Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Rapid In Vitro Screening of Polymers for Biocompatibility

Charles A. Homsy[a]; Krystyna D. Ansevin[a]; William O'bannon[a]; Sara A. Thompson[a]; Roosevelt Hodge[a]; Marcella E. Estrella[a]
[a] Bio-Medical Engineering Laboratory and Biology Department, Rice University, Houston, Texas

To cite this Article Homsy, Charles A. , Ansevin, Krystyna D. , O'bannon, William , Thompson, Sara A. , Hodge, Roosevelt and Estrella, Marcella E.(1970) 'Rapid In Vitro Screening of Polymers for Biocompatibility', Journal of Macromolecular Science, Part A, 4: 3, 615 — 634
To link to this Article: DOI: 10.1080/00222337008074366
URL: http://dx.doi.org/10.1080/00222337008074366

J. MACROMOL. SCI.–CHEM., A4(3), pp. 615-634, May, 1970

Rapid In Vitro Screening of Polymers for Biocompatibility

CHARLES A. HOMSY, KRYSTYNA D. ANSEVIN, WILLIAM O'BANNON, SARA A. THOMPSON, ROOSEVELT HODGE, and MARCELLA E. ESTRELLA

Bio-Medical Engineering Laboratory and Biology Department
Rice University
Houston, Texas

SUMMARY

A rapid in vitro screening protocol to guide selection and monitoring of polymers for implant or extracorporeal applications is described. There are four main procedures under the protocol: 1) and 2) measurement of physical property change over thermal and simulated biochemical stress, 3) infrared spectrophotometric assay of migratible species developed over simulated biochemical stress, and 4) cell culture sensitivity to those migratible species. In vivo biochemical stress was simulated and accelerated by exposing polymer specimens to a pseudoextracellular fluid (PECF) at 120°C for 62 hr.

Results from procedures 3) and 4) are presented. These show a good correlation between cell culture response and the total carbon-hydrogen bonds developed in the PSF over the exposure period. Correlation of these data with available clinical and animal implant experience is consistent and is discussed in detail. Infrared assays for a candidate polymer may be obtained at relatively low cost within 4 days and stand as a rational basis for rejection or retention for preclinical in vivo studies.

*This work was supported by Public Health Service Grant HE 09251-04.

INTRODUCTION

The versatility of elastomers and polymers for prosthetic implant applications has accelerated interest in their biomedical use. The authors were assigned responsibility for selection of polymeric materials for artificial heart development. Examination of published data on biocompatibility of polymers showed these to be generally inadequate as a basis for objective selection [1-5].

A general scheme has been developed for rational selection and monitoring of polymers for any long-term implantation use. This system is shown in Table 1. Results from use of protocol 3 have already been reported [6]. This protocol involves infrared spectrographic identification of carbon-hydrogen bonds in a simulated body fluid to which candidate materials had been exposed.

In this paper we present results obtained in use of protocols 3 and 4, infrared analysis and tissue culture, respectively, with pseudobody fluid of identical experimental history. The results support the use of methodology for rapid and relatively low-cost selection of candidate materials for preclinical animal implantation studies.

RATIONALE

Implantation of a prosthetic material necessarily involves surgical trauma at the implant site. If the surgeon decides against actual implantation of a foreign material and closes the incision using his best technique, a complex series of biochemical processes directed to healing the incisional site begins. According to a simplified scheme and in the absence of infection, there is acute cellular activity with an influx of polymorphonuclear cells and edema which is soon followed by the appearance of monocytes and fibroblasts [7]. The latter cells undertake reintegration of the operatively disturbed area by generation of collagenous tissue (scar tissue). This fibrous tissue provides a framework on which reconstitution of preoperative tissue structure may proceed. In time a major part of the scar tissue may be replaced by normal tissue structure [8]. The exuberance of scar tissue development and its persistence is quite variable among individuals and is greatest in epithelial or skin tissue [9].

The presence of an implant is an impediment to the normal healing sequence just described. If the implant surface is sufficiently extensive (of the order of centimeters in length and breadth) to present an impervious

Table 1. Materials Screening Schema

Protocol	Polymer form	Exposure conditions	Properties measured
1	(a) Micro-tensile bars (ASTM D 1708-66)	150°C, dry heat	Weight, dimensions, tensile strength,
	(b) Flex-test Strips (ASTM D 2176-63 T)	150°C, dry heat	flexural fatigue strength
2	(a) and (b) above	115°C, 30 psia PECF[a]	As above
3	(a) and (b) above (c) Resin as supplied by manufacturer	115°C, 30 psia PECF[a] 62 hr	(C–H) containing moieties in PECF[a]
4	(a), (b), and (c) above	115°C, 30 psia PECF[a] 62 hr	Tissue culture response of solids and PECF[a]

Pseudoextracellular Fluid (PECF)

($NaHCO_3$, K_2HPO_4, NaCL, KCl)[b]

Ion	Concentration (meq./liter)	
	Physiological	PECF
Na^+	145	145
K^+	5	5
Cl^-	113	118
HCO_3^-	30	30
HPO_4^{2-}	2	2

[a]Pseudoextracellular fluid.

