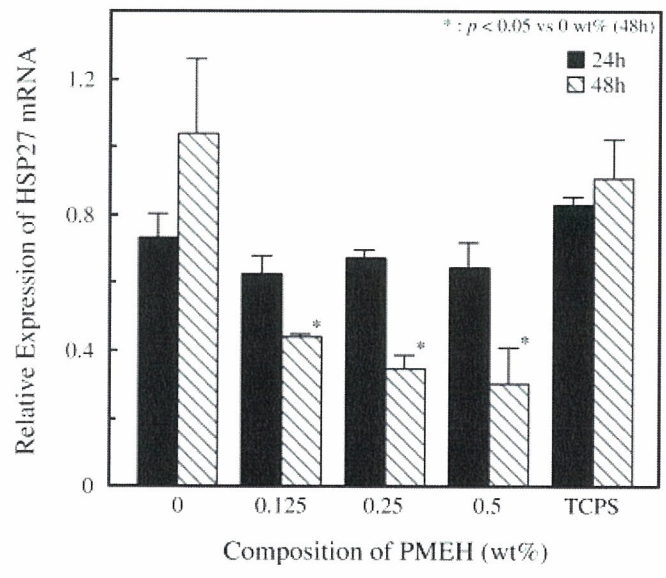


Figure 7. Expression of HSP27 mRNA transcripts in HeLa S3 cells on the SPU and the SPU/PMEH films as a standard of β-actin by RT-PCR after incubation for 24 and 48 h. Mean values of three measurements and standard deviations are indicated.

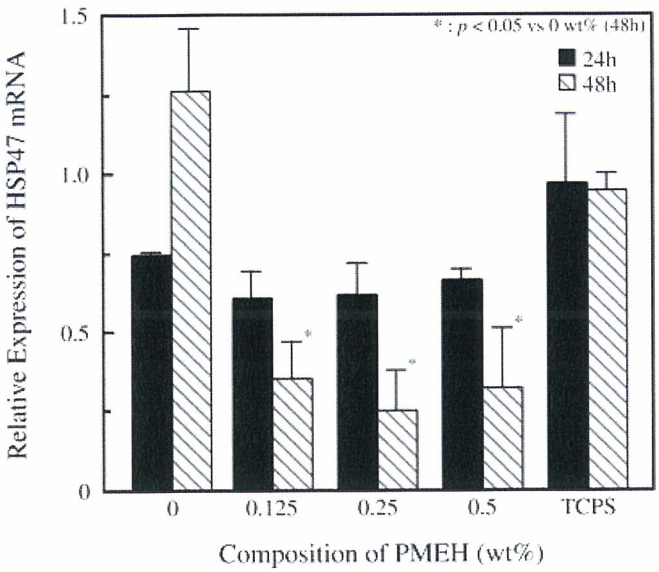


Figure 8. Expression of HSP47 mRNA transcripts in HeLa S3 cells on the SPU and the SPU/PMEH films as a standard of β-actin by RT-PCR after incubation for 24 and 48 h. Mean values of three measurements and standard deviations are indicated.

films for 24 and 48 h, respectively. The vertical axis is the relative amount of mRNA that had been normalized by an amount of β-actin mRNA. In incubation for 24 h, both HSP mRNA expressions showed no significant differences among all samples. In the HeLa S3 cells that adhered to SPU films for incubation of 48 h, the relative expressions of the HSP27 and HSP47 mRNAs obviously increased when compared with those for incubation of 24 h. In contrast, the HSP27 and HSP47 mRNA expressions decreased in the HeLa S3 cells that adhered to the SPU/PMEH films for 48 h. On TCPS, no significant difference based on incubation period was observed in the expressions of HSP27 and HSP47 mRNAs from the HeLa S3 cells.

DISCUSSION

We have previously reported that the blending of the MPC polymer with SPU shows excellent biocompatibility such as suppression of protein adsorption and inhibition of platelet adhesion and activation.[5,6] Interactions between different types of cells and MPC polymer surfaces were reported.[35,36] In these studies, MPC polymers suppressed cell adhesion and proliferation. However, detailed studies of the influence on cell adherence to MPC copolymer surfaces are lacking. The purpose of this research was to collect more information on the interaction between cells and MPC copolymers. Especially, we focused on the stress response of the cell on the MPC materials.

