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TISSUE RESPONSE TO PERITONEAL IMPLANTS

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CONTENTS

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INTRODUCTION

The purpose of this contract was to provide a basis for characterization of cellular interactions with a foreign surface placed into the peritoneal cavity. The peritoneal cavity is an abdominal space that has been extensively utilized by others to study cytological responses under a variety of circumstances.[1,2] The principal reasons for selecting this region are ease in access, a suspended cell population, and an absence of a direct hematologic response. The abdominal cavity is continuously bathed and exchanged of its fluid through osmotic and hydrostatic forces from surrounding vasculature and lymphatic system. In addition, cells can freely migrate either directly from the vascular space, from the abdominal walls, or omentum into the peritoneal space. This versatility affords the experimenter a system analagous to a tissue culture model with suspended cells in a physiologically adjusted fluid, but its potential extends beyond this analogy. Cell culture requires great care, appreciable expense, and the experimenter is always reminded, by virtue of the protocol, that the system is in vitro suggesting if a continuum of events is desired such as recruitment of other cell types or protein systems, this recruitment is generally not available. In contrast, the peritoneal system, being in vivo, has the potential and capability of calling forth a full range of body responses.

The cells that are found either freely floating or lining the walls and organs are primarily macrophages and mesothelial cells and, to a lesser extent, mast cells, eosinophils, and lymphocytes. The mesothelial cell as the macrophage is a multipotential cell that remains to a greater extent attached to a reticular network that covers all the organs and lines the abdominal wall. The macrophage, however, is less likely to be attached but freely floating in the peritoneal fluid.

It was the intent of this contract to implant into the peritoneum a variety of materials varying in their surface chemistry and morphology in an effort to test the feasibility of studying cell attachment kinetics and their associated responses.

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MATERIALS AND METHODS

Materials

The selection of the materials was influenced by their ability to be translucent and texturable if not directly, then indirectly, through casting techniques. In addition to these prerequisites, a range in surface energy was also considered important. The materials selected were:

Polytetraflorethylene (PTFE) (Hydrophobic)
Polyetherurethane (PEU)
2 Hydroxyethylmethacrylate (HEMA) (Hydrophilic)

PTFE: This material was supplied by NASA-Lewis Research with four different surface conditions: as received, ion polished, natural textured, and pitted with an array of holes 50μ x 50μ x 100μ deep. The pitted surface was generated by using a nickel screen overlying the untreated PTFE and exposing this assembly to the ion beam. Some samples were facricated in a half and half sample, one half having natural texture and the other half being as received.

PEU: The polyurethane selected for this study previously had been shown capable of altering the hematological response to the formed elements and proteins in the blood. It is synthesized from 1000 molecular weight polytetramethylene glycol (PTMG) with end caps of methylene diisocynate (MDI) and chain extended with ethylene diamine. The result is a block copolymer of flexible soft segments of PTMG and hard segments of MDI that tend to phase separate. The separation in phases can be significantly altered by processing as either the annealed state (ANN) which enhances phase separation and order or the quenched state (fast air dried, FAD) which limits long range order. The increase in long range order and short range order respectively were found to correlate with increased platelet attachment and shortened partial thromboplastin time (PTT). It was then considered of interest to observe whether similar changes in kinetic cellular response occurred in a non-hematological environment.

HEMA: This polymer was synthesized from 2-OH ethylmethacrylate (opthalmic grade from Polyscience) and crosslinked with 2% ethyleneglycol dimethacrylate. The concentration of the HEMA monomer was 65% in a 60/40 v/v ratio of ethylene glycol and distilled water. The reaction was initiated with 0.1% ammonium persulfate and activator sodium metabisulfate (0.05%). It was then vacuum cast between two glass plates and placed at 60° C. for 30 minutes, after which the sheet was floated in double-distilled deionized water and changed daily for two weeks. The solution was then replaced by sterile Ringer's lactate or 0.9% NaCl pH 7.4 buffered with dibasic sodium phosphate for two weeks prior to implantation.

Implantation Method

All implant samples for this experiment were 0.8 cm in diameter and approximately 1 mm thick.

The selected implantation times were chosen on the basis of cell kinetics and response from preliminary experiments. These times were 30 minutes, 6 hours, 24 hours, 72 hours, 7 days, and 2 weeks.

The animal model was the male Sprague Dawley rat ranging in weight from 250 grams to 300 grams. The animals were anesthetized with ether, shaved over the abdomen with electric clippers, washed with Phisohex, and then shaved with a safety razor. They were then placed on the surgical table, and the abdomen was opened with a midline incision down the rectus abdominus. An incision was made in the linea alba and entry into the abdomen completed. The implants were inserted in the upper and lower quadrates of the peritoneal cavity. Occasionally other samples at times were placed among the intestines, omentum, and liver lobes. The wound was closed with 4-0 Vicryl suture in tissue planes, and wound clips were placed on the skin. The wound was then coated with Betadiene.

Upon completion of the experiment the animal was reanesthetized with ether and the abdomen was quickly entered through the previous incision, at all times care being taken to retract the tissues and minimize bleeding into the surgical site.

The abdominal contents were carefully displaced to allow visualization of the implants. The implants were removed with blunt tweezers and placed in the appropriate solution. Histological samples were put into 4% buffered (pH 7.4) formaline and placed in the refrigerator. Cell-count samples were placed in methanol for 5 to 7 minutes and then stained with May Glunwald/Giemsa. Scanning electron microscopy (SEM) samples were rinsed twice in Ringer's with 5% glucose and pH adjusted with H_2PO_4. Two rinse solutions for the SEM samples were made using 0.2% and 2.0% glutaraldehyde. The first solution with 0.2% glutaraldehyde was used for approximately two hours. The samples were then transferred to a 2.0% glutaraldehyde solution for twenty four hours. Upon removal the samples were washed in the following solutions at 0^{o} C for 15 minutes. The first solutions were deionized water and the graded increments of H_2O/ethanol with increasing concentrations of ethanol at 20% increments with two final changes of absolute alcohol. This same sequence of changes in solutions occurred with ethanol and freon-113. Some samples were critically point dried while others were simply placed in room air after double washings in 100% freon. This was intentionally done to observe if the ommission of the critical point drying significantly altered cell morphology as evidenced by fine cellular surface structures. For the purposes of this study critical point drying was not found essential for fine cellular detail.

The cell counts were performed on all materials at least up to and including the 24 hour period when indicated. A 72 hour period was also counted but only for the PEU's. Nine 4000 mm^2 areas were counted per implant material. There were between 6 and 30 implants per material per time period. These cell counts were then analyzed on a "t" distribution for standard error. A comparison of the means to determine significant differences at ($p \leq .05$) was also performed for the PEU.

The principal cells observed were the macrophage, lymphocyte, and mast cell as determined by morphological characteristics. Other cells, but far less frequent in occurrence, were the eosinophil and polymorphonuclear leukocyte (PMN). The mesothelial cell, as referred to previously, infrequently occurred on the surfaces.

Further reference to this cell will be made at a later time.

RESULTS

Cell Adhesion

The results from the cell adhesion studies are presented in Table I with implantation times ranging from 30 minutes to 24 or 72 hours. These data are plotted in Figure 1. The standard deviations are omitted for ease of interpretation.

The cell attachment on the PTFE untextured vs. PTFE textured differs by an order of magnitude within the first 30 minutes. The textured surface continues to incorporate cells up to 24 hours at which time confluence has occurred and cells begin to pile onto each other. If one assumes that the approximate area covered by a cell is 15μ x 10μ, which is an underestimate for the spread macrophage, then a value of approximately 250 cells per unit area represents confluence. Thus in the case of the natural texture, confluence is achieved at approximately 6 hours. In contrast, the ion polished material attaches cells at a much slower rate and no confluence is obtained for the time periods studied.

The number of cells attached to the PEU's more closely resemble the result of the PTFE ion polished. The FAD sample appeared not to enhance cell attachment to the same degree as the ANN. This difference was significant at the ($P = 0.05$) level up to 24 hours after which their respective cell counts were not significantly different. This result would suggest that the material processing influences primarily the initial attachment kinetics since cell type and degree of spreading were similar.

TABLE I

CELL ATTACHMENT, number of cells/40,000 m^2

	30 Min	6 Hr	24 Hr	72 Hr
PTFE Ion Polish	4.16 ±2.0	10.0 ±7.1	20.0 ±7.8	
PTFE Natural Texture	46.9 ±5.8	219 ±47	257 ±36	
PEU ANN	13.9 ±7.5	9.9 ±7.5	11.1 ±7.0	18.2 ±7.3
PEU FAD	3.6 ±2.7	2.8 ±2.0	5.3 ±2.6	12.8 ±10.0
HEMA R.L. pH6.8	3.0 ±1.7	20.6 ±8.3	157 ±29	
HEMA 0.9% NaCl pH6.8	10.9 ±5.9	134 ±62	202 ±70	

PEU ANN: Polyurethane annealed
PEU FAD: polyurethane fast air dried
HEMA R.L.: 2 Hydroxyethylmethacrylate equilibrated in Ringer's lactate

The hydrogel data were particularly interesting in light of the PTFE control and textured responses. The first series of experiments were done with the gels equilibrated in Ringer's lactate which has lactate ions present and is at a pH of 6.8. In the second series of experiments the HEMA was equilibrated in 0.9% NaCl at a pH of 7.4. The cell attachment rate for HEMA 0.9% NaCl attained confluence at a comparable rate to the ion textured samples. The HEMA R.L. did not enhance cell attachment to the same degree but attained near confluence levels at 24 hours versus approximately 6 hours for the HEMA 0.9% NaCl. Not only is there a difference in kinetics for HEMA in different solutions, but also more significantly the attachment rates of HEMA and natural texture PTFE are similar. The comparison is only reflected in cell numbers, because the response to the two materials in cell type, morphology, and subsequent response appears to differ dramatically.

The percent composition of cells is listed in Table II and graphed in Figure 2. The HEMA sample is not included because of its absorption

of stain, making identification of cell morphology uncertain. However, for the HEMA sample the mast cell population was easily identified and was found to increase up to 6 hours and maintain this level at 24 hours.

The following criteria were used to categorize cells: cells which had an equal to or greater than one cytoplasmic to nuclear area ratio, had pale blue cytoplasm with vacuoles, and had pink nucleus approximately 8μ in diameter were called macrophage cells. The monocytic cell that most closely resembled the lymphocyte had a nondiscernible to perhaps marginal blue cytoplasm with a dark blue to deep pink nucleus approximately 5μ in diameter. There was an intermediate cell population ascribed to the macrophages that had a large cytoplasmic to nuclear area ratio, dark blue and at times granular cytoplasm, and the nuclear diameter was greater than the lymphocyte's. These cells were felt to either represent activated lymphocytes or young macrophages. Their numbers were not great, approximately 3%, but noticeable. These cells may represent a B-lymphocyte; however, it has been shown to possess many characteristics typically assigned to the macrophage.[3] The macrophage's nuclear diameter ranged from 8 to 10μ whereas the nuclear diameter for the mesothelial cell was 15 to 20μ. In addition, the cytoplasmic diameter of the macrophage was 20μ whereas the diameter of the mesothelial cell was 30 to 50μ. The control peritoneal lining cell population was stained for nonspecific esterase (NSE) activity for which 10% were NSE positive. This stain is unique for cells usually ascribed to the macrophage population with appropriate cell morphology.

TABLE II

Mean Values of Percent Composition
for Cells on Material Surfaces

Time	30 Min			6 Hr			24 Hr			72 Hr		
Material	Mc	L	Mast	Mc	L	Mast	Mc	L	Mast	Mc	L	
PTFE Nat Text	62	35	4	50	48	2	48	48	5			
PTFE Control	78	20	2	63	36	1	70	30	0			
PEU FAD	92	8	0	57	38	5	75	24	1	68	30	1.8 0.5% (MNGC)
PEU ANN	69	28	3	54	40	1	74	25	1	50	50	0.5

Mc Macrophage
L Lymphocyte
MNGC Multi Nucleated Giant Cells

The other cells such as PMN's, eosinophils, and mast cells were less frequently encountered though easily identified. The PMN's has a multilobular nucleus; the eosinophils had a ringed nucleus, and the mast cell cytoplasm was darkly stained (deep blue) because of its cytoplasmic granules and the cell diameter ranged from 20 to 30μ. On occasion these granules could be observed freely floating and at times within the macrophage's cytoplasm.

The results from the differential count for the natural textured PTFE (PTFE NT) indicated the principal cell type adhering to the surface was the macrophage which with time incorporated an equal number of lymphocytes. The smooth PTFE initial cell composition was similar to the PFTE NT; however, at 6 hours and 24 hours the macrophage and lymphocytes populations were not proportionally similar.