[b]Reagent Grade Chemicals.

barrier to body fluid, the healing process described above leads to a fibrocartilagenous membrane which isolates the implant from normal tissue. This membrane is the body's response to the stimulus of an inert foreign material grossly impervious to body fluid.

On the other hand, it has been shown that perforated structures and porous structures (of the order of millimeters or less in dimensions) through which sufficient body fluid movement may occur become more or less thoroughly infiltrated with fibrous tissue [10-12]. The important element for this invasive action appears to be permeability sufficient for effective transimplant metabolic activity.

The above scheme relates to changes that occur in the absence of biochemical toxicity associated with the presence of the implant. It has been observed that solid metallic implants are incapsulated by membranes, the thickness of which appears to be proportional to the degree of metallic dissolution, i.e., biochemical irritation adjacent to the implant causes more vigorous sequestration response [13]. Thicker membranes are also observed when fracture fixation appliances become loose and allow increased relative motion between the appliance and adjacent tissue or bone. One may infer, then, that cellular injury either of traumatic or biochemical origin stimulates fibroblastic response to encapsulate the offending locus within the body. This sequence is followed for any foreign body implant, metallic or polymeric.

In view of the above, we may consider polymer compatibility to result from biochemical inertness of the polymer such that few or no low molecular weight chemical moieties are released by the implant. These moieties may have existed in the polymer before implantation, e.g., as stabilizers and plasticizers, or may have developed through degradation of the bulk polymer. In either case, the presence of such moieties is likely to result in cytotoxicity.

This picture of polymer biocompatibility is supported by clinical experience with polymer implantation over the past two decades. Only two families of high polymers, the polyperfluorocarbons (TFE and FEP resins) and polymethylsilanes (MS resins) have shown a consistent pattern of tolerance within the body [1-5]. Both of these polymer families share characteristics which mediate strongly against generation of low molecular weight chemical species with active functional groups that may induce cytotoxic tissue and blood reactions. Both families exhibit: 1) very high molecular weight (6-10,000,000 for TFE and infinite for cross-linked MS), 2) narrow molecular weight distribution, 3) little or no catalysis residues, 4) very high resistance to the chemical degradative effect on body fluid without added stabilizers.

PROCEDURE

Based on the above rationale of polymer cytoxicity, protocols 3 and 4 of Table 1 were developed. These protocols are designed to objectively grade candidate polymers by degree of degradation to low molecular weight, migratible compounds over exposure to simulated body fluid (PECF, Table 1), and by apparent cytotoxicity of these compounds in tissue culture.

A test temperature of 115°C was used to simulate the effects of long-term in vivo implantation. It is believed that elevated temperature efficiently models the influence of long-term implantation in terms of degradation chemistry and migration of additive chemicals from the implant material. The protein constituents of body fluid were not simulated since these would be expected to contribute but little to the chemical stress upon an implant. The absence of protein buffers caused a slight elevation from physiological pH of 7.2-7.3 to 8.2; the anionic strength of the proteins was compensated by a slightly greater chloride ion concentration over physiologic. While the possible effects of enzyme chemistry are not included, the omission is not considered significant since such effects should become apparent during subsequent animal implantation studies.

Protocol 3 provided for detection of low molecular weight moieties in the PECF by infrared (ir) spectrophotometric detection of primary, secondary, and tertiary carbon-hydrogen bonds. Protocol 4 made use of aliquot portions of PECF from a given experiment for preparation of a nutrient medium for tissue culture. In this way direct comparison could be made of carbon-hydrogen bond development in PECF and tissue culture cytotoxicity.

Sample Handling

Twelve manufacturers of high polymers provided 15 generic types of resin for this study. Many types were represented by several commercial versions. Each polymer was identified by manufacturer's lot number and date of purchase. It was found that the molding powders varied widely in individual particle shape; however, the shapes could be identified closely either with the rectangular parallelopided, the right circular cylinder, the right elliptical cylinder, or the sphere. Statistical values for each of three characteristic dimensions corresponding to the particle shape in question were used to determine an average ratio for area to volume. This ratio was then combined with resin density to yield the weight of a particular resin type which corresponded to 1200 cm^2 surface area. This weight was approximately 100 g for most resins.

The appropriate weight of a resin was washed in Haemo-sol* solution to remove any deposited foreign matter. The resin was exhaustively rinsed in distilled, deionized water. The resin was placed in 500-ml round-bottom flasks which had been washed according to tissue culture standards: Haemo-sol wash and exhaustive rinsing followed by autoclaving at 115°C for 62 hr while filled with double glass-distilled water. Two hundred and fifty milliliters of pseudo-extracellular fluid were added and the flasks flame-sealed.

Groups of 10 or 12 flasks were placed in a standard laboratory autoclave for 62 hr. Following removal and cooling to room temperature, the flask seals were broken and the supernatant fluid decanted. The fluid was divided into aliquots for the ir and tissue culture studies.