XPS analyses of SPU/PMEH film surfaces indicated that the surface concentration of MPC increased with an increase in the PMEH composition in a solution mixed for film preparation. This tendency was observed in previous studies,[4] and it was thought that the surface concentration of MPC units could be controlled. In this experiment, the side of the SPU/PMEH film in contact with the glass was used because more of the PMEH polymer in the blended film was on that side than on the side exposed to air.[4]

Both the initial adhesion and proliferation of HeLa S3 cells decreased significantly in a PMEH composition of 0.5 wt % or more, as shown in Figure 4. When the PMEH composition was less than 0.5 wt %, the adherent HeLa S3 cells were spread whereas the cells adhering to the SPU/PMEH film that had a PMEH concentration above 1.0 wt % were round (Fig. 5). The cell adhesive proteins adsorbed on the film surface, such as fibronectin, promote cell adhesion and spreading. HeLa S3 cells have fibronectin receptors. However, the number of HeLa S3 cells that adhered to the SPU/PMEH film surface was independent of the amount of fibronectin adsorbed. Horbett and coworkers reported that fibronectin adsorption on the material surfaces changes the conformation of the protein and influences cell adhesion, spreading, and migration.[37] We have already reported that the interactions between the MPC copolymer and the proteins are weak, and the proteins can be reversibly adsorbed.[38] Therefore, we suppose that the adhesive behavior of cells on the SPU/PMEH films might be elucidated in detail by investigating the conformation of adsorbed fibronectin on the SPU/PMEH films.

Different types of cells rapidly induce HSPs under stress conditions such as heat shock. Consequently, the evaluation of HSPs in the cells that adhered to the biomaterials requires consideration of the stress response of cells to biomaterials. The direct detection of HSPs from a small number of cells is difficult. For that reason, we employed the RT-PCR that is highly sensitive and can detect various mRNAs from a very small number of cells adhered to polymeric materials. To optimize cell culture and PCR conditions, cultivate of HeLa S3 was performed under heat treatment. When HeLa S3 cells were heated at 45°C for 20 min, expressions of HSP 27 and 47 mRNA were significantly enhanced when compared with those of control (non heated) cells. As shown in Figures 7 and 8, the relative expressions of HSP27 and HSP47 mRNAs in HeLa S3 cells on the SPU/PMEH films after incubation of 24 h showed no significant difference. Trypsin treatment of the HeLa S3 cells might be related to these results. In the early stages of adhesion, the cells were exposed to stress by treatment with trypsin.[22] On the SPU/PMEH films, the expression of HSP47 mRNA after 24 h decreased markedly when compared with the expression of those after 48 h. Kishida and coworkers published the HSP47 mRNA expressions in

the L929 cells that adhered to various lipid films.[27] They found that the expression of HSP47 mRNA in the L929 cells that adhered to DPPC films increased for the first 24 h and then decreased. In this study, the results of HSP47 mRNA expressions in HeLa S3 cells that adhered to the SPU/PMEH films were similar to that of the L929 cells that adhered to the DPPC film. In addition, HSP27 mRNA expressions on the SPU/PMEH films were also identical with those of HSP47. On the SPU/PMEH films, the expressions of HSP27 and HSP47 mRNAs did not correlate with the number of adherent HeLa S3 cells. We suggest that both protein adsorption and conformational changes influence cell adhesion to materials and that the biocompatibility of materials does not adversely affect the expression of HSPs.

From this study, It has been found that HeLa S3 cells adhered to and proliferated on the SPU/PMEH films had a low concentration of PMEH. The expressions of stress genes such as HSP27 and HSP47 were suppressed in the HeLa S3 cells that adhered to the SPU/PMEH films. It became apparent that the SPU film showed excellent biocompatibility by being blended with PMEH. Further study is necessary to clarify the influence between the conformational changes of adsorbed protein and the expressions of stress genes. We also intend to investigate the stress response of cells exposed to MPC polymer materials *in vivo*.

We gratefully acknowledge the valuable discussions provided by Mr. Shujiro Sakaki, NOF Co., Tokyo, Japan.