The cell population on the PEU FAD in the first 30 minutes was primarily macrophages. At 6 hours there were more lymphocytes than macrophages. At 24 and 72 hours the population was again dominated by the macrophage cell population.

The PEU ANN cell population was similar to that of the control PTFE for the first 24 hours. At 72 hours the relative compositions of macrophage and lymphocytes approached each other. This increase in lymphocyte cell type at 72 hours primarily represents clumping on the surface. There was at 3 days an occasional multinucleated giant cell (MNGC) on the order of a half of a percent maximum and usually containing only two nuclei. The mast cell population generally increased at 6 hours and then diminished except in the case of HEMA and PTFE NT which continued to maintain a significant percentage.

On the smooth surface, of the "as received" PTFE control there were primarily macrophages at 30 minutes then a slight increase in the lymphocytic type cell only to return to a predominantly macrophage cell type. The 3-day PTFE control resulted in a predominantly macrophage population with some cell clumping and occasional binucleate MNGC's. The cell numbers were comparable to the PEU's. The presence of a textured surface continues to incorporate cells with clumping occurring at 24 hours. At 72 hours the surface is covered with vaculated MNGC's having 10 to 20 nuclei. Thus at 72 hours on a (1/2, 1/2) sample one observes that the natural-texture surface responds as predicted, but the "as-received" surface does not. The "as-received" half has enhanced cellularity, increased clustering, and a far greater number of MNGC's. The lymphocytic cell type is frequently found encircling a mesothelial-macrophage type cell (Fig. 3). During this time period cell clumping also takes place (Fig. 4). Though these cells appear morphologically to be lymphocytes for reasons that will be mentioned later, they can not be discounted as representing young macrophage. On the (1/2, 1/2) PTFE samples, the occurrence of clumping and MNGC's is significantly enhanced by the presence of a textured surface. If one were to count the number of MNGC's as a function of distance away from the textured surface, there would be a continued reduction in their number (Fig. 5). In spite of this fact none of the materials tested induced MNGC's at three days except in the presence of a textured surface.

The clumping and encircling of cells are felt to represent a preliminary step in the formulation of the MNGC form. Similar observations have been made on other materials which would suggest that these cells are macrophages in the early phases of fusion.[4] However, their morphology is not typical of the macrophage thus precluding the need for further work and identification.

PTFE Smooth Surface

Cells on the surface of ion-polished and as-received PTFE remain spherical with occasional flattening after 24 hours. Though these samples are untextured, the material contours play a significant role in cell attachment as evidenced by their predilection for machining defects (Figs. 6 and 7).

After 3 and 7 days the number of cells on the surface increases with a greater degree of flattening (Fig. 8). As observed in the light microscope, cell clumping is readily observed on the (1/2, 1/2) PTFE sample at 3 days (Fig. 9).

At 14 days the ion-polished PTFE material continues to be exposed and incompletely covered by cells (Figs. 10 and 11). There also appears to be an amorphous deposit as suggested by the cracks in the surface between and around cells. The smooth surface on the (1/2, 1/2) PTFE sample appears to attract more cells per unit area than the control sample (Fig. 12).

Natural-Texture PTFE

The natural-texture surface dramatically alters the cells' interaction as contrasted with the ion-polished surface. This difference in cell attachment was observed to take place within 30 minutes of implantation (Fig. 13). Some of the findings include rapid alteration in shape resulting in flattening, elongation, extensive pseudopodia formation, clumping, and a loss of cell contact inhibition (Figs. 14 and 15). There is an additional change in the membrane surface structure which can best be described as membrane ruffling. This will be referred to in the discussion section (Figs. 16 and 17).

The 24-hour sample persisted in like fashion by continued accumulation of cells onto the surface which developed into multiple layers and sheets of cells. There is also a fine fibrillar structure approximately 0.1 to 0.2μ in diameter. It is felt that these are cytoplasmic processes (Figs. 18 to 23).

After 14 days the natural-texture material is completely covered by cells taking on an appearance that approaches that of the natural mesothelial lining (Figs. 24 to 26). In marked contrast the control material remained exposed and relatively free of cells (Figs. 24 to 26).

PTFE Pits

The pitted surfaces raised several interesting questions regarding the texturing procedure as well as the cells' response. The pits ($50\mu^2$W x 100μ D) are not simply surface defects but are also textured. Thus one is not dealing with a smooth surface and pits but instead a regular array of surface microdepressions in conjunction with a submicra natural-texture. A few samples had the natural texture extend beyond and between pits. This occurs during the sputtering process if the mask is not tightly pressed against the sample surface.

The sample after 30 minutes showed very little attachment in those areas that did not have the natural texture. However, when the texture was present, it controlled the cell's interaction (Figs. 27 and 28). After three days there was extensive cell attachment over the entire surface with growth into the pits (Figs. 29 to 31). The cellular response in all aspects appears to be similar to that of the natural texture. In addition, the cells appear to display a preferred orientation when either on the surface or in association with the pit. Many of the cells appear stretched either normal to the pit or encircling it at the edges. Once the pit is filled with cells many others will flatten and extend themselves radially from the center of the hole.

At 7 days the cells continue to fill the pits while the smooth portion of the surfaces maintains a uniform cell number (Fig. 32). The lack of cell contact inhibition continues to control the cell's surface interaction with cell layering in the pit and at its edges (Figs. 33 and 34). Once again a fine fibrillar texture develops which eventually results in a loss of individual cell indentity within this matted surface (Fig. 35).

This process apparently continues as with the natural-texture surface resulting in a uniform cell layer at 14 days. This cell layer also appears similar to the natural-texture implants (Fig. 36).

PEU

The polyurethane is similar in many respects to the smooth PTFE; however, it has increased cell spreading (Figs. 37 to 41). There is not the extensive pseudopodia formation nor the degree of cell clumping as observed on the natural texture. The cell morphology between the FAD and ANN samples did not suggest that, at the time intervals sampled, differences could be discerned. There was complete sample coverage by cells at 7 and 14 days on the ANN samples, but the results were more variable for the FAD samples (Figs. 42 to 44). However, this result could not be considered significant based upon the number of samples evaluated. At 14 days there was not a uniform coverage as found with the pitted and textured PTFE surfaces.

HEMA

The HEMA sample at 30 minutes showed extensive cell flattening and spreading to a significantly greater degree than the PEU or PTFE control surfaces. Occasionally there is cell clumping (Figs. 45 and 46). The cell attachment continues and at 7 days many of the cells form a uniform layer with no apparent further attachment of cells (Figs. 47 49). However, the 14-day sample had a unique cell form that was not observed previously with any of the materials (Figs. 50 to 53). These cells ranged in size from 30 to 50μ in diameter. Isolated, they were raised in the center and tapered at the edges. These cells were observed to be in intimate contact with newly arrived cellular elements. It was felt that these cells most likely represent the mesothelial cell form.

HISTOLOGY RESULTS

As previously described, the mesothelial cell was larger than the macrophage and Figures 54, 55, and 56 allow for this comparison. All three pictures were taken at the same magnification. The first two photographs show mesothelial cells from the abdominal lining, with their 15μ, lighter-staining nucleus. The dark particles in Figure 54 represent mast cell granules.

The cells on the PEU-FAD at 30 minutes represent the macrophage's morphology with a smaller nucleus and darker staining cytoplasm. It should be noted that the cells at 30 minutes are still round with pseu-

dopods just beginning to form. The intermediate cell form between that of the macrophage and lymphocyte can be seen in Figure 56 marked "I". Its nucleus is similar to the macrophage's in staining characteristics but is smaller. This cell has been described as a surface-adherent, non-phagocytizing macrophage with B-lymphocyte characteristics.[3]

PTFE

The (1/2, 1/2) samples most clearly reflect the influence of texture on cell attachment. Within 30 minutes there is clearly a significant difference between the control and textured surface (Fig. 57), and at 6 hours this difference has developed to a greater extent (Fig. 58). The control surface selected for the photo represented a worst case. Note that clustering has already occurred at 6 hours on the textured surface.

At 72 hours the low power magnification displays the presence of the MNGC's on the smooth surface in proximity to the natural texture (Fig. 59). At higher magnification the smooth surface has increased cellularity with clumping which, as previously discussed, may represent precursors to the formation of MNGC's (Fig. 60). The textured surface is now covered with MNGC's. These cell forms are diffuse and highly vaculated (Fig. 61). Again, it should be noted that the occurrence of MNGC's is an infrequent event at three days for all of the nontextured materials.

The 7-day and 14-day implants for smooth PTFE were similar except for the increased capsular collagen at two weeks. These figures (62 and 63) for the control are shown primarily to demonstrate the edge effect that an implant has upon its capsule. These edge effects correlate with similar findings for subcutaneous implants. (Note the increased cellularity and MNGC formation (Figures 62 to 64)). The remainder of the capsule (upper half of figure) was lined by a layer of cells with minimal reactive cell forms. The bottom half of the capsule is not representative since there was another implant adjacent to it.

The influence of the natural texture upon the capsule in many respects was similar to that observed in soft tissue[10]. The most obvious similarity being the formation of sheets of MNGC's adjacent to the capsule. It was difficult to ascribe a relative proportion of cellularity to the capsule or thickness since the time period for this difference to manifest itself was insufficient. In addition, the sample's orientation could not be clearly discerned at all times due to artifacts from the histological preparation. However, this difficulty could easily be overcome with special embedding techniques in the future (Fig. 65).

In Figure 66 the cells at the interface are in intimate contact with the texture and in close proximity to the vascular supply. The collagen capsule is highly cellular and the fiber packing is loose.

The fourteen day textured implants are similar except there is increased cellularity, collagen deposition, and vascularity at the outer margins of the capsule (Figs. 67 and 68).

Figure 69 is an intact interface showing a plump cell form with nuclei at the textured interface. This layer rapidly transfers across a sharp boundary into a highly cellular capsule with dark staining flattened nuclei typical of fibroblasts. These cells most likely represent the surface cell in the SEM pictures.

The conclusions from the textured implants at 7 and 14 days were not as definitive as they might have been had the interface been preserved. However, the overall response was similar to the subcutaneous implants suggesting that it can be considered as an analagous system for future work[10].

Pitted PTFE Surface

The pitted surfaces represented a mixed response. A reliable cell count could not be performed due to irregularities in sample fabrication. In general, there was increased cell attachment compared to the smooth PTFE, but less than the textured surface (Fig. 70). Cells rapidly entered the pits, provided the samples were degassed and the pits filled with saline (0.9%) prior to implantation. It was not unusual to find this surface adherent to the tissue of the peritoneal wall as early as 6 hours. One also occasionally observed a regular array of tissue pillars at three days. It was felt that the mesothelial lining was adhering to the pitted surface and conforming to it. At three days a sheet of cells could be peeled away from the pitted surface (Fig. 71). The 3-day sample also displayed the influence of the natural texture at the pit's edges resulting in a regular array of cell deposition (Fig. 72). This process could be employed to control surface deposition of cells.

The 7- and 14-day implants again were similar to the soft tissue response with cells at the base of the pits. They were plump with large nuclei and without collagen deposition. However, once again the interface was significantly disrupted making the analysis difficult.

PEU'S

The cell morphology on the PEU's did not change significantly at the chosen time periods. At 30 minutes there was minimal spreading for both ANN and FAD. The principal cell was the macrophage (Figs. 73 and 74). At 24 hours these cell forms are closely adherent and display a vaculated cytoplasm (Figs. 75 and 76). Their area of surface contact has increased several fold by this time. Other darkly stained cells with little or no cytoplasm are lymphocytic cells. No clumping or MNGC's are present at 24 hours. Clumping begins to occur at 72 hours but at levels significantly less than those found on the smooth part of the (1/2, 1/2) samples.

The capsule, when present, at 7 and 14 days was much thinner than the capsule around the PTFE control or natural texture. In contrast to the PTFE NT, there was no vascularity or MNGC formation and only a few cells in a very loose matrix of ground substance and collagen (Figs. 77 and 78).

HEMA

The 7- and 14-day capsules for HEMA were variable as depicted in Figures 79 and 80. Both of these samples represent a 7-day implantation period.

The only apparent difference between the two samples was that Figure 79 shows one of several samples which had inadvertently come in contact with each other and were enclosed by the same capsule. These samples were also adherent to the omentum at time of removal. The other sample was free floating. The free-floating capsule is more representative of the other HEMA implants.

The 14-day samples were characteristically without the presence of a fibrous capsule. Presumably there was a disruption of the interface at the time of staining, or the samples are representative of the freely floating type. The most characteristic finding was a uniform flattened layer of cells approximately 30u in diameter encircling the implant.

These cells may reflect those found at 14 days by SEM but further work is required for definitive identification (Fig. 81).

The results from the peritoneal implants suggest that it is a system which models its tissue response in a manner consistent with soft tissue and exhibits responses which are sensitive to chemical and morphological properties of the material with which it interacts.