Infrared Studies

Fifty milliliter aliquots of supernatant solution were extracted with 5 ml of spectrographic grade carbon tetrachloride in a Squibb funnel. The CCl_4 was dried by contact with anhydrous $MgSO_4$. Approximately 3 ml of CCl_4 were recovered for loading the spectrograph cell. These cells were Perkin-Elmer precision demountable types; however, path length was increased to 1 cm by introduction of a spacer fabricated from polytetrafluoroethylene polymer.

A model IR-8 Beckmann ir spectrophotometer was used. The entire procedure was calibrated using CCl_4 which had been used to extract a prepared aqueous solution of n-hexanol. Absorption bands centered at approximately 2962, 2926, and 2870 cm^{-1} corresponding to C–H stretching in $-CH_3$, $-CH_2$, and –CH bonds, respectively, were well articulated in calibration and test spectra. The expected linear calibrations were readily developed and related logarithm per cent transmission to moles per million of primary, secondary, and tertiary C–H groups in the prepared solutions of n-hexanol. These relations were

$$\log T = 4.61 - 0.0033C \ (-CH)$$
$$\log T = 4.61 - 0.012C \ (-CH_2)$$
$$\log T = 4.61 - 0.069C \ (-CH_3)$$

where T = % transmission and C = moles/million radical.

*Haemo-sol, Meinecke Co., Baltimore, Maryland.

Table 2. Tissue Culture Medium
(80 ml Solution A, 20 ml Solution B, 48 ml Solution C)

Solution A		Solution B		Solution C	
NaCl[a]	6.68 g	$CaCl_2$	0.42 g	NCTC 109[c]	70 ml
KCl	0.22 g	$MgSO_4 \cdot 7H_2O$	0.60 g	Calf serum[d]	23 ml
$NaHCO_3$	2.50 g	Glucose	3.00 g	Chick embryo	
H_2O[b]	1000 ml	H_2O[b]	5000 ml	extract	7 ml

[a]All Reagent Grade chemicals.
[b]Glass distilled twice.
[c]Microbiological Association, Bethesda, Maryland.
[d]Colorado Serum Co., 4950 York St., Denver, Colorado.

Tissue Culture Studies

The following requirements were the guidelines in devising the experimental method.

1. The technique should be easy, rapid, and very repeatable.
2. Screening should be done on primary cultures rather than on established cell lines since the latter are genetically and metabolically different from tissue cells and are known to be more tolerant to environmental conditions [19].
3. The culture medium should be so composed as to support excellent "growth" of the cultures and healthy appearance of the cells. A salt solution that would closely resemble ionic composition of mammalian body fluid should constitute part of this medium.

The explants for each experiment were derived from several pooled newborn mouse hearts. Six explants were used in each culture dish. Five milliliters of cultured medium was then added. Table 2 shows the composition of the culture medium.

Solution A was the PECF to which polymer had been exposed as described above.

Fifty millimeter disposable Falcon petri dishes were used as culture dishes. The bottom of the dish was covered with a thin chick plasma-chick embryo extract clot which immobilized the tissue explants and provided a satisfactory substrate for cell migration. Figure 1 shows a culture dish after preparation for incubation.

The cultures were kept for 1 week in a CO_2 incubator at 38°C. The medium was changed twice during that time and the cultures were examined under the inverted microscope every second day. At the end of the week the cultures were fixed in formaline, stained with hemotoxylin, and, sometimes, counterstained for photographic purposes with methylene blue or Giemsa stain. The extent of cell migration around particular tissue explants was determined on the fixed cultures by outlining the zone of growth in standardized conditions with the help of a projection and drawing mirror attached to an inverted microscope.

Each plastic was tested on four petri dishes in two different experimental runs. The controls which accompanied each experiment involved cultures in which Solution A had not been contacted with polymer and had been: 1) only briefly autoclaved (control series C), or 2) autoclaved in sealed flasks for 60 hr alongside test flasks containing the solution and a test plastic (control series CF). This arrangement provided checks on two potential artifacts: 1) that abnormal condition of some experimental cultures was in fact induced by the culture medium itself because of its

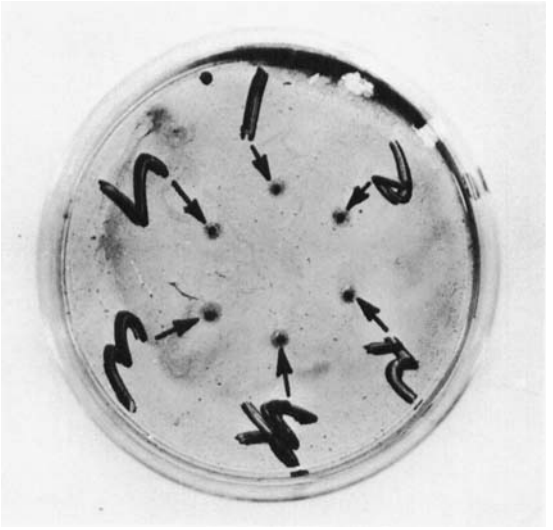

Fig. 1. An overall view of a culture dish. Approximately 1X.

unsuitable composition; and 2) that prolonged exposure of glassware to high temperature, increased pressure, and ions caused release of some cytotoxic materials from the glassware.