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Highly Wettable Polyethylene Films Generated by Spontaneous Surface Enrichment of Perfluoroalkylated Phosphorylcholines

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Received 22 February 2006; accepted 13 April 2006
DOI 10.1002/app.24676
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: We report here a new feature for highly wettable polyethylene films prepared by spontaneous surface enrichment of perfluoroalkylated phosphorylcholine (PC) additives via a simple heat-press technique. Perfluoroalkylated PCs were newly synthesized from monohydroxyethyl ether compounds with hexafluoromethylene (C_6F_{13}PC), octafluoromethylene (C_8F_{17}PC), and decafluoromethylene ($C_{10}F_{21}$PC) chains. Hexadecyl phosphorylcholine (C_{16}PC) was synthesized as a control. These PC additives were mixed well with low-density polyethylene (LDPE) microparticles (ϕ = 6 μm), placed between stainless plates, and pressed at 120°C. Perfluoroalkylated PCs effectively improved the surface wettability of the composite film compared with that of the alkylated PC. C_8F_{17}PC is extremely surface active in the LDPE matrix and occupies ~ 95% of the outermost ~ 10 Å. The water contact angle data for the LDPE film was decreased from 94°/81° (θ_A/θ_R) to 28°/8° by the addition of an approximately low concentration of C_8F_{17}PC (3.3% w/w) because of spontaneous enrichment on the surface. When the elongation to break value of the films was slightly reduced with the PC additives, Young's modulus and the tensile strength of the composite films were similar to those of pure LDPE film. In conclusion, fluoroalkylated PCs have good potential as additives to improve the wettablility of thermoplastic polymers.
 J Appl Polym Sci 102: 2868–2874, 2006

Key words: perfluoroalkylated phosphorylcholine; surface modification; wettability; low-density polyethylene (LDPE)

INTRODUCTION

Recently, there has been a great deal of interest in the control of surface wettability in both research and technology because of the possibilities of many potential applications.[1] Improvements in the hydrophilicity of polymer surfaces can be accomplished by several methods[2] including chemical treatment, plasma-, corona-, photo-irradiation, etc. While these approaches have been quite successful, new processes for surface modification for use with specific equipment are always needed.

To this end, surface modification via surface enrichment of one component of a multicomponent system, the driving force of surface modification in such a system, is largely thermodynamic where the component with the lowest critical surface tension rises to the air/polymer interface, thereby lowering interfacial free energy. Consequently, fluorochemicals and fluoropolymers have often been studied as surface-modifying molecules for various applications.[3–8] To enhance additive efficiency, suitable conditions such as solvents, annealing temperature, and chemical structures were optimized. The surfaces normally show a highly hydrophobic and lyophobic nature. In contrast, Yuan and Shoichet demonstrated that the application of trifluorovinyl ether polymers could enhance the surface wettability of poly(styrene) films.[9] They prepared blend film by the solvent casting method; the surface hydrophilicity on the film depended on the type of solvent.

We have been studying 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers synthesized as biomimetics of biomembrane structures.[10–13] MPC is a highly hygroscopic monomer because of the zwitterionic phosphorylcholine group. MPC polymers have a surface that resists nonspecific protein adsorption and cell adhesion, i.e., "biofouling."[14,15] Biofouling reduces a material's functionality and can induce an unexpected bioreaction. Further, it has been shown that cells in contact with MPC polymers do not exhibit activation or an inflammatory response.[16,17] Using MPC polymers, the surface modification of conventional polymers such as polyurethane,[18] polysulfone,[19] and polyolefine[20] by blending has been studied. Although these blend polymers showed excellent "nonbiofouling" properties, solvents and their evaporation conditions for casting polymers were found to be important for enriching the surfaces of MPC polymers.

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Recently, Nederberg et al. reported on the synthesis and characterization of phosphorylcholine-end capped poly(ε-caprolactone) (PCL-PC) as polymeric additives in a poly(ε-caprolactone) (PCL) membrane. Surface rearrangement and enrichment of the PCL-PC occurred on the PCL membrane by use of the hot-water treatment and resulted in improved surface wettability.[21,22] An environmentally responsive surface (smart surface) with a polymer blend was also demonstrated by Anastasiadis et al.[23] They synthesized polystyrene-*block*-polyisoprene diblocks with hydrophilic groups such as a sulfobetaine- and a dimethylamine group at the end of a low energy polyisoprene block and mixed with polystyrene homopolymer.[23] The wettability of the blend polymer changed with environmental humidity. The surface response demonstrated good harmonization of a hydrophilic functional group and the polyisoprene block. To make additives having PC groups and low energy chemicals or polymers, spontaneous PC-enriched surfaces would be obtained from polymer blending.