Continued study is indicated with prolonged implantation times and careful retention and preservation of the tissue-implant interface.

DISCUSSION

The results from the analysis of the peritoneal implants provided interesting and potentially very useful information. The peritoneum as a body cavity is bathed with an extracellular fluid that is similar in composition to serum and conforms to a Donnan equilibrium (Table III).[5,6] The composition, surprisingly, has not been thoroughly investigated with respect to protein content. There is, however, protein in the fluid but reduced in comparison to the plasma. The Albumin/Globulin ratio is slightly higher than that of plasma. The total free floating cell number for the rat's peritoneum is approximately 20 million, of which the majority are macrophages and lymphocytes.

All of the cell types present in the blood are also represented, but their relative proportions differ. Table IV lists the relative percentages of these cells.[7]

The cell turnover is unique when compared with soft tissue but similar to the respiratory system. The macrophage and lymphocyte population is indigenous, but their numbers are capable of both multiplication and/or recruitment from the surrounding lymphatic and vascular system.[8,9] Recruitment usually occurs only as a response to an inflammatory incident.

With this background, the results from the implants will be discussed with regard to cell attachment, SEM, and histology. There will also be included a suggested protocol for implantation, further experimentation, and implications of this work with reference to clinical significance.

TABLE III
CHEMICAL COMPOSITION OF
PERITONEAL FLUID AND SERUM

Chemical	Peritoneal	Serum
Phosphorous	6.5	6.3
Uric Acid	8.7	8.7
Creatinine	13.7	13.1
Potassium	4.7	5.1
Sodium	141.0	140.6
Chloride	108.5	102.7
Bicarbonate	19.4	20.4
Calcium	6.74	8.44
Glucose	126.5	122.0
Protein	3.03	5.74

These values were taken from patients on peritoneal dialysis.

Kelton et al.
Annals of Int. Med., 89, 67 70. 1978.

TABLE IV
DIFFERENTIAL COUNT OF
PERITONEAL CELLS

	Percentage
Polymorphonuclear leukocytes	0.12
Lymphocytes	17.75
Macrophages	67.30
Eosinophils	14.8

Gardner, Leroy
Society of Exp. Bio. Med., XXVI, 690-693, 1929.

PTFE

The influence of surface chemistry and morphology on cell attachment was dramatically displayed by the use of textured and untextured PTFE. The enhanced cell attachment attained with the natural texture is apparently due to the PTFE's altered surface morphology. The influence of ion sputtering upon chemical composition is minimal based on ESCA and I.S.S. Both analyses suggest a slight increase in fluorene concentration.[10]

The influence of texture on attachment kinetics can be approached in several ways. If one considers the electrostatic repulsion theory, then the energy of repulsion for two surfaces decreases with decreasing radius of curvature assuming the surface potential of the untextured and textured surfaces do not change. This theory would suggest that for a cell interacting with the natural textured surface, less work would be required to overcome the repulsive barrier during attachment.

The energy of attraction, however, is not enhanced but reduced with the reduction in the radius of curvature. The repulsion term is generally the larger of the two, thus the reduction in the repulsive term would be greater than that of the attractive term.[11] The attractive term, however, could conceivably be enhanced by the Hamaker constant through an inverse dependence of the radius on the interacting particles.[12]

In parallel with this argument, the textured surface may also alter fluid volume displacement at the interface during the course of cell attachment. The textured surface would offer a unique situation, with cones of small radii of curvature extending into the bulk making a point contact, thus reducing the initial surface area and shearing force required for fluid displacement. Therefore, the force required to bring into apposition two surfaces at a given velocity would decrease with the decreasing radius of curvature.[13]

Concerning the issue of cell contact and inhibition, frequently during the early time periods, cells on the natural texture were found to have ruffled membranes. This morphology has been considered unique to the macrophage in a study that compared surface structure by SEM with histology.[14] In addition, and perhaps more relevant to the textured surface, is the observation that similar wave patterns have occurred when a lack of contact inhibition is displayed.[16]

HEMA

The unexpected observation that the rate of cell attachment for HEMA was comparable with the textured material was in part thought to result from the sample being equilibrated in Ringers' Lactate which not only has a lactate ion but is slightly acidic. The experiment was repeated in 0.9% NaCl buffered to pH 7.4 with dibasic sodium phosphate. This treatment further enhanced cell attachment, thus reinforcing the importance of the equilibration solution and its interaction with the cells.

More significant is the comparable attachment rates between a physically modified hydrophobic polymer and a smooth hydrophilic material.

In general, HEMA is considered a relatively biocompatible material with respect to blood and soft tissue.[17,18] For example, tissue cultures using myoblasts or fibroblasts indicate that there is a relatively low percentage of cell attachment. In addition, the soft tissue response, when compared with other biopolymers, is judged to be mild having a small capsule thickness, minimal cellularity, and no reported multinucleated giant cells.

However, there are indications that the HEMA polymer may in fact encourage membrane attachment. Experiments utilizing HEMA-coated Kusserow rings have resulted in relatively thrombus-free implants after exposure to the blood. However, examination of the kidneys has shown moderate to heavy infarction secondary to emboli, thus suggesting the surface of HEMA is more nonthrombus-adherent than resistant.[19]

The interaction of platelets and HEMA was also studied in vitro suggesting that there is increased adherence leading to aggregation. This reactivity was comparable to polymethylmethacrylate, which is the hydrophobic form of the methacrylate polymer. These data would tend to uniquely support the peritoneal data.[20] However, they do represent isolated observations.

Another point to consider when comparing the tissue culture results of HEMA with the peritoneum, is the fact that the in vitro system cannot as yet fully reflect the in vivo environment as to all of the required cellular nutrients and biochemical factors. In addition, the cell lines used in culture were neither macrophages nor lymphocytes, and it is well recognized that the different cell lines and types have unique requirements for growth and attachment.[21] These considerations could significantly influence cell contact.

.he other, and most important, consideration is the misguided concept that cell adhesion and compatibility are inversely related. This relationship need not be the case since it is not the rate of cell adherence to the surface that is important, but how the cell "perceives" that surface subsequent to attachment.

The other materials, i.e., PTFE control and the PEU's, generally responded in like fashion differing only in kinetics. The reason for this may be their differences in surface energy. However, improved sample handling is required with the PTFE before comparisons can be made. There was also a trend of increased lymphocyte attachment up to 6 hours and a subsequent decrease in attachment. The lymphocyte cells may be reflecting different attachment kinetics as compared to the macrophage for the same surface, or during their early adherence phase they are truly macrophages mistaken for lymphocytes.

In addition to these considerations, and in light of the PEU data, one may ask if there is any similarity between the cell attachment in soft tissue and blood. The data would support that both the structural

and chemical influence of the material in soft tissue has a correspondingly similar response in the blood.

For example, the PEU's have been shown to enhance platelet attachment in the annealed state. This relationship appears to persist in the peritoneal system, though with time the surfaces become equally covered with cells at 72 hours. This similarity suggests an initial rate-dependent phenomena or a secondary response which is occurring with time.

The HEMA polymer, as previously discussed, further supports the concept that there are analagous responses to soft tissue and formed blood element attachment.

The influence of morphology has long been recognized as playing a significant role in cell attachment in blood. Again, the peritoneal implants only further support these findings. Thus, models developed for cell adhesion in the soft tissue may very well extend to the vascular system.

Combining the short term cell attachment results with the 7-day and 14-day results, one finds that apparently the initial events in cell interaction can influence the subsequent response.

The PTFE NT and HEMA results clearly demonstrate this relationship. The PTFE NT had multiple cell layers, no apparent contact inhibition, collagen deposition, and the absence of mesothelial-type cells at the surface. In spite of HEMA attracting comparable numbers of cells, their response was entirely different showing flattening, few pseudopodia, and displaying contact inhibition. This reponse resulted in a monolayer of cells, many of which appeared to be typical of the mesothelium.

The PEU's and smooth PTFE for the most part did not develop a cellular response similar to PTFE NT or HEMA, which is believed secondary to their reduced cell attachment and activation. However, when a capsule was present, there was little apparent difference between the PTFE and PEU's.

Natural Texture PTFE

The natural texture, by comparison with other soft tissue work on PTFE, selectively induces macrophage fusion over its surface. This is a process usually associated with engulfment. As observed, the peritoneal macrophage responds in a similar fashion to the soft tissue macrophage by forming multinucleated giant cells which apparently is a morphologically induced phenomena.[10]

Work with bacteria and phagocytic cells suggests that the surface energy of bacteria can strongly influence their interaction with the white blood cell. There appears to be a relationship between high contact angle or low surface energy and enhanced phagocytosis. The phagocytized bacterial number ranged from 0.2 bacterium per phagocyte

for a critical contact angle of 16.5° to a maximum of 6.4 bacteria per phagocyte for a contact angle of 70°. The calculated free energy for opsonization was -0.15 ergs/cm^2 and -3.1 ergs/cm^2, respectively. This model has been extended to other systems such as surface proteins and soluble proteins, i.e. gamma globulins with and without compliment, and the trend of increased hydrophobicity with enhanced phagocytosis persists. Thus, it was felt that an attempt to model the natural texture with its 0.1μ radius of curvature at the peaks as compared with the bacterial 0.4μ radius of curvature would be appropriate. In addition, the PTFE should represent an extreme hydrophobic model.[10,23]

The calculations were performed using the model developed for the bacteria-monocyte interaction but replacing the bacteria values with appropriate values for PTFE. The net free energy for the PTFE was -3.8 ergs/cm^2 yielding a relatively large driving force for phagocytosis.

This model admittedly ignores specific cell type interactions and protein components on the surface of the polymer which could alter the cell's interaction. However, enzyme studies on both rat macrophages and human monocytes on natural textured PTFE indicate that their enzyme systems, for example esterase activity, are induced to extremely high levels of activity.[10,22] It would be of interest to test this model with materials that are extremely hydrophilic and have a surface morphology similar to the natural-texture PTFE.

This elevated enzyme activity may in fact be reflected in the enhanced clumping and MNGC formation on the control surface for the PTFE (1/2, 1/2) sample. These observations suggest a diffusional component from the textured surface cells or in fact the cells are migrating from the textured surface resulting in MNGC formation. Lymphokines such as the macrophage inhibitory factor and the macrophage fusion factor could also explain many of these phenomena but further work is needed to confirm this.

There are other similarities between the peritoneal implant and soft tissue response, for example the edge effect in which there is a narrowing and compression of the capsule at the corners. Further, as with the soft tissue, an increase in cellularity regularly occurs and in some cases giant cells are formed at the edges. Both of these phenomena were observed in the peritoneum. These results further suggest that these characteristics are functionally related to the material and the capsule, while less dependent upon the local tissue type.

SUGGESTED PROTOCOL FOR PERITONEAL IMPLANTS

Based upon this peritoneal implant experiment, there are some improvements which are felt to enhance the retrieval and reliability of the information from the implants.

Surgery: Instead of using a scalpel for the incision, it is suggested that electrocautery be used. It reduces the chance of contaminating the samples with blood, both operatively and postoperatively. When suturing the peritoneal cavity one should do it in such a manner that the mesothelium is anastomosed and folded outward. Do not keep

the animals under continuous ether blow-by for the 30 minute experiment; it alters cell attachment. When placing the samples into the gut, try not to put them into the region of the liver or spleen. Either placement makes for a difficult dissection at the end of an experiment. Instead, place them into the gutters and lower quadrants (region of illiac artery), also beneath a loop of bowel or stomach; this will help to keep samples separated. The 250 gm male rat is an ideal size and has reduced vascularity at the time of surgery. It has been determined that female rats alter their abdominal cytology during their menstrual cycles.

Sample: It should not be greater than 0.25 mm thick (ideally 1 mm if it is clear) and not greater than 1 cm in diameter. Seven millimeters appears to be an ideal diameter. Translucent samples can be thinner. Prior to implantation the sample should be immersed in 0.9% NaCl. This procedure helps to lubricate the sample so that it does not immediately stick to the mesothelium. In the case of the "as-received" control PTFE one should place those samples between glass plates under compression and heat for 18 hours prior to ion-polishing.

Stain: May Grunwald/Giemsa is felt to be a good staining system. However, problems will be encountered with any of the hydrogels in retaining the stain. The alternative may be to shorten the staining times or thinner samples. Other stains that are water based or rapid staining may be helpful, for example Rapid Wright's. Of great importance is to have the histological samples embedded in epoxy to preserve the interface at the time of sectioning. It was also helpful on removing a sample to cut it in half, one part for histology and the other part for SEM.

Implantation Times: There are two phases, one is the acute range from 30 minutes to 3 days and then the range of 7 days and above. The times chosen to date were felt to be representative of the events at the surface. However, longer times and perhaps sampling at 5 and 10 days might be of interest since some of the samples changed dramatically between 3 and 7 days as well as between 7 and 14 days. However, for the histological samples one month and two month periods are strongly suggested for studying the fibrous capsule and interface cells.