Early in our study it became apparent that this method was giving unambiguous and reproducible results; therefore, it was adopted as standard procedure for the entire study.

Figure 2 shows a typical culture of the control series C. Note the extensive cell migration around the original explant. The cells form a monolayer. As will be seen, the extent of outgrowth is one of the diagnostic measures for evaluating relative cytotoxicity.

Figure 3 is a magnification of a portion of the C-type control culture and shows a sheet of cells that look normal and healthy. Cytoplasmic cell boundaries are not very discernible. The cytoplasm is clear, free of granules or vacuoles, and the cells are well spread. Most of the cells that constitute the outgrowth appear to be epithelial; thus, they must have derived from the endothelial lining of the heart cavities and possibly also of the entering blood vessels. Fibroblasts were sometimes present in control cultures, but more often they seemed to be completely overgrown by the endothelium as illustrated in Fig. 3.

Figures 2 and 3 are also typical for most of the control CF cultures. On

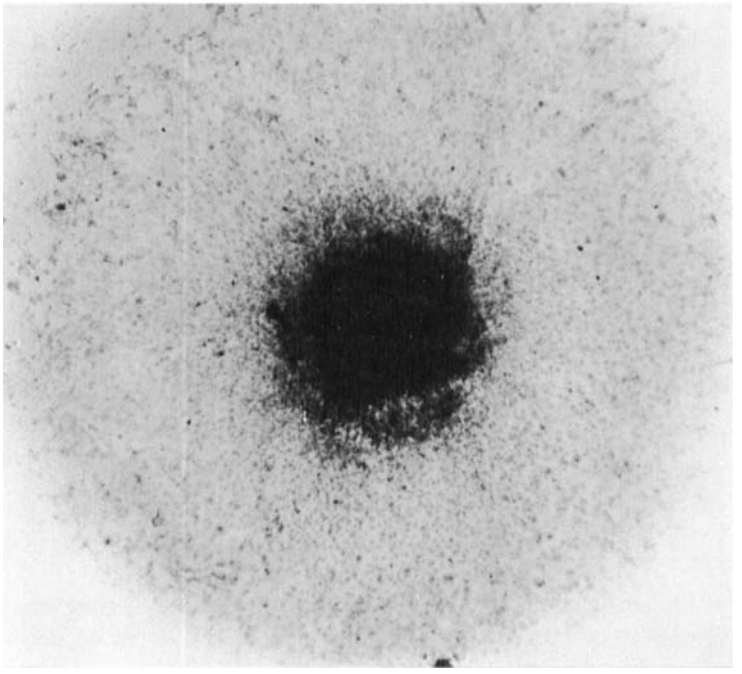

Fig. 2. A typical culture of the control series: 30X.

one occasion the CF culture cells appeared vacuolated; in this case it is presumed that a cytotoxic factor had been released from the glassware during the long autoclaving procedure.

Cytotoxic effects for different polymers ranged from a very mild reaction to total inhibition of the culture's growth. In addition to a varying degree of reduction of cell migration (as represented by the zone of outgrowth), morphological abnormalities could be found in the cells. Arbitrarily the cells' response was classified into five categories:

0. excellent outgrowth, normal cell morphology (typical for control cultures); Figs. 2 and 3.
+1. outgrowth comparable to that of the control, but some vacuolization of the cells; Fig. 4.
+2. distinct reduction in growth usually accompanied by extensive vacuolization; Fig. 5.

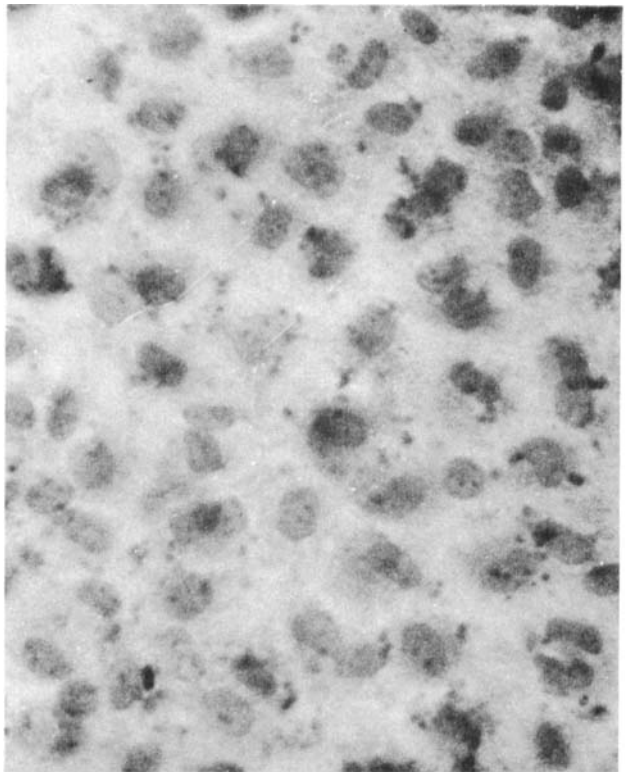

Fig. 3. A closer view of a portion of a control culture. Note the healthy appearance of epithelial-like cells. 430X.