We report here on the preparation of highly wettable polyethylene films with small amounts of perfluoroalkylated phosphorylcholine (PC) additives by the heat-press technique. The effects of chemical structure and fluoroalkyl chain length on surface modification were studied.

EXPERIMENTAL

Materials

2-(Perfluorohexyl)ethanol ($C_6F_{13}OH$), 2-(perfluorooctyl)ethanol ($C_8F_{17}OH$), and 2-(perfluorodecyl)ethanol ($C_{10}F_{21}OH$) were purchased from Daikin, Tokyo, Japan. 2-Chloro-2-oxo-1,3,2-dioxaphospholane (COP) was obtained from NOF, Tokyo, Japan. Tetrahydrofuran (THF), acetonitrile (MeCN), triethylamine (TEA), and trimethylamine (TMA) were purified by distillation. All other chemicals were used without further purification.

Synthesis of perfluoroalkylated phosphorylcholines ($C_nF_{2n+1}PCs$)

Perfluoroalkylated phosphorylcholines (n = 6, 8, 10) were synthesized by an improved process of MPC synthesis.[24] Briefly, a solution of 6.43 g (45.0 mmol) of 2-chloro-2-oxo-1,3,2-dioxaphospholane in 25 mL of dry THF was added dropwise to a mixture of 16.38 g (45.0 mmol) of $C_6F_{13}OH$ and 4.55 g (45.mmol) of TEA in 120 mL dry THF at 0°C for 1 h and at room temperature for another 1 h under a nitrogen atmosphere. The triethylamine hydrochloride salt precipitate was filtered off. The filtrate was evaporated under reduced pressure up to half its volume. The concentrated solution of 1-(2-oxo-1,3,2-dioxaphospholan-2-yloxy)-2-(perfluorohexyl)ethane and 150 mL of dry MeCN were placed in a glass pressure bottle. After the mixture was cooled to −20°C, 15 mL of anhydrous TMA was added, and the reaction was allowed to continue at 70°C for 12 h. The reaction mixture was again cooled to −20°C to precipitate 2-(perfluorohexyl)ethyl phosphorylcholine ($C_6F_{13}PC$).

^{1}H NMR (CD_3OD) δ = 2.59 (m, CF_2CH_2, 2H), 3.21 (s, $-N^+(CH_3)_3$, 9H), 3.63 (t, $-CH_2N^+$, 2H), 4.19 (m, $-CH_2OP-$, 2H), 4.27(br, $-OPCH_2-$, 2H); ^{19}F NMR (CD_3OD) δ = −81.40 (CF_3, 3F), −113.39 (CF_2CH_2, 2F), −121.75 ($(CF_2)_3CF_2CH_2$, 6H), −122.58 ($CF_3CF_2CF_2CF_2CF_2$, 2F), −123.49 ($CF_3CF_2CF_2$, 2F), −126.11 (CF_3CF_2, 2F); IR (cm^{-1}): 2910 (—CH_2—), 1300 (P=O), 1235 (—OPO—), 1085 (—$OPOCH_2$—), 970 ($N^+(CH_3)_3$).

$C_8F_{17}PC$ and $C_{10}F_{21}PC$ were also synthesized by a method similar to that described above. Hexadecyl phosphorylcholine ($C_{16}PC$) was synthesized as previously reported.[25] Figure 1 shows the chemical structure of the PC additives synthesized in this study.

Preparation of composite low-density polyethylene films with PC additives

The low-density polyethylene (LDPE) used as the base material in particle form was purchased from Sumitomo Seika Chemicals (Flow beads LE-1080, Osaka, Japan). The average diameter was 6 μm.