PROPOSED FUTURE WORK

The peritoneal cavity as indicated by this work can respond to and has a degree of sensitivity that can detect minor differences in material composition. Further it lends itself to studying short or long term periods, thus providing a correlation between them. It differs from the soft tissue response in some respects since there is no acute hematologic response with the kinin's release, etc. and chemical mediators, though this is not to suggest that it does not occur in a very local sense. Another difference is the lack of the polymorphonuclear response within 24 hours. However the macrophage attachment to the surface and associated responses to different materials with the fibrous capsule and its cellular components suggest that the peritoneum may offer some analytical advantages to the subcutaneous implantation.

For example, the implantation and removal of implants can be performed in a very controlled fashion with minimal disruption of the cells at the interface. This would permit the entire surface to be characterized and not simply in 5μ cross sections. The macrophage could be studied from the time of attachment as it begins to respond to the surface via enzyme conversion to the time it fuses with other macrophages or interacts with fibroblasts in the early phases of capsule formation. This system is also amenable for studying in a single sample histology, histochemistry, SEM, and transmission electron microscopy, allowing one to correlate a variety of parameters uniquely contributed by each technique.

A more imaginative approach would be to deposit cell forms, for example from a human onto the material of interest, i.e., monocyte on a PTFE (1/2, 1/2) sample and then place this into a chamber to be bathed by the peritoneal fluid. This technique would allow for the characterization of the cells by karyotype and enable them to respond in a more physiologic environment. There are many unanswered questions regarding cell metabolism and transformation during the course of implantation and this system may be used to study these phenomena. There appears to be an even more crucial question when considering the textured material as to what is the critical morphological dimension that the cells respond to for both adherence and the fusion of macrophages. An additional area is whether there is a diffusional product produced when cells are on the textured material that enhances multinucleated giant cell formation. This question initially could be investigated by means of a Boyden chamber.

The rat also has further capabilities. One could deplete certain cell forms by radiation or chemical means to observe their respective role in tissue interaction.

Finally, the system has shown itself to be a good in vivo system for studying cellular kinetics or attachment and correlating material characteristics. This system lends itself to studying a full range of materials combining techniques in sputtering and casting which accurately reproduce the textured surface with varied chemical composition, for example a series of polymers ranging from pure HEMA to pure polymethylmethacrylate. One could also compare collagen content and capsule density with careful dissection of the sample combining thickness and hydroxyproline content. The peritoneum is also amenable to studying the presence and influence of enzymes secreted from the cells on the surface or in the capsule which can modify its characteristics. This ability could lend itself to characterize the interaction between the surface macrophage and the modulation that occurs between it and the fibroblast.

CLINICAL IMPLICATION AND POTENTIAL APPLICATION

The natural texture and the pitted structure have lent themselves to some exciting and new applications, many of which will not be discussed except as it relates to the peritoneal results.

Some of the general observations suggest that the avid adherence of cells to the texture could be used to extract them from body fluids in either diseased states such as leukemia or routine removal in separation from plasma.

One could also use gradations in texture which could evaluate diseased states characterized by the lack of adherence to surfaces. This is primarily recognized in hematological disorders with platelet disfunction or perhaps blood protein disorders. Continuing in the vein of cell attachment, it would be interesting to achieve an adherent but nondisruptive morphology on materials other than polytetrafluoroethylene and grow liver cells, pancreatic cells, or other metabolically active cells that could perform an enzymatic function. These cells could then be placed in the implant pore morphology allowing blood or plasma to pass over them, giving a much higher surface area to volume ratio and achieving an enzymatic level comparable to the intact organ. One could also envision blood vessels extending into a pit of the proper material composition and interfacing with the gut epithelium to function as an artificial gut. Another area of application for textured surfaces is to monitor and characterize patients with defective phagocytic cells with subsequent lack of enzymatic conversion. The textured surface may afford a sensitivity of response previously unavailable to the biological sciences. This characteristic would assist in defining subpopulations of genetically aberrant groups by increasing the signal to noise ratio.

Pursuant to the idea of phagocytosis, bacterial or polymeric spheres could be sputter coated with a variety of polymers to further develop and understand the phagocytic model.

An additional area for application would be the development of an artificial ureter and a colostomy device. One could use the pitted and pore structure for anchorage and tissue ingrowth at the anastomsis site. In contrast, the external surface should be smooth and compatible, minimizing the opportunity for adhesion formation.

Current needs for understanding the biological response in the peritoneum include the application of a defined morphology and material for the peritoneal shunted hydrocephalic and peritoneal dialysis patient. Further work is needed based upon the observations from this study. However, given the current data, it would appear that to maintain patency (non occlusion) one would like to use a greater than 50μ pore. This is suggested by the pits filling with cells and spanning its width. However, the pore morphology with associated fluid movement could alter this response. Secondly, the pit should not be textured at least in the case of Teflon, and the corners should be rounded to reduce the tendency for fibrous capsule formation and adhesions. Thirdly, the material should be coated either with a urethane or HEMA. The silicones should be considered as well, in light of adhesion formation.

There is also a need for further work that investigates cell interactions with materials of different morphologies. It is only through the support of agencies who are familiar with this technology that the fruition of ion technology and its application can be achieved.

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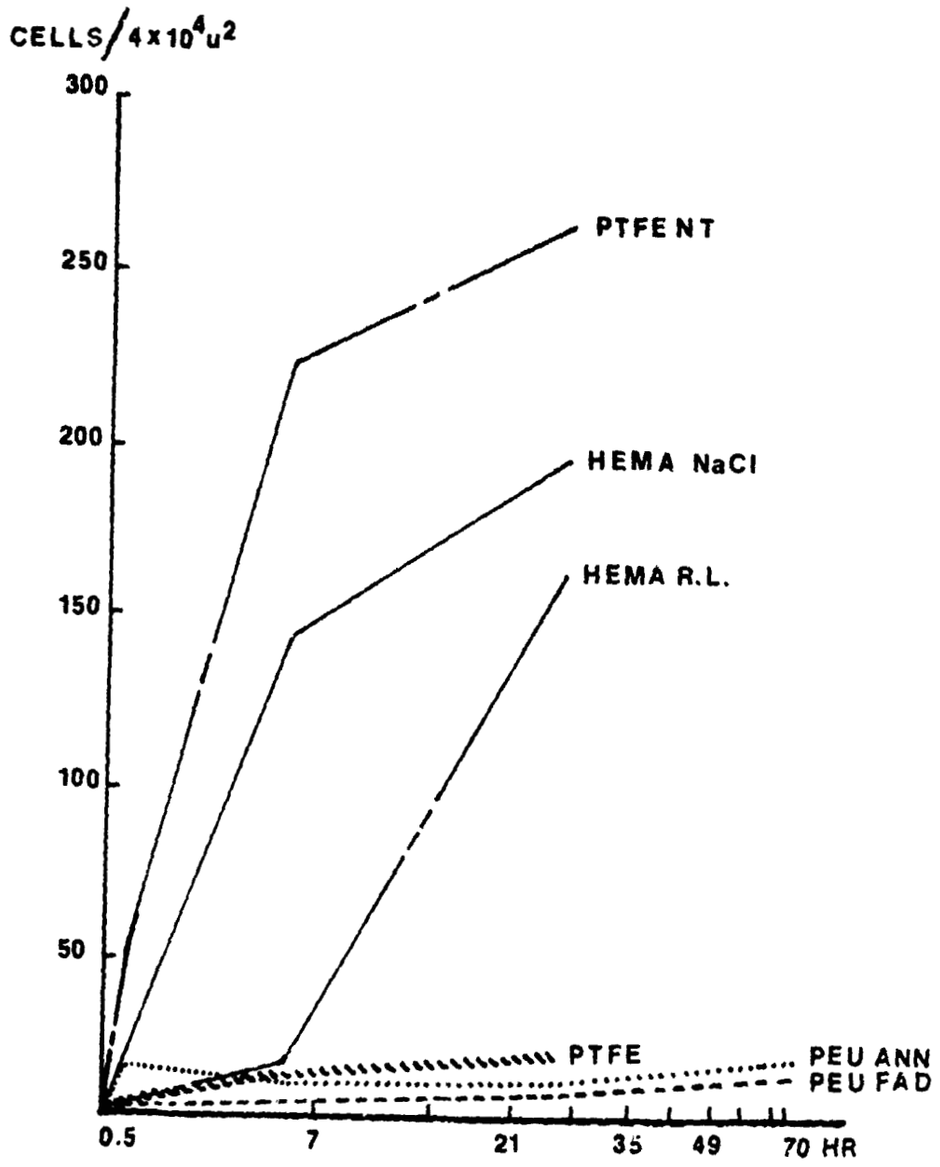

Figure 1. - Cell attachment for PTFE NT, HEMA (NaCl), HEMA (R.L.), PTFE (control ion polish and as received) PEU ANN, and PEU FAD

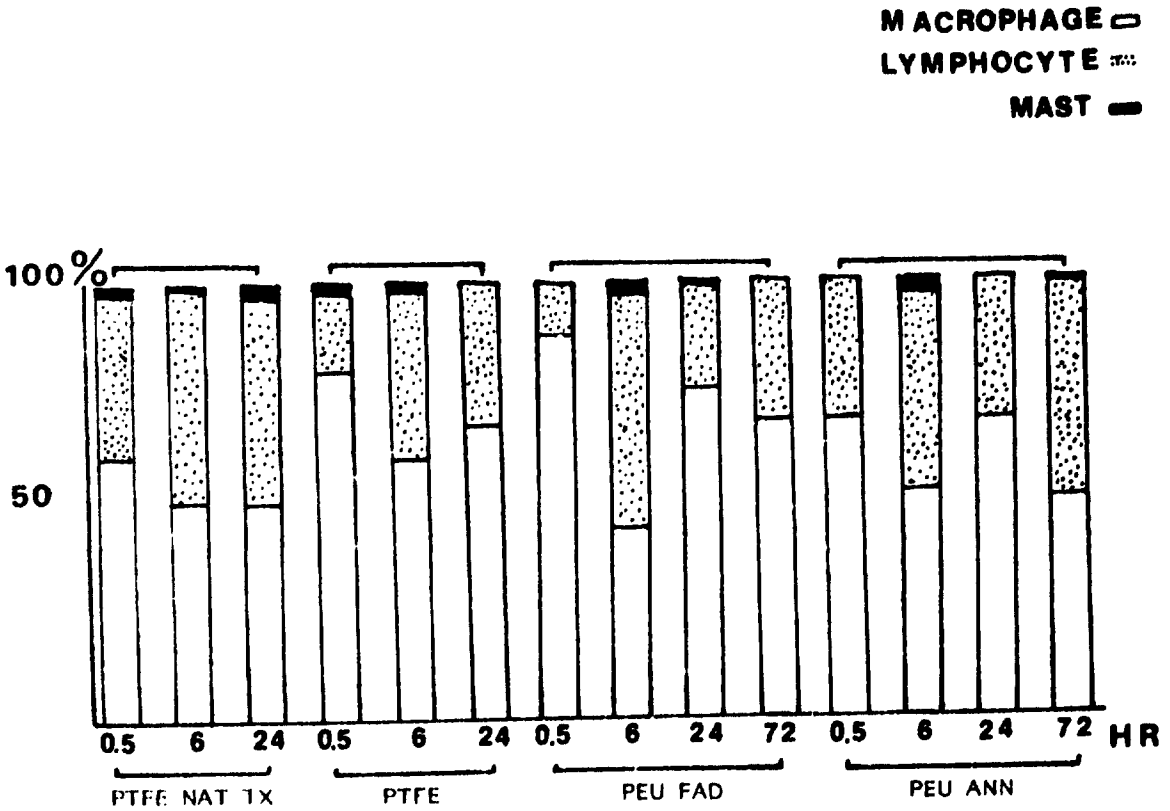

Figure 2. Percent cell composition as a function of time and material.

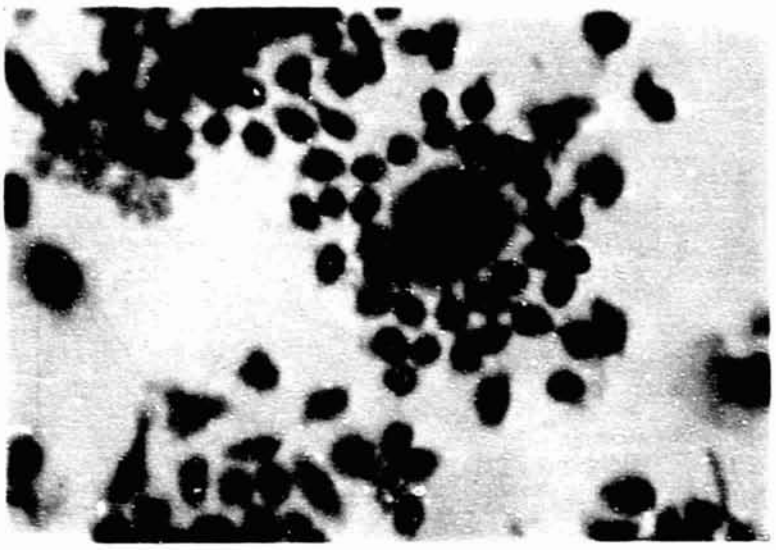

Figure 3. - PTFE (1/2,1/2), 72 hours, untextured half. Lymphocytic cell form encircling mesothelial macrophage cell type. 400X.