+3. growth almost totally inhibited, and in most cases only fibroblastic; severe vacuolization or granular degeneration of cells; Figs. 6 and 7.
+4. growth totally inhibited, although the culture is not necessarily dead (as indicated in rare cases by rhythmic contractions of the explant).

RESULTS AND DISCUSSION

Data on ir and tissue culture response from the same experiment [i.e., for aliquots of supernatant pseudobody fluid (PECF)] are presented in Table 3 for 22 polymers. Tissue culture response is seen to vary between the unremarkable "+1" category to "+4" or total inhibition of cell migration. The moles per million moles of methyl ($-CH_3$), methylene ($-CH_2$),

Table 3. Biocompatibility Screening Results
(Resin Exposed to PECF, 62 hr, 115°C, 30 psia)

Polymer	Tissue culture response[a]	Total CH_3, CH_2, and CH in PECF via ir analysis mpm[b] equivalent n-hexanol
1. Silastic (Dow Corning 372 Nonreinforced)	+1	5
372 Reinforced	+1	5
2. Polyethylene (Univ. of Texas)[c] (SG: 0.96)	+1	17
3. Fluorinated ethylene propylene (FEP T-160)	+1 to +2	n.d.[d]
4. Polyphenylene oxide (Grade 731)	+1 to +2	27
5. Polyethylene (SG: 0.96)	+2	n.d.
6. Acrylic molding powder (V - 415)	+2	n.d.
7. Polyphenylene oxide (Grade 534-801)	+2	17
8. Polyethylene (SG: 0.925)	+2	17
9. Fluorinated ethylene propylene (FEP T-100)	+2	23
10. Ionomer (1550)	+2	142
11. Polypropylene (Grade 114, Food Grade)	+2	198
12. Vinylidene fluoride (Grade 200)	+2 to +3	3

13. Nylon (Grade 101)	+2 to +3	14
14. Ionomer (AD 8043)	+2 to +3	30
15. Cellulose propionate	+3	81.7
16. Polystyrene (HH401) (Grade 300)	+3	168
17. Nylon (Grade 38)	+4	12
18. Polyvinyl chloride (Grade 5430)	+3 to +4	277
19. Polyurethane (Grade 58093)	+4	89
20. Polyurethane (Grade 16139)	+4	328
21. Polyvinylchloride (Univ. of Texas)[e]	+4	514
22. ABS (Grade X7-1000)	+4	516

[a]Scale: +1. Some vacuolization and growth inhibition but nominally as control cultures.
+2. Moderate vacuolization, morphological changes, and growth inhibition.
+3. Severe growth inhibition and vacuolization.
+4. Total growth inhibition.

[b]Moles per million.

[c]University of Texas, Austin, Drug/Plastic Research Laboratory, negative standard.

[d]Not detectable.

[e]University of Texas, Austin, Drug/Plastic Research Laboratory, intensely toxic standard.

and methyne (—CH) structural groupings detected within the PECF is seen to correlate generally with the degree of tissue culture pathology.

Polymers Nos. 2 and 21 were polyethylene (PE) and polyvinyl chloride (PVC) obtained from the University of Texas' Drug/Plastic Reasearch Laboratory. These were used as external control materials since they had been established as "negative" and "cytotoxic" standards, respectively, in that laboratory's extensive polymer toxicity screening methodology [14]. The present results are in agreement with that assessment. These polymers were provided in sheet form and were diced to provide 1200 cm^2 of surface area. The negative control was identified as "high density" polyethylene, i.e., the Ziegler catalysis product of high molecular weight with little or no chain branching, narrow molecular weight distribution, and pronounced hydrophobicity.

The medical grade Silastic materials (No. 1) represent a polymer family that has seen extensive implantation applications over the past 10-15 years. In the tissue culture test the cell response to these materials was low, as would be expected.

Two other PE polymers (Nos. 5 and 8) with "high density" resin and "low density," respectively, gave a relatively low response.

The nylon resins (Nos. 13 and 17) were aberrant from the correlation and were seen to cause inhibition of cell migration in the culture dishes. This exceptional behavior and that of polyvinylidene fluoride (No. 12) and of Ionomer grade AD 8043 (No. 14) are believed to reflect toxic degradation or migration moieties from these polymers which do not contain appreciable C—H bonds. This illustrates a weakness in relying exclusively upon simple (C—H) bond ir assay for acceptance screening or monitoring. On the other hand, the assay is believed to be efficient for rejection screening. It is hoped that our ir studies will be extended to elucidate functional group(s) and other carbon bonds which may be associated with cytotoxic response.

The relatively high ir response for the samples of food grade polypropylene (No. 11) and Ionomer grade 1550 (No. 10) is puzzling and may indicate the presence of a relatively innocuous antioxidant which migrated from the polymer.