The LDPE and composite films with PC additives were processed by a heat-press technique. Typically, the LDPE particles (0.5 g) and 2-(perfluorodecyl)ethyl phosphorylcholine ($C_{10}F_{21}PC$, 0.05 g, 6.86 × 10^{-5} mol) were mixed and thoroughly ground in a mortar. The mixed powder was then placed between two stainless steel plates and pressed at a pressure of about 5 MPa at 120°C for 3 min. To determine the appropriate $C_{10}F_{21}PC$ composition, we prepared films with different blend ratios [$C_{10}F_{21}PC$/LDPE = 1/10 (0.05 g, 6.86 × 10^{-5} mol/0.5 g); 1/25 (0.02 g, 2.74 × 10^{-5} mol/0.5 g); 1/50 (0.01 g, 1.37 × 10^{-5} mol/0.5 g); and 1/100 (0.005 g, 6.86 × 10^{-6} mol/0.5 g) by weight]. Other PC compounds were mixed with LDPE particles with molar compositions similar to that of $C_{10}F_{21}PC$.

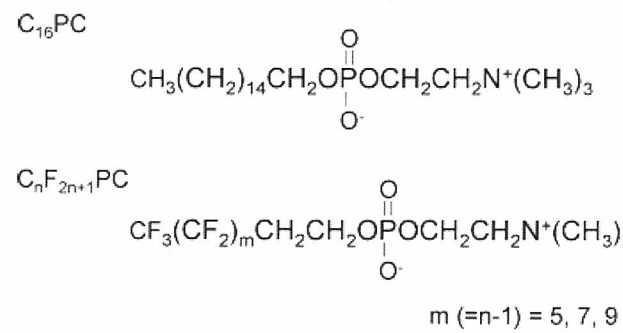

Figure 1 Chemical structure of alkylated and perfluoroalkylated PC.

To determine the elution amount of the PC additives from the composite films, the films were immersed in water or ethanol with gentle shaking. The solvents were changed three times at 2-h intervals, and the concentration of the PC additives was determined by phosphorus analysis.

Surface analysis

X-ray photoelectron spectroscopy (XPS) was performed on a Scienta ESCA-200 spectrometer with Al Kα. Survey scan spectra of C_{1s}, O_{1s}, N_{1s}, P_{2p}, and F_{1s} were obtained. All XPS data were collected at takeoff angles of 15° and 75° (between the specimen surface and the detector).

The dynamic contact angles for the samples were recorded as the probe fluid, water (deionized to 18.2 MΩ), using a First Ten Angstroms FT-125 goniometer and Gilmont syringes. The advancing (θ_A) and receding (θ_R) contact angles were measured at addition to and withdrawal from the drop, respectively.

Evaluation of mechanical properties

Tensile strength measurements were performed using an STA-1150 (ORIENTEC, Tokyo, Japan). The samples were cut into dog-bone shape (size, 12.5 mm × 2.5 mm). The crosshead speed was 2 mm/min. Four specimens were tested. Within the region of elongation from 0 to 5%, the strain–stress curves were linear. Young's modulus was then obtained from this initial elastic region.

RESULTS AND DISCUSSION

Surface activity of perfluoroalkylated PCs

The perfluoroalkylated PCs with different perfluoroalkyl chain lengths were obtained as a white powder. C_6F_{13}PC was readily soluble in water and ethanol. C_8F_{17}PC and C_{16}PC were soluble in water, but the solutions at this concentration were very viscous, resembling hydrogel, indicating that the intramolecular interaction of these molecules is high in an aqueous solution. In addition, the C_{16}PC aqueous solution was turbid. These additives were easily soluble in ethanol. In contrast, $C_{10}F_{21}$PC was not soluble in water or ethanol.

Several groups have shown that perfluoroalkyl groups that are incorporated into polymers are surface active[7,26–33] and adsorb at free polymer interfaces due to minimization of surface free energy. We tested the incorporation of the perfluoroalkyl groups with the PC groups to enhance the efficiency of the