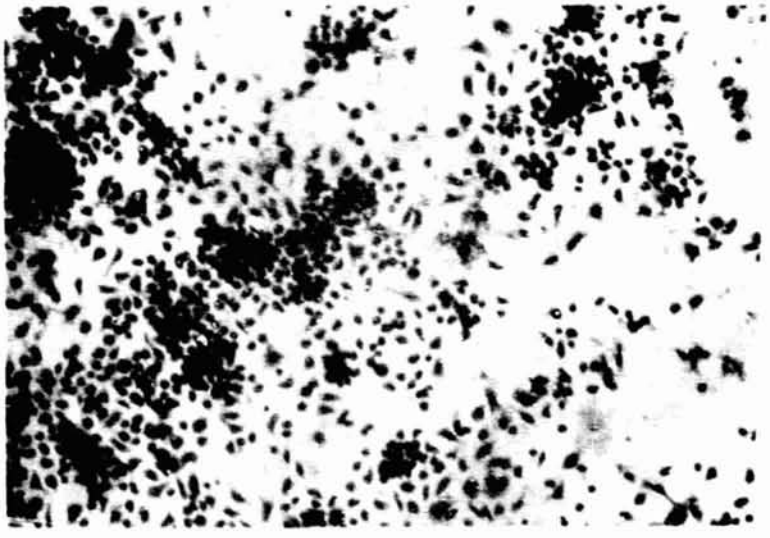

Figure 4. - PTFE (1/2,1/2), 72 hours. Cell clumping on smooth PTFE. 100X.

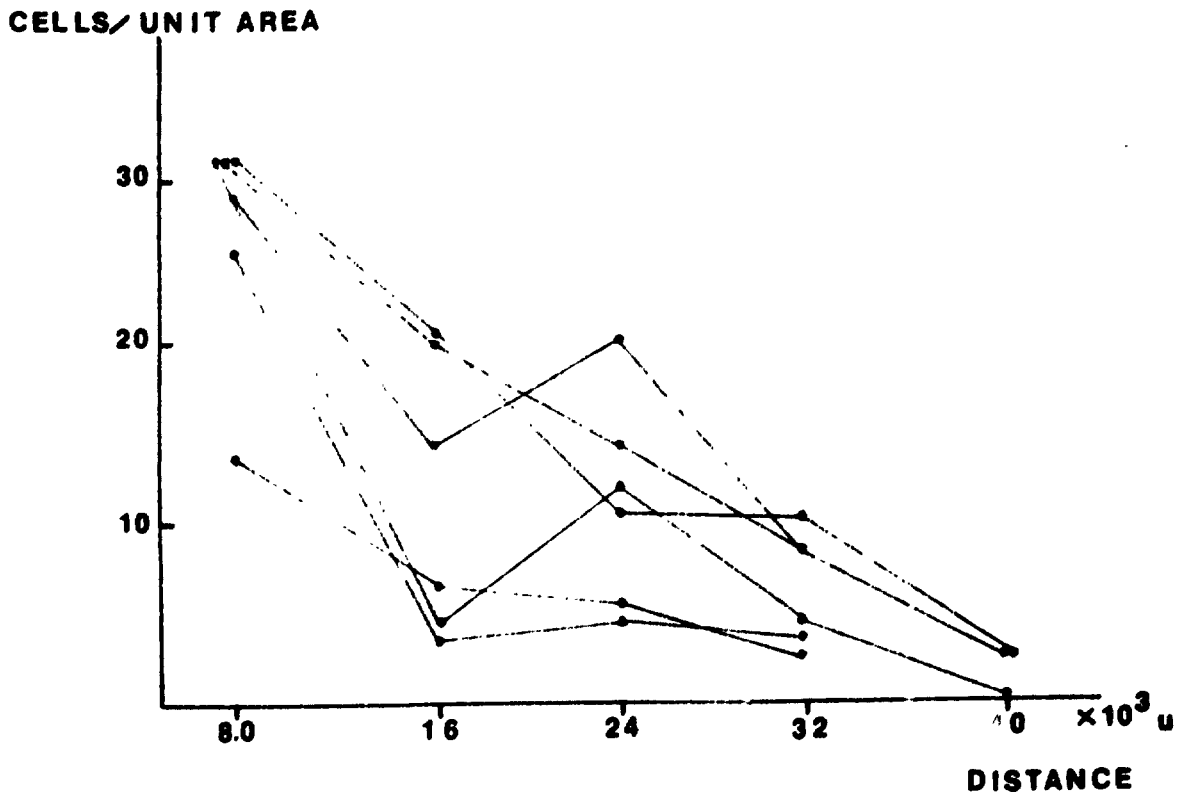

Figure 5 - Multinucleated giant cell number per unit area (64,000 u^2) as a function of distance from the textured PTFE surface, for 3-day sample.

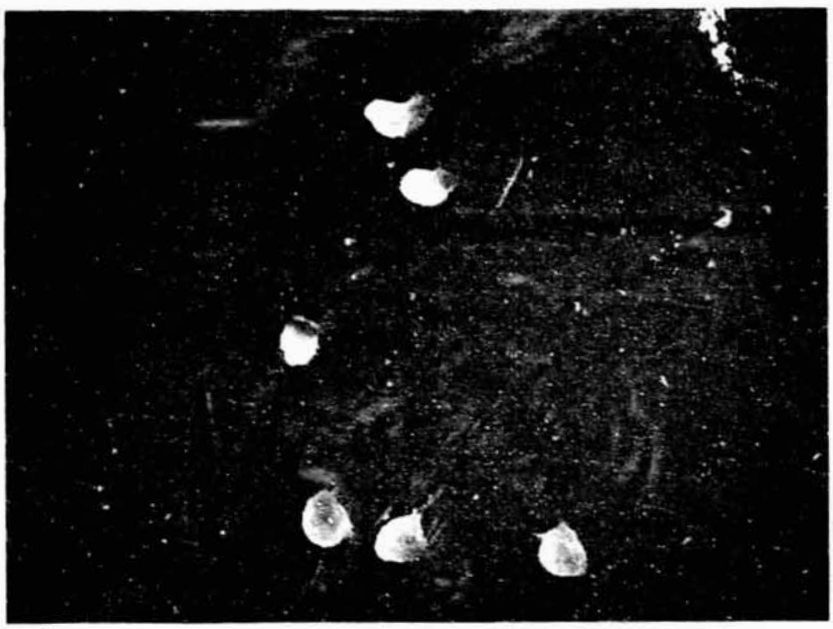

Figure 6. - PTFE ion polished after 24-hour implantation. Note the lack of spreading and pseudopod formation. The SEM photograms have a scale in the lower half of the picture. It corresponds to the following dimensions:

50 u .5u 5u

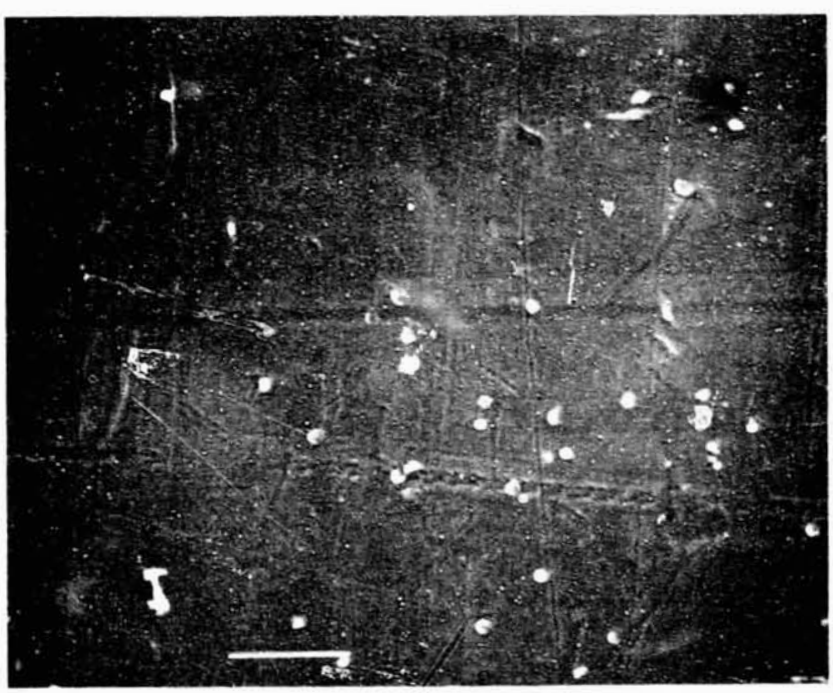

Figure 7. - PTFE ion-polished after 24 hour implantation.

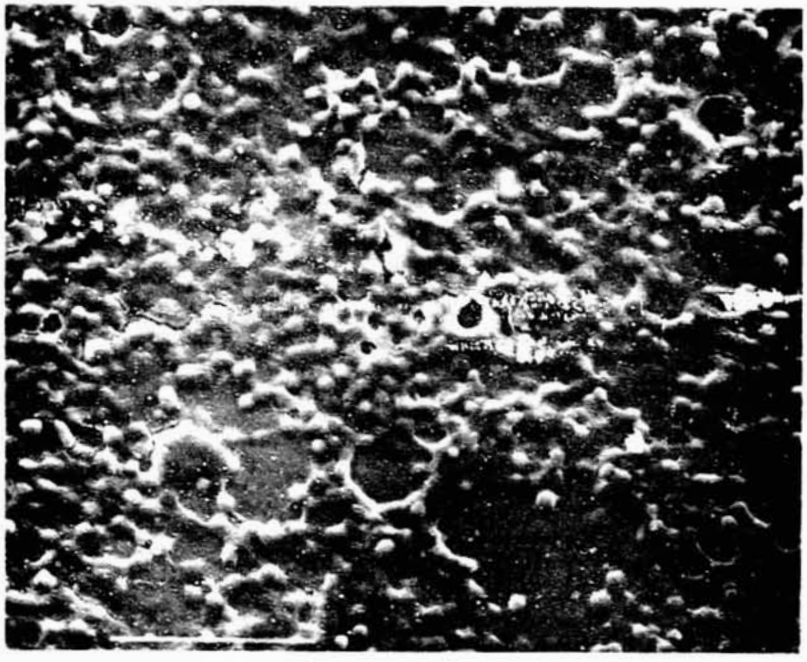

Figure 8. - PTFE control, 7 days' implantation. Note increased cellularity and amorphous deposit. Cells appear more spread.

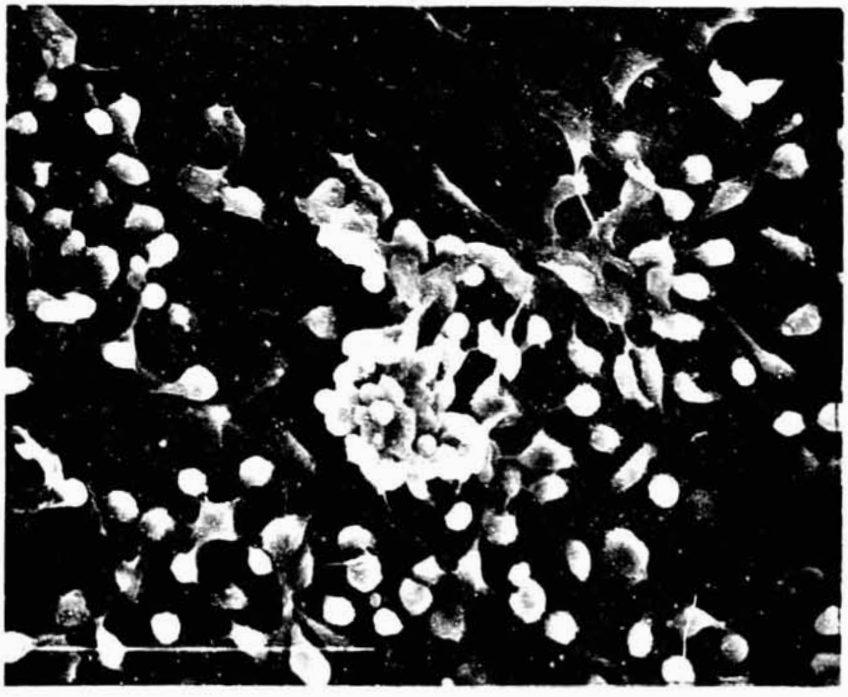

Figure 9. - Smooth half of PTFE (1/2, 1/2) sample at 7 days. The cell clumps correspond to the light microscope findings.