Polytetrafluoroethylene was not included in this study since its bioinertness was accepted on the basis of long-term clinical usage and unremarkable animal implant studies [15]. The perfluorinated copolymer of tetrafluoroethylene and hexafluoropropylene (FEP) (Nos. 3 and 9) was included since it is a more recent article of commerce with limited biocompatibility evaluation [16]. One may expect that FEP resin could only

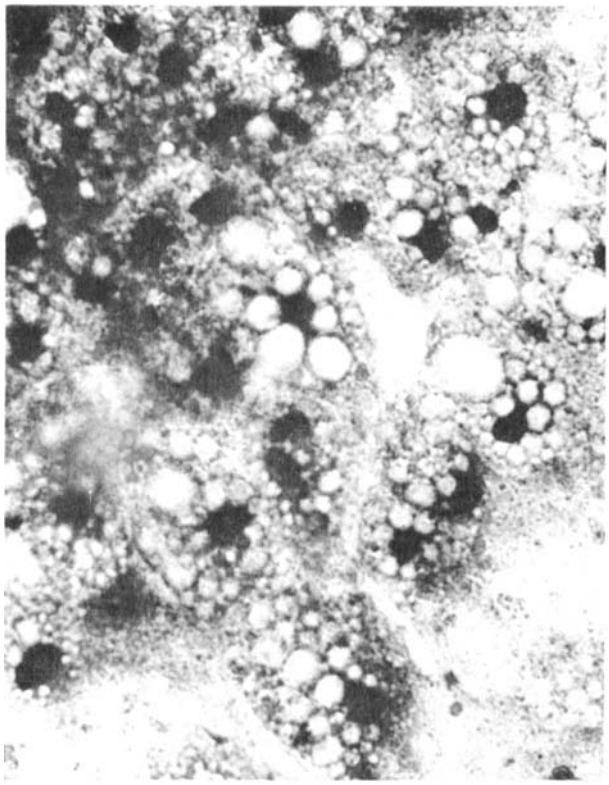

Fig. 4. Vacuolated epithelial-like cells which were characteristic for all those cultures of the experimental series in which cell migration had occurred (polyethylene, Univ. of Texas). 430X.

be less biocompatible than the homopolymer based on the perfluoromethyl side groups (i.e., more strained C–C side chain bond) and lower molecular weight (i.e., greater concentration of end groups). In fact, in Table 3 FEP resin is seen to be quite innocuous.

Several polymers exhibited relatively high responses. Polyvinyl chloride (Nos. 18 and 21) is usually formulated with a variety of low molecular weight organic ingredients which confer stability and flexibility. These have been recognized to migrate from the polymer into proximal liquids with potential toxicity for implant and drug container applications [17]. The relatively high responses of the two polyurethane samples (Nos. 19 and 20) are consistent with clinical observations of acute inflammation after use of this class of polymer for internal fixation of fractures. The

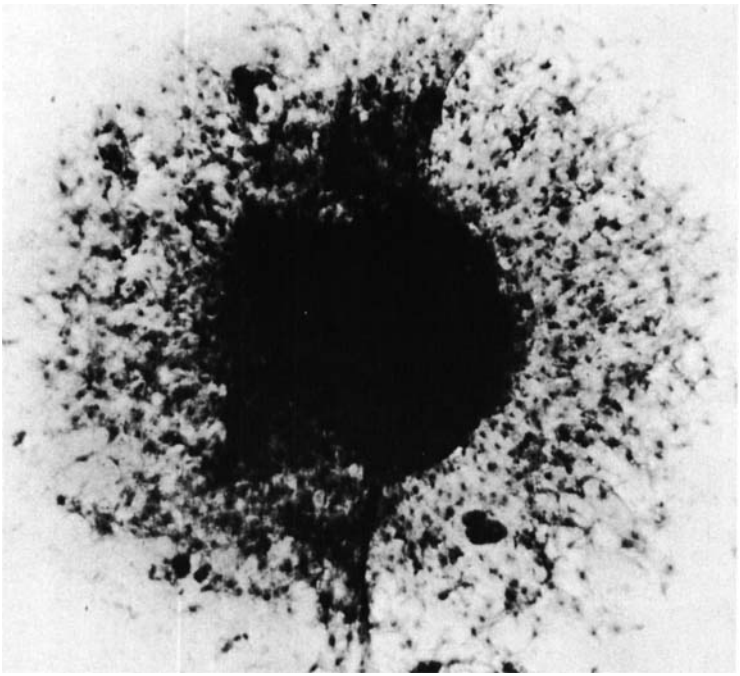

Fig. 5. The type +2 response to a test plastic (polypropylene, Grade 114, Food Grade). 30X.

polyurethane family of polymers has been identified as susceptible to degradation by body fluids [18].

Both of the in vitro screening protocols discussed above should be viewed as preliminary to in vivo implantation studies. The ir spectrographic response is most rapidly obtained at modest cost. Results may be obtained for a large number of candidate polymers or a series by monitoring the same polymer (limited primarily by autoclave capacity) within four days. However, the ir results should be used only as a basis for rejection when response exceeds about 50 moles per million total C–H moiety.

Materials for which ir response is less than this threshold may be favorably considered for tissue culture studies and eventual animal implantation. In this way the cost and time increments associated with the latter techniques can be avoided in screening or monitoring a large number of candidate materials.