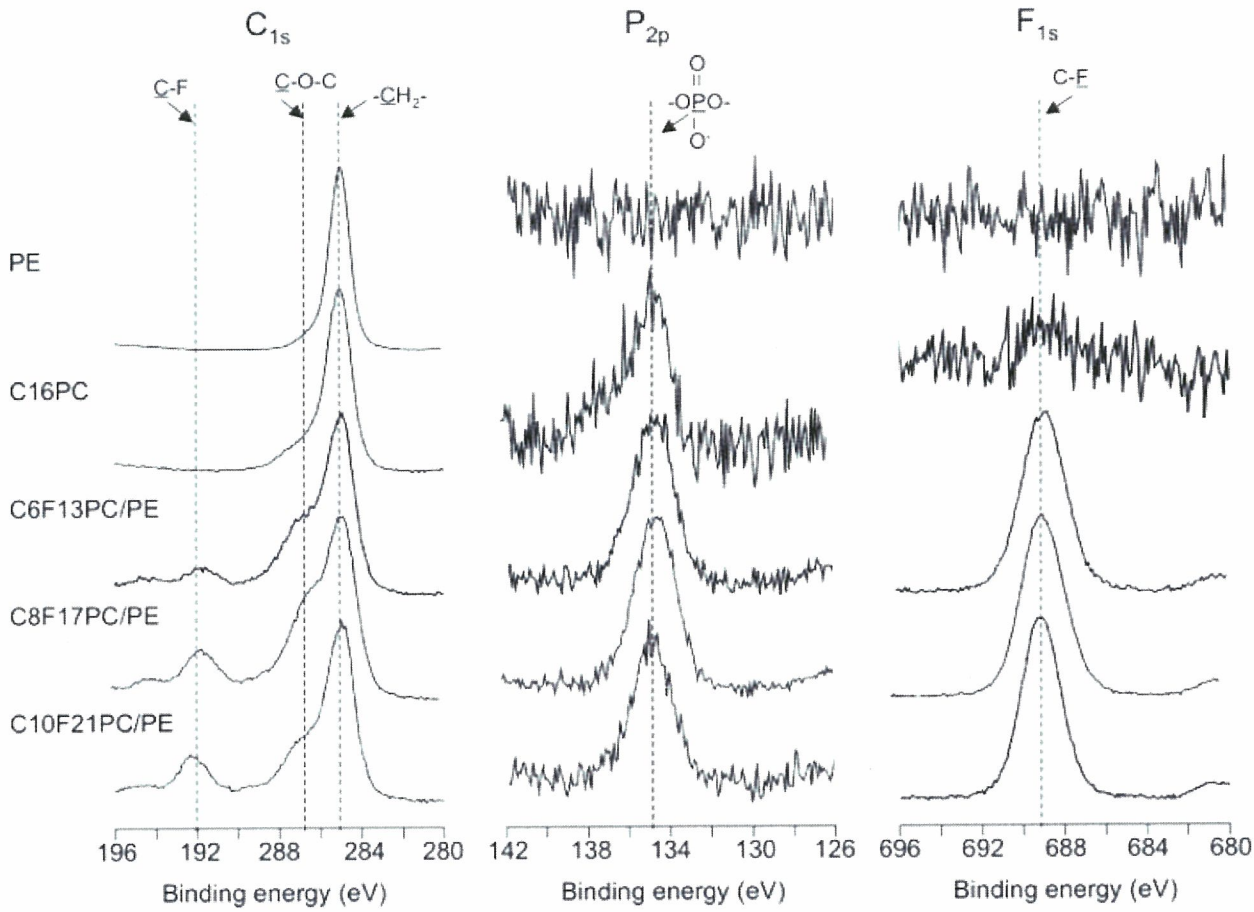

Figure 2 XPS spectra of PE and composite polymer films.