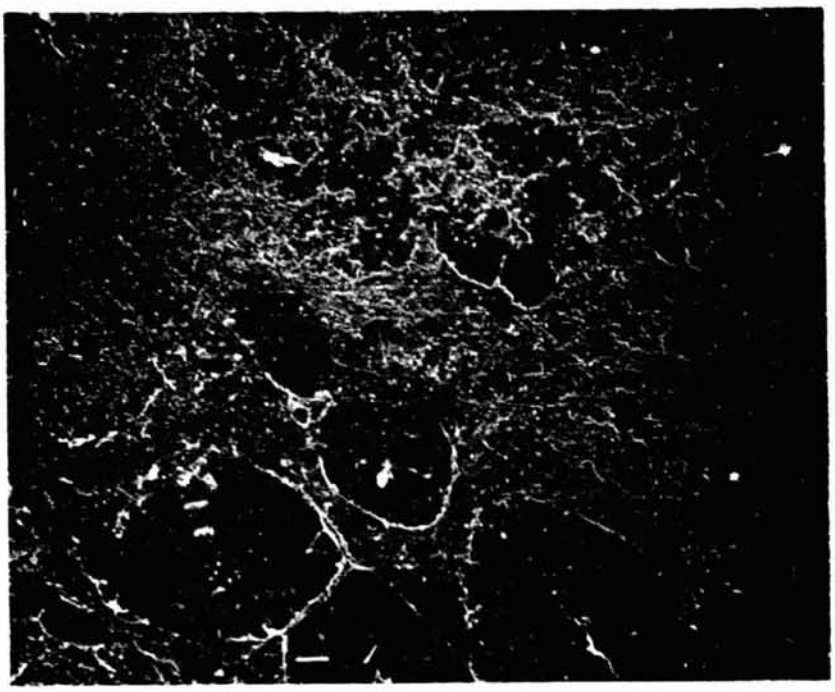

Figure 10. - PTFE control, ion-polished at 14 days. Note the lack of a uniform cell coverage.

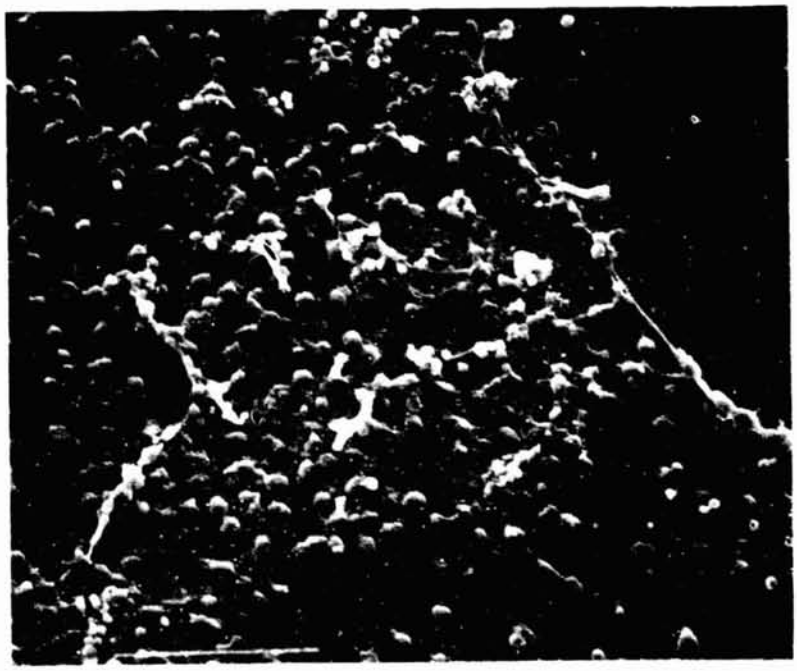

Figure 11. - PTFE control, ion-polished at 14 days. Note the amorphous deposit associated with the cells.

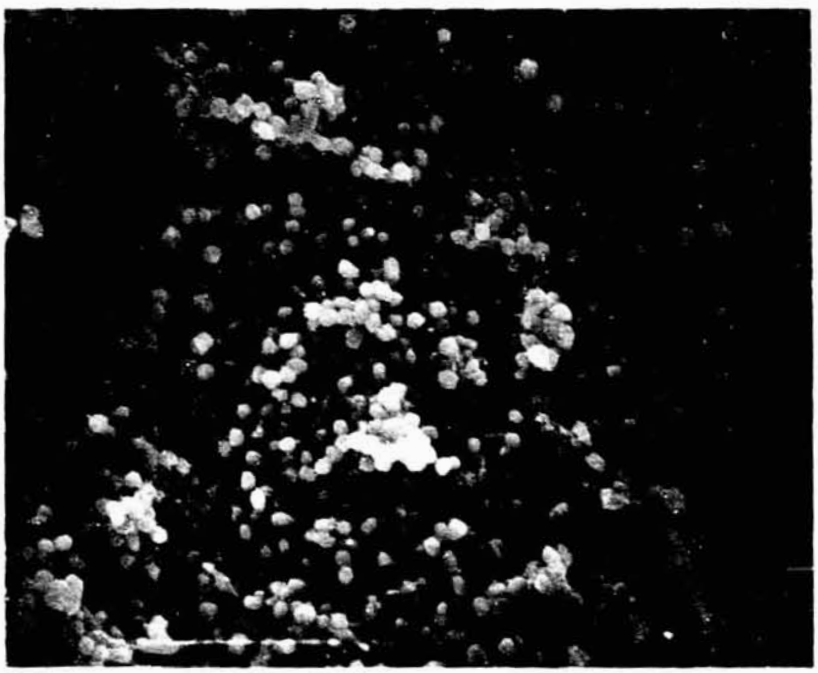

Figure 12. - PTFE (1/2,1/2) smooth side at 14 days. Note increased cellularity, spreading and clumping as compared to the 24-hour sample.

Figure 13(a). - PTFE NT, 30 minutes. Notice altered cell morphology as compared to the PTFE control at 30-minutes (fig. 13(b)). Many cells remain spherical while the larger 40 u cells are more elongated.

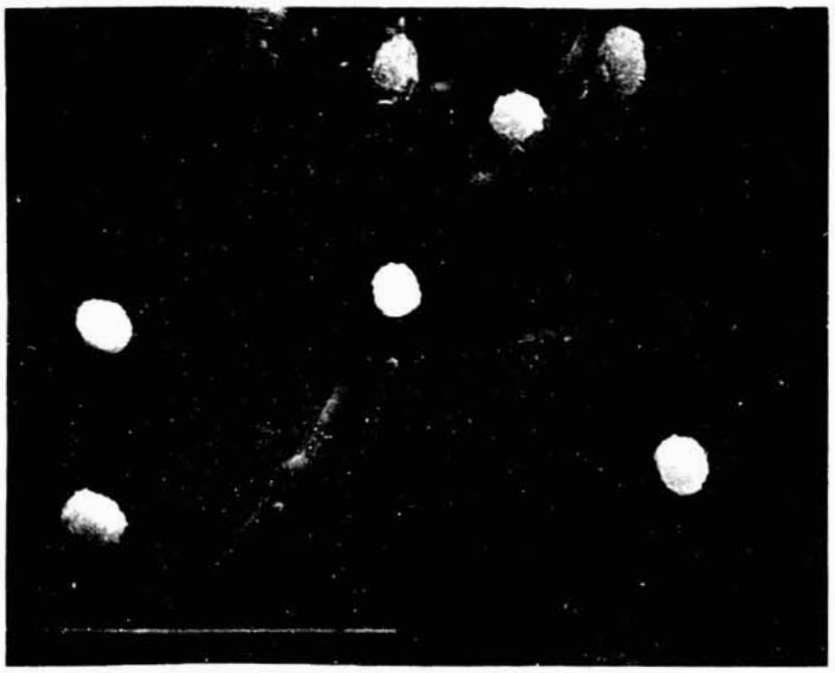

Figure 13b. - PTFE-smooth control, 30-minutes. Compare these cells with figure 13a. These cells do not display the extensive pseudopod development. Some flattening is present but not as extensive as on the natural-texture surface.

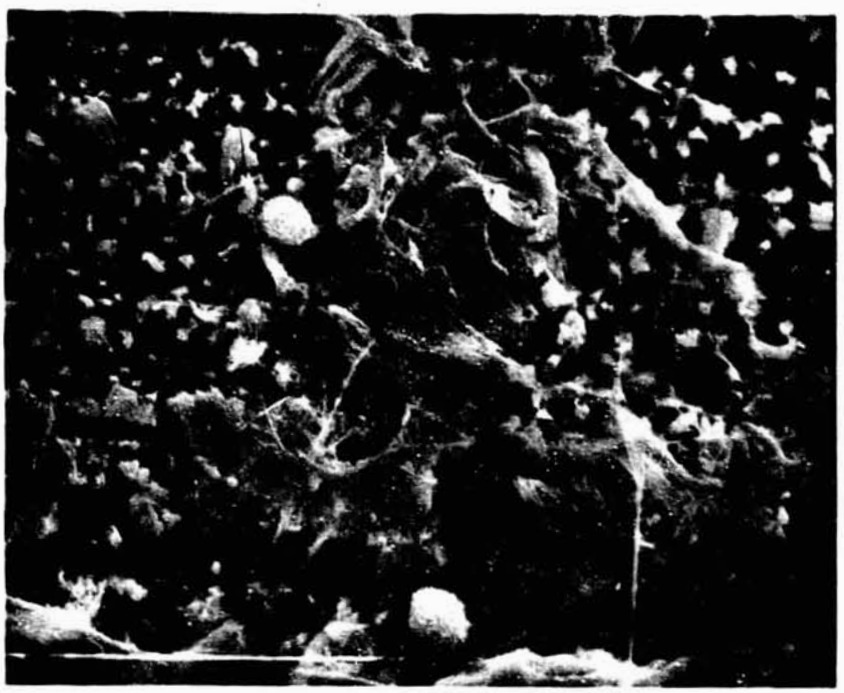

Figure 14. - PTFE NT, 30-minute. Flattened cells with layering displaying a lack of contact inhibition.

Figure 15. - PTFE Ni, 30-minutes. Cell's surface is lightly ruffled with intimate contact between membranes. Natural-texture surface is in the background. Mast cell with granules is in the lower right.

Figure 16. - PTFE NT, 30-minutes. Ruffled membrane on cells with extensive pseudopodia and filipodia.

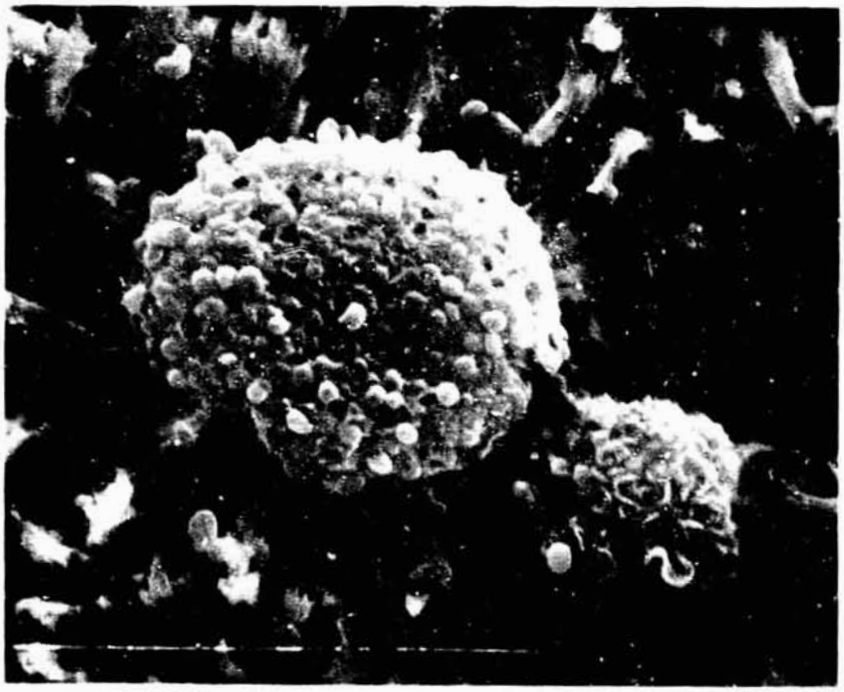

Figure 17. - PTFE NT, 30-minutes. Mast cell with granules. Some granules are being released.