Previously reported attempts at developing a reliable cell culture technique for implant screening purposes do not appear to have been fully successful. In no study were primary mammalian tissue cultures used; in all

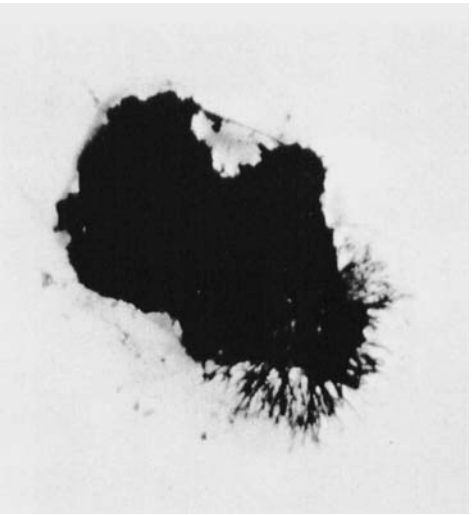

Fig. 6. The type +3 response to a test plastic. Only fibroblasts have migrated out of the explant (polyvinyl chloride, Univ. of Texas). 30X.

investigations cell lines [20-26] or embryonic chick cells [24] were employed. In no previous work has cell migration ("culture growth") been utilized as an indicator of cytotoxicity of the cells' environment; in the present study this parameter has been found very effective for this particular purpose. In several reports the techniques of observation were such as to permit only gross discrimination between mass cell death and apparently unaffected cultures. No attempts were made to detect lower grades of toxicity which manifest themselves in changed cell morphology.

The present technique that employs primary mammalian tissue culture has appeared highly sensitive and reproducible; thus it is a reliable screening tool for detecting various grades of cytotoxicity. However, the acute sensitivity of this method necessitates the adoption of criteria for grading the acceptability of a particular material.

Those polymers that exhibit a response of <50 mpm total (C−H) groups and +1 tissue culture reaction would be expected to yield migration products at very low rates. Moreover, it is likely that in vivo concentrations of these would be lower than the values obtained in the fixed volume of the culture medium. Therefore, it can be expected that these polymers are harmless in vivo and could be safely used even for long-term implantation in humans.

Those materials which exhibit a +2 reaction would require more caution since migration products from these polymers appear distinctly harmful to living cells. Although, as noted above, in vivo concentrations of these prod-

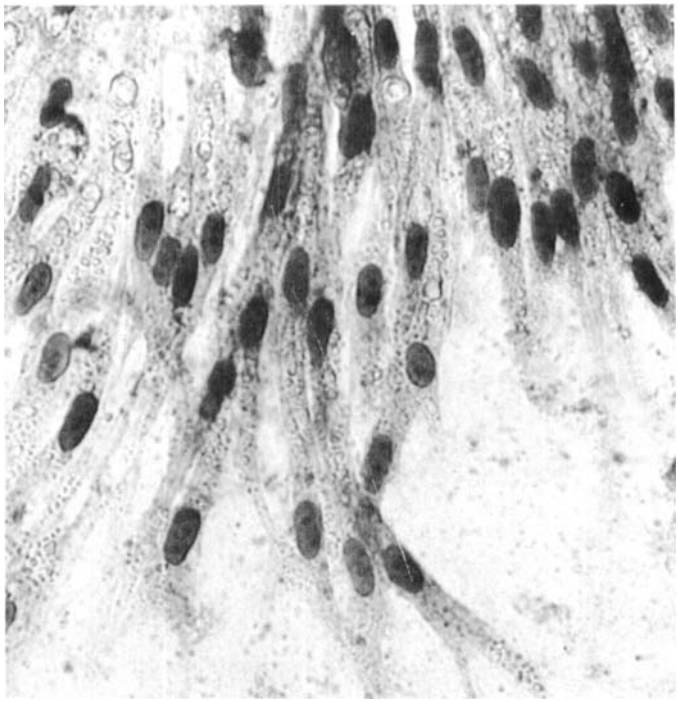

Fig. 7. A portion of this culture at a higher magnification. Note the granular appearance of the fibroblasts. 430X.

ucts would not accumulate to the same extent as in the culture dish, and although several materials in the category have been found to be well tolerated in animal and human use, any +2 plastic should be thoroughly screened in vivo before it is used in humans.

Those materials which score +3 or +4 should not be contemplated for medical use and would be inappropriate for most kinds of animal work with implants.

We want to emphasize that for medical purposes the preliminary screening with ir and tissue culture should always be followed by animal experimentation. A certain amount of the latter is necessary even with those materials which have scored very well with the ir and tissue culture tests. Infrared analysis allows rejection of unacceptable candidates; tissue culture screening is the most unambiguous test for cytotoxicity. For this reason, we consider the latter a necessary part in qualifying any material for implantation in humans. In addition to chemically-induced cytotoxicity, however, physical factors may also render a material unacceptable for implantation; too great rigidity or the wrong shape of an implant may provide

mechanical trauma. Animal tests in vivo are necessary for detection of those factors. Also, long-term implantation in animals is probably the only sure way to ascertain that a particular plastic is completely noncarcinogenic; in its present form the tissue culture test gives no information on this possible aspect.