TABLE I
XPS Data for Polymer Films

Films	Composition of PC additives (mol)	Composition of PC additives (% w/w)	Takeoff angle (°)	XPS elemental data (%) C	O	N	P	F
$C_{16}PC/PE$	6.86×10^{-6}	0.6	15	90.07	8.96	0.34	0.63	
			75	98.71	1.07	0.19	0.03	
	1.37×10^{-5}	1.1	15	94.21	4.43	0.45	0.91	
			75	96.54	2.65	0.45	0.36	
	2.74×10^{-5}	2.2	15	88.74	8.84	1.18	1.24	
			75	91.40	6.71	0.82	1.07	
	6.86×10^{-5}	5.3	15	93.75	3.20	1.50	1.55	
$C_{10}F_{21}/PC/PE$	6.86×10^{-6}	1.0	15	84.45	7.06	0.64	0.58	7.27
			75	80.73	4.97	0.33	0.54	13.43
	1.37×10^{-5}	2.0	15	76.17	7.03	0.42	0.93	15.45
			75	82.93	4.07	0.33	0.43	12.24
	2.74×10^{-5}	3.8	15	56.30	9.17	0.67	1.48	32.38
			75	42.49	10.33	1.19	1.95	44.04
	6.86×10^{-5}	9.1	15	65.10	7.20	2.40	2.30	23.00
$C_8F_{17}/PC/PE$	6.86×10^{-6}	0.9	15	84.51	7.30	0.37	1.18	6.64
			75	89.04	2.87	0.23	0.36	7.50
	1.37×10^{-5}	1.7	15	36.37	6.71	1.07	2.62	53.23
			75	47.67	10.04	1.20	1.73	39.36
	2.74×10^{-5}	3.3	15	38.26	8.02	1.76	2.64	49.32
			75	48.32	10.62	1.07	1.94	38.05
	6.86×10^{-5}	7.9	15	37.50	8.00	2.00	2.80	49.70
$C_6F_{15}/PC/PE$	6.86×10^{-6}	0.7	15	80.77	8.89	0.47	1.08	8.79
			75	75.34	7.38	0.68	0.81	15.79
	1.37×10^{-5}	1.4	15	79.32	3.88	0.49	1.36	14.95
			75	85.63	3.14	0.46	0.57	10.20
	2.74×10^{-5}	2.8	15	58.17	13.27	1.25	1.80	25.51
			75	60.98	11.03	1.14	1.80	25.05
	6.86×10^{-5}	6.8	15	52.90	10.90	2.50	2.10	31.60

additives in the LDPE films that resulted in highly hydrophilic surfaces.

Figure 2 shows the XPS spectra of LDPE and composite films, which are corrected at a takeoff angle of 15°. For the original LDPE film, a strong intensity at 285 eV was observed and attributed to carbon atoms in the methylene chains of the polyethylene backbone. Mixing the PC additives with the PE particles dramatically changed the XPS spectra of the fabricated films. In every C_{1s} spectrum of the composite films, a shoulder was observed at 286.5 eV. This shoulder could be attributed to the ether bonds (C—O) of the PC additives. In addition, for films mixed with fluoroalkylated PCs a peak was observed at 290 eV. This peak corresponded to the carbon atom bonded to fluorine (C—F). A peak ascribed to phosphorus was observed at 134.1 eV. This could be attributed to the phosphorylcholine group in the PC additives. The spectra of the phosphorus of the films containing fluoroalkylated PCs were clearer than that of those containing $C_{16}PC$. This means that enrichment of fluoroalkylated PC is preferred. At 680 eV, a fluorine signal was also observed on the fluoroalkylated PC composite film.

The XPS elemental concentrations for the polymer films are summarized in Table I. The nitrogen and phosphorus concentrations increased with an increase in the composition of the PC additives in the feed. In particular, the surface concentrations for these elements were remarkably high when $C_8F_{17}PC$ was used as the additive. Figure 3 shows the effect of the PC additives on surface enrichment of the composite films. While the P/C ratio did not change remarkably with the bulk composition of $C_{16}PC$, the ratio did increase with an increase in the composition of perfluoroalkylated phos-

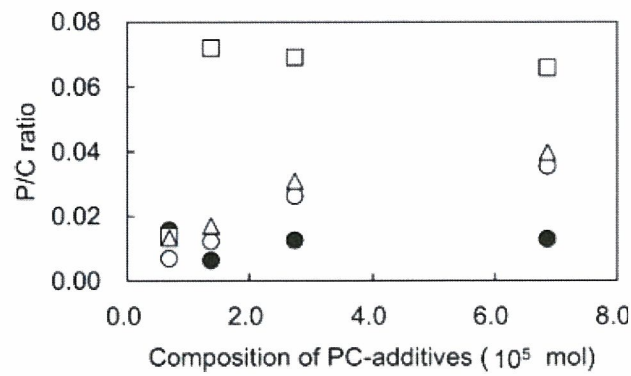

Figure 3 Surface P/C ratio versus bulk composition films. Filled circle: $C_{16}PC/PE$, Open circle: $C_6F_{13}PC/PE$, Open square: $C_8F_{17}PC/PE$, Open triangle: $C_{10}F_{21}PC/PE$.