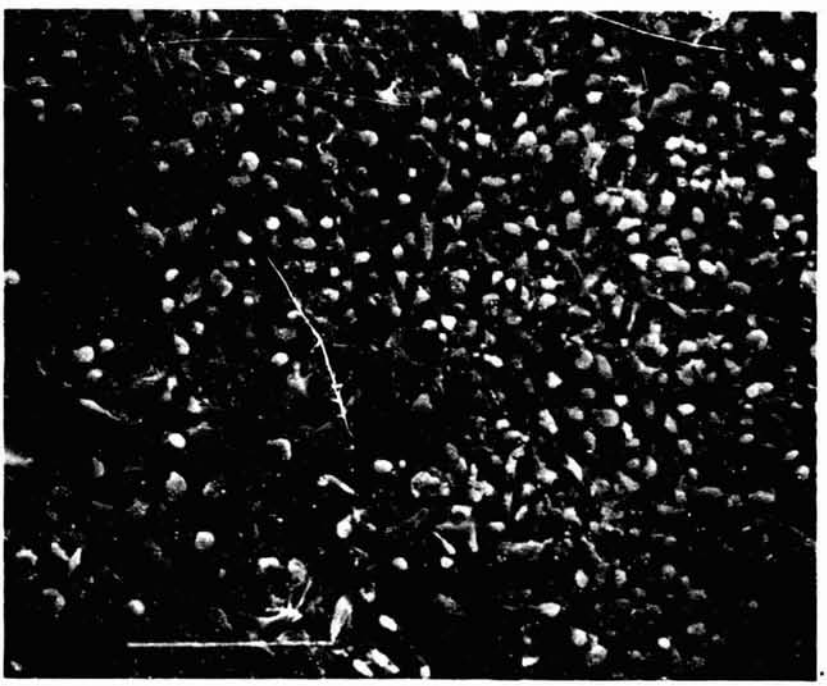

Figure 18. - PTFE NT, 24-hours. This sample displays two markedly different surfaces. The lower left shows spreading and layering while the upper right has less spreading with more individual cell forms. The natural-texture can also be observed in this region.

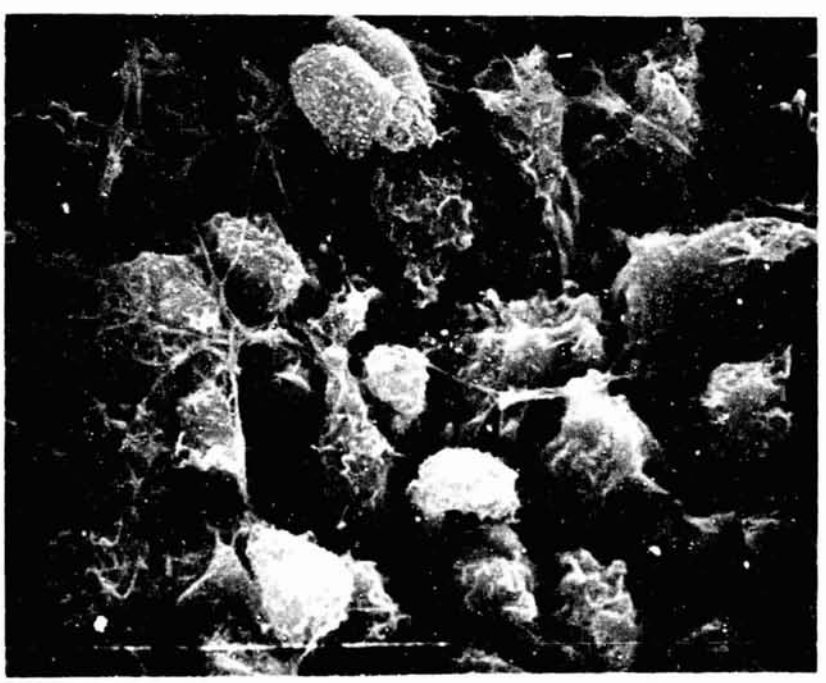

Figure 19. - PTFE NT, 24-hours. Upper right region of figure 18 showing cells enveloping and closely adhering to the natural texture.

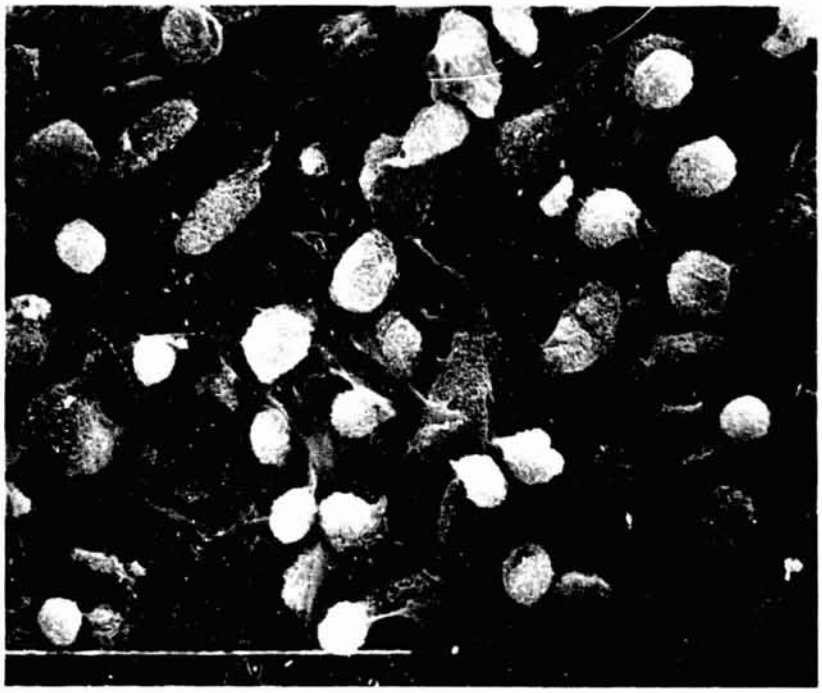

Figure 20. - PTFE NT, 24-hours. Lower left region of figure 18 depicting cell flattening and merging.

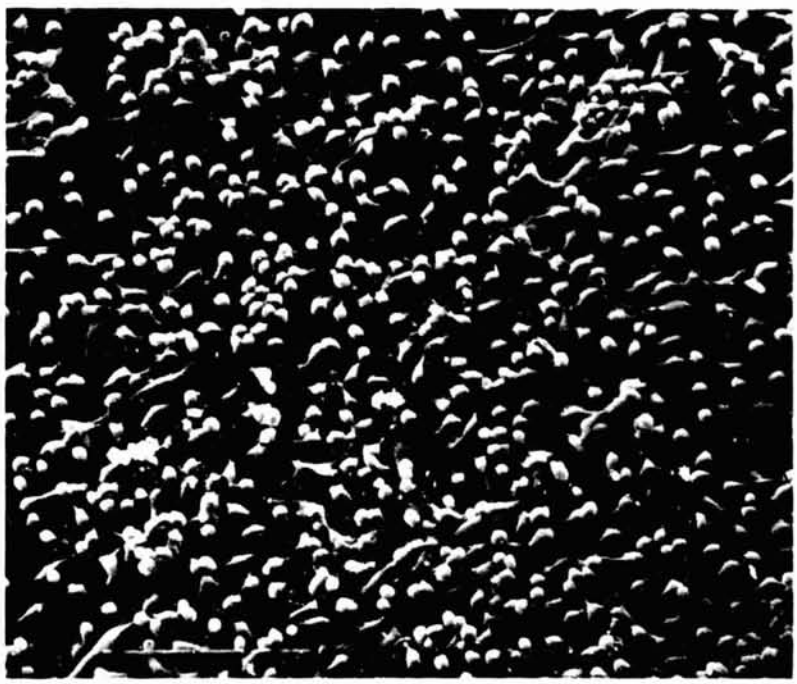

Figure 21. - PTFE (1/2, 1/2), 3-day sample, smooth surface. Notice cell clumping.

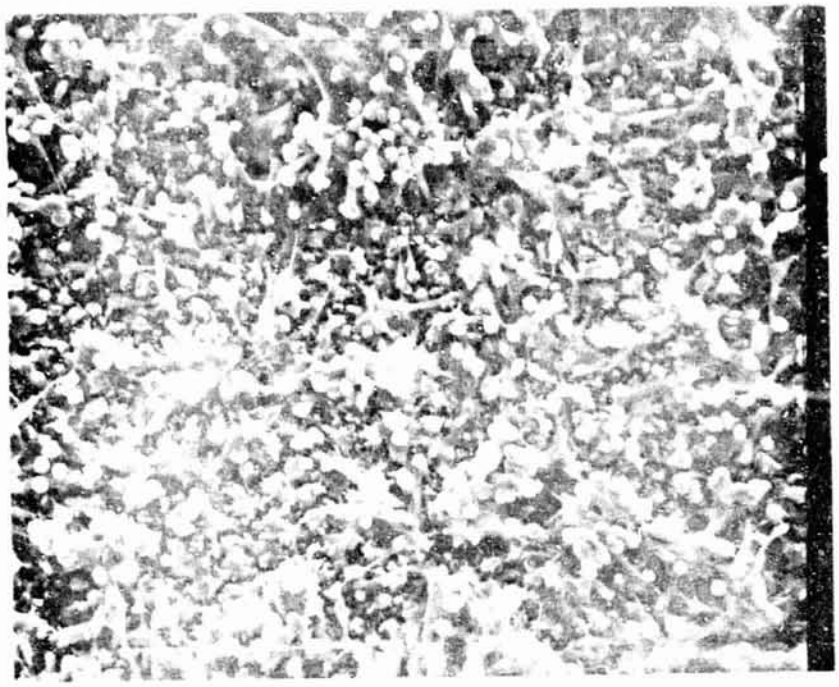

Figure 22. - PTFE (1/2, 1/2) 3-day natural textured surface. There is extensive clumping and increased numbers. Also note the extensive pseudopodia formation.

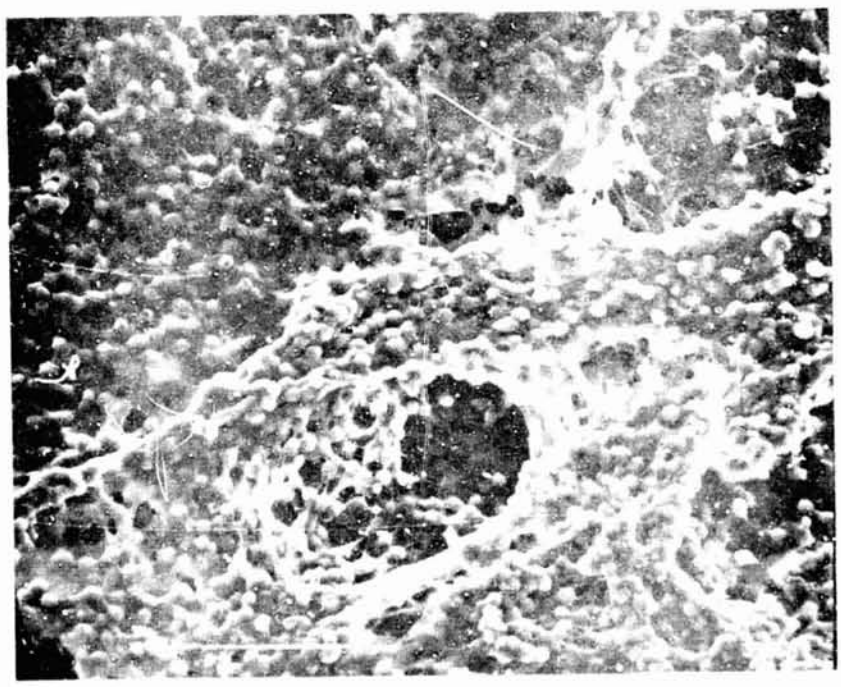

Figure 23. - PTFE NT, 7-day. Compare this figure with figure 8, PTFE control. There is increased cellularity, intimate contact and cell layering.