In conclusion, we suggest that selection of any material for medical implantation should always be made on the basis of two biological tests; an in vitro and an in vivo test. If these are preceded by the ir protocol, new information is added and the expenditure in effort, time, and cost of the screening and monitoring of polymers becomes considerably reduced.

REFERENCES

[1] S. N. Levine, ed., "Materials in Biomedical Engineering," *Ann. N. Y. Acad. Sci.*, **146** (1968).

[2] T. Akutsu and A. Kantrowitz, "Problems of Materials in Mechanical Heart Systems," *J. Biomed. Mater. Res.*, **1**, 33-54 (1967).

[3] J. Autian, "Toxicological Aspects of Implants," *J. Biomed. Mater. Res.*, **1**, 433-449 (1967).

[4] R. I. Leininger, "Plastics in Surgical Implants," *ASTM Publ.*, **386**, 71-76 (1964).

[5] S. A. Wesolowski, A. Martinez, and J. D. McMahan, "The Use of Plastic Materials in Surgery," *SPE RETEC, New York Section SPE*, 58-85 (1967).

[6] C. A. Homsy, R. Hodge, N. Gordon, E. E. Braggs, and M. Estrella, "Biochemical Engineering Screening and Monitoring," *J. Biomed. Mater. Res.*, **3**, 235-245 (1969).

[7] A. F. Szezypinski, "Present Status of Suture Materials," *Amer. Surgeon*, **32**, 372-374 (1966).

[8] E. J. Kamenski, R. Oglesby, N. K. Wood, and J. Sandrik, "The Behavior of Biological Materials at Different Sites of Implantation," *J. Biomed. Mater. Res.*, **2**, 81-88 (1968).

[9] L. V. Ordman and T. Gillman, "Studies in Healing of Cutaneous Wounds," *Arch. Surg.*, **93**, 857-911 (1966).

[10] E. T. Oppenheimer et al., "Observations on the Effects of Powdered Polymer in the Carcinogenic Process," *Cancer Res.*, **21**, 132-134 (1961).

[11] S. F. Hulbert et al., *Materials of Construction for Artificial Bone Segments*, Clemson University, 1968.

[12] M. E. DeBakey et al., "The Fate of Dacron Vascular Grafts," *Arch. Surg.*, **89**, 755 (1964).

[13] P. Laing, A. Ferguson, Jr., and E. S. Hodge, "Tissue Reaction in Rabbit Muscle Exposed to Metallic Implants," *J. Biomed. Mater. Res.*, **1**, 135-149 (1967).

[14] S. A. Rosenbluth et al., "Tissue Culture Method for Screening Toxicity of Plastic Materials to be Used in Medical Practice," *J. Pharm. Sci.*, **54**, 156-159 (1965).

[15] F. Usher and S. Wallace, "Tissue Reaction of Plastics," *Arch. Surg.*, **76**, 997-999 (1958).

[16] C. A. Homsy, H. S. Tullos, and J. W. King, "Corrosion of SMO Stainless Steel Fracture Fixation Plates," *Proc. Western Orthopedic Assn.* (1968).

[17] W. L. Guess and S. Habeman, "Toxicity Profiles of Vinyl and Polyolefinic Plastics and Their Additives," *Proc. SFE RETEC, New York Section SPE*, 15-27 (1967).

[18] V. Menkovitch et al., "Polyurethane Aortas in Dogs–Three Year Results," *Amer. Soc. Artif. Internal Organs*, 8, 79-84 (1962).

[19] M. Harris, "Resistance to Extrinsic Factors," in *Cell Culture and Somatic Variation*, Holt, Rinehart and Winston, New York, 1964.

[20] W. L. Guess and J. Autian, "Toxicity Evaluation of Lexan, Kynar and Vilsan–Short Term Studies," *J. Oral Ther. Pharm.*, 3, 116-23 (1966).

[21] W. L. Guess et al., "Agar Diffusion Method for Toxicity Screening of Plastics on Cultured Cell Monolayers," *J. Pharm. Sci.*, **54**, 1545-1547 (1965).

[22] W. L. Guess et al., "Characterization of Subtle Toxicity of Certain Plastic Components Used in Manufacture of the Polyvinyls," *Amer. J. Hosp. Pharm.*, **24**, 495-501 (1967).

[23] R. Johnson and A. F. Hegyeli, "Tissue Culture Techniques for Screening of Prosthetic Materials," *Ann. N. Y. Acad. Sci.*, **46**, 193-202 (1968).

[24] R. K. O'Leary and W. L. Guess, "Toxicological Studies on Certain Medical Grade Plastics Sterilized by Ethylene Oxide," *J. Pharm. Sci.*, **57**, 12-17 (1968).

[25] S. A. Rosenbluth et al., "Tissue Culture Method for Screening Toxicity of Plastic Materials to be Used in Medical Practice," *Amer. J. Hosp. Pharm.*, **54**, 156-159 (1965).

[26] R. F. Sisca et al., "Responses of Epithelial-like Cells in Tissue Culture to Implant Materials," *J. Dent. Res.*, **46**, 248-252 (1967).

Received for publication January 20, 1970