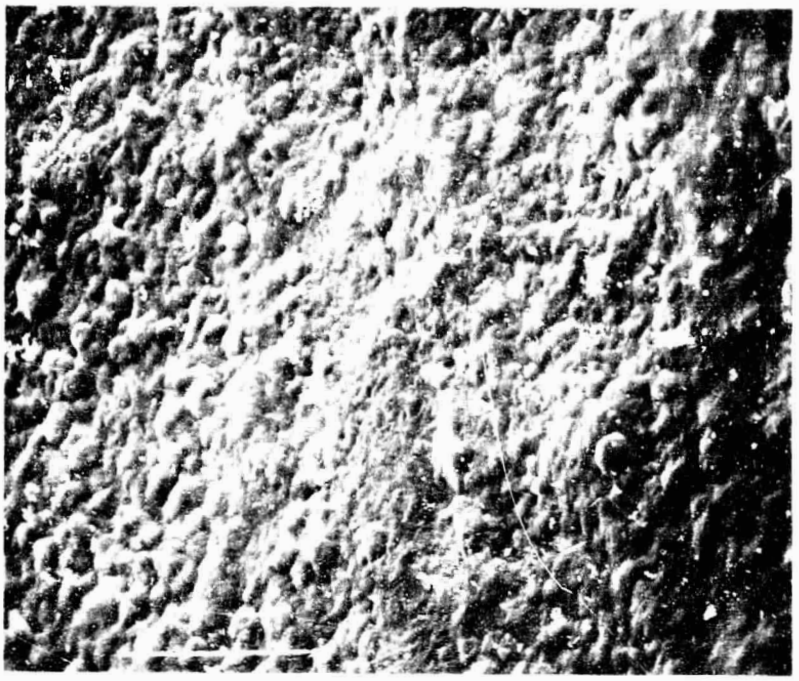

Figure 24. - PTFE NT, 14-days There is a confluent cell coverage. However the cellular dimensions do not compare with the control mesothelium.

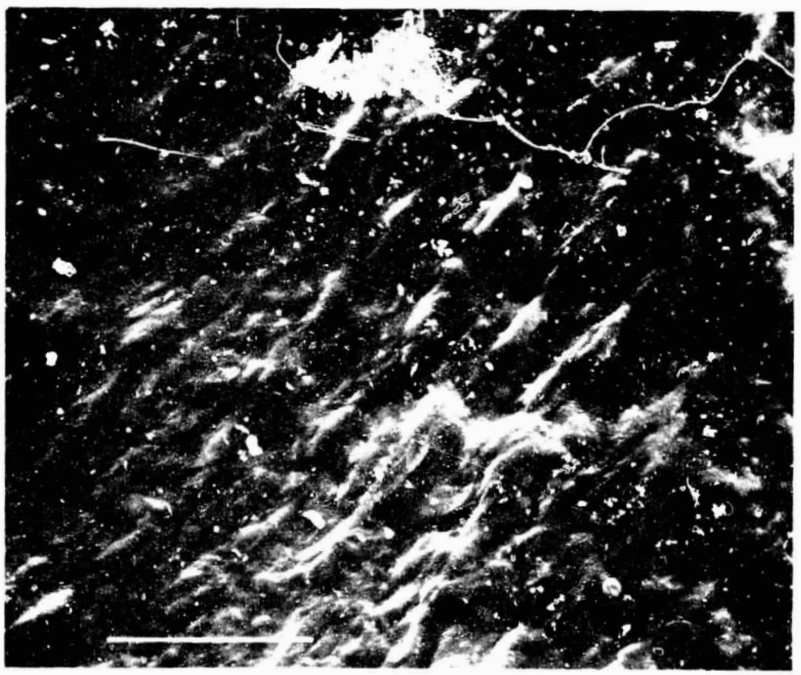

Figure 25. - Control mesothelial lining. The raised areas are felt to represent mesothelial nuclei.

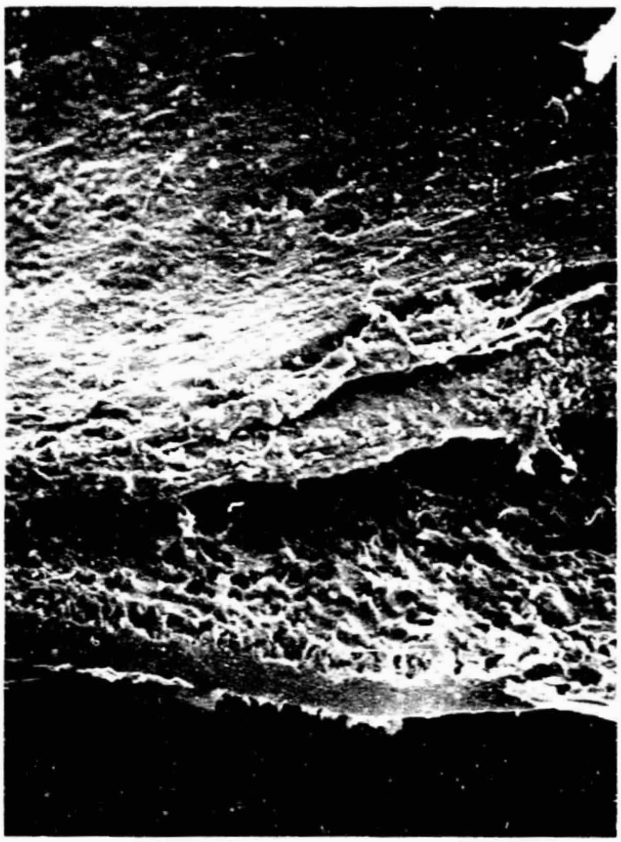

Figure 26. - PTFE NT. Cross section of 14-day implant. There are multiple cell layers, and the natural texture appears to be filled in with an amorphous material.

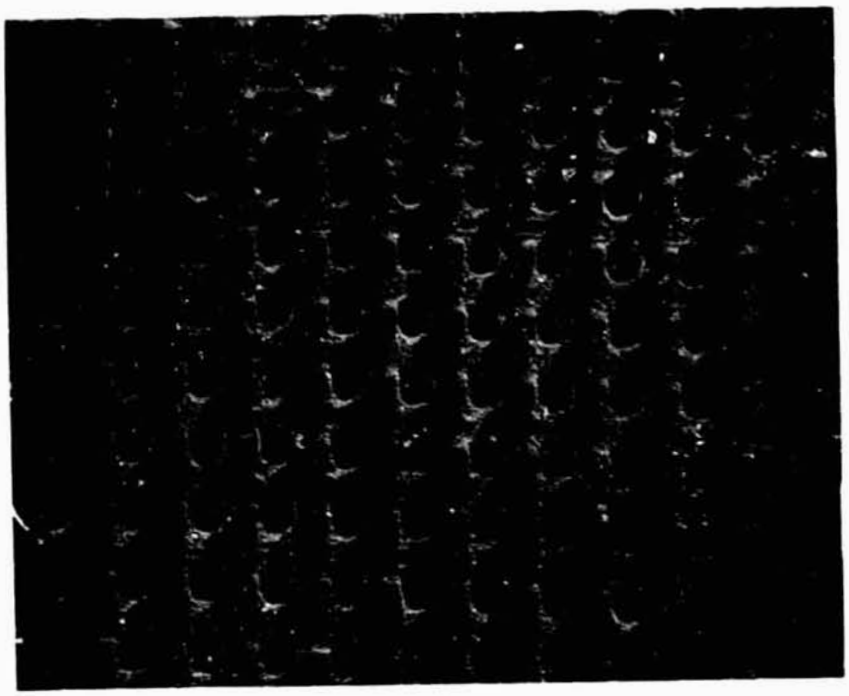

Figure 27. - PTFE, pits $(50\ u)^2$ x 100 u deep. Notice the natural texture at the corners extending diagonally.

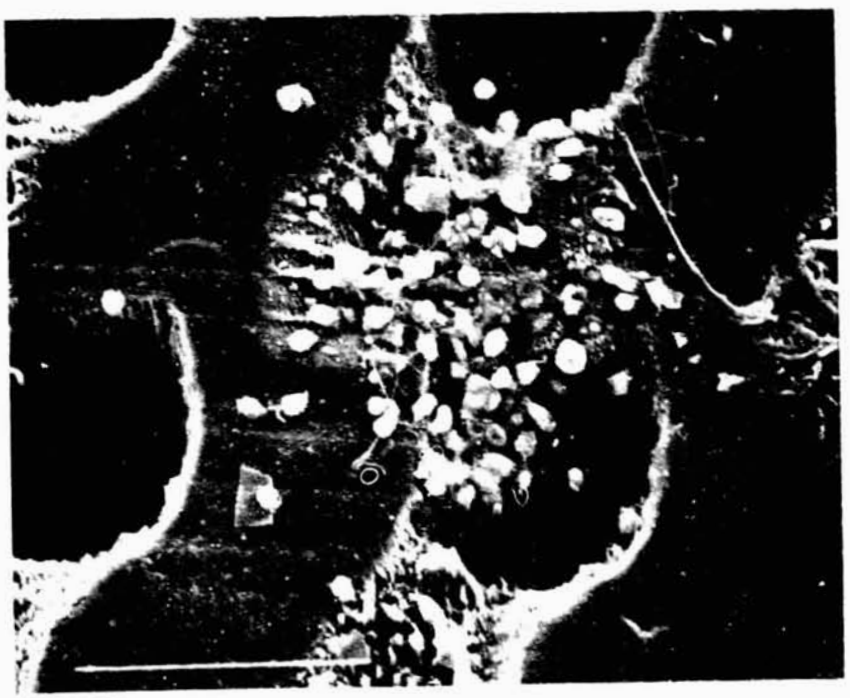

Figure 28. - Higher magnification of figure 27 showing the influence of the natural texture near the pit edge.

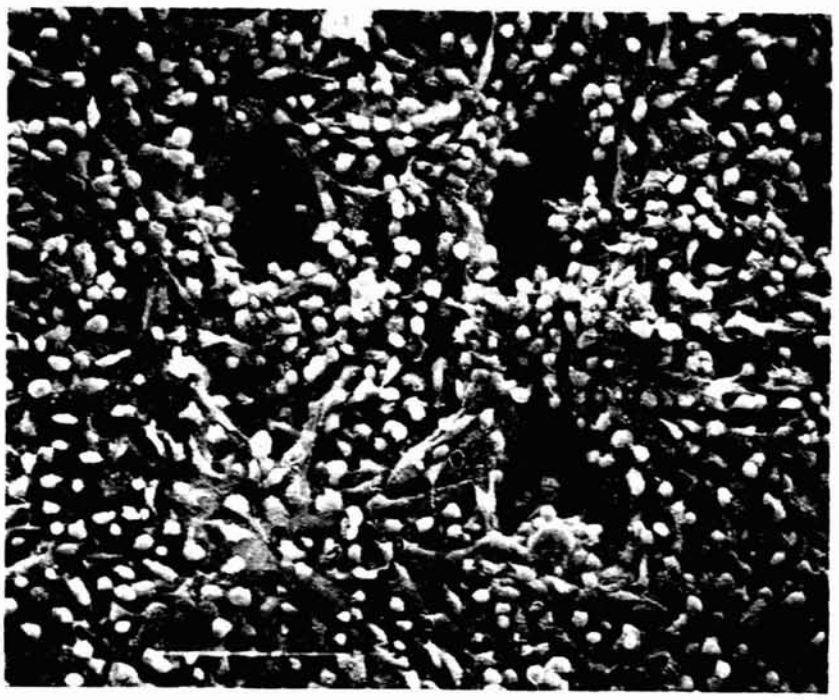

Figure 31. - PTFE pits, 3-days. The pit in lower left is filled with cells. Also of note is the cells' radial orientation from the center of the hole.

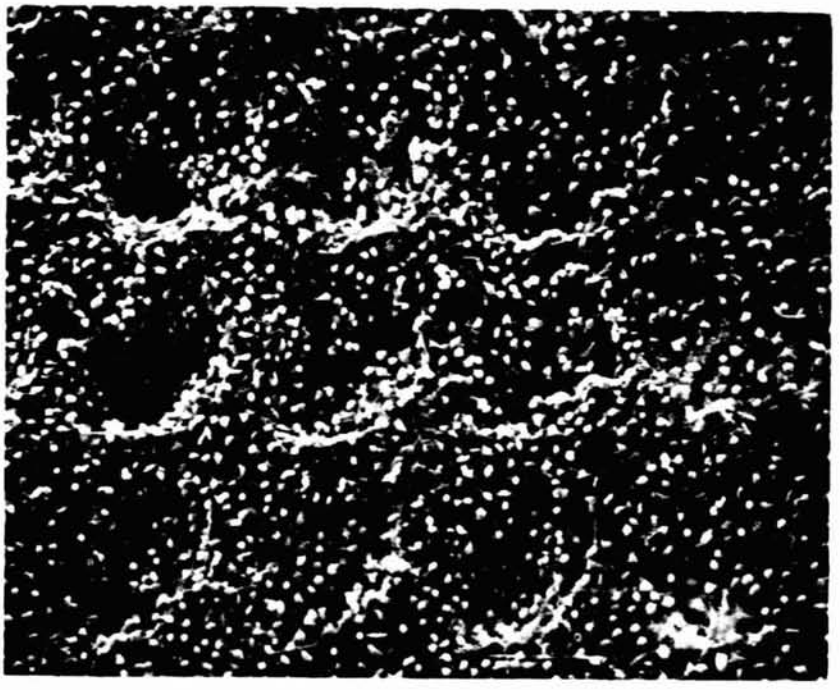

Figure 32. - PTFE pits, 7-days. Pits are filled with cells, maintaining an actively incorporating cell population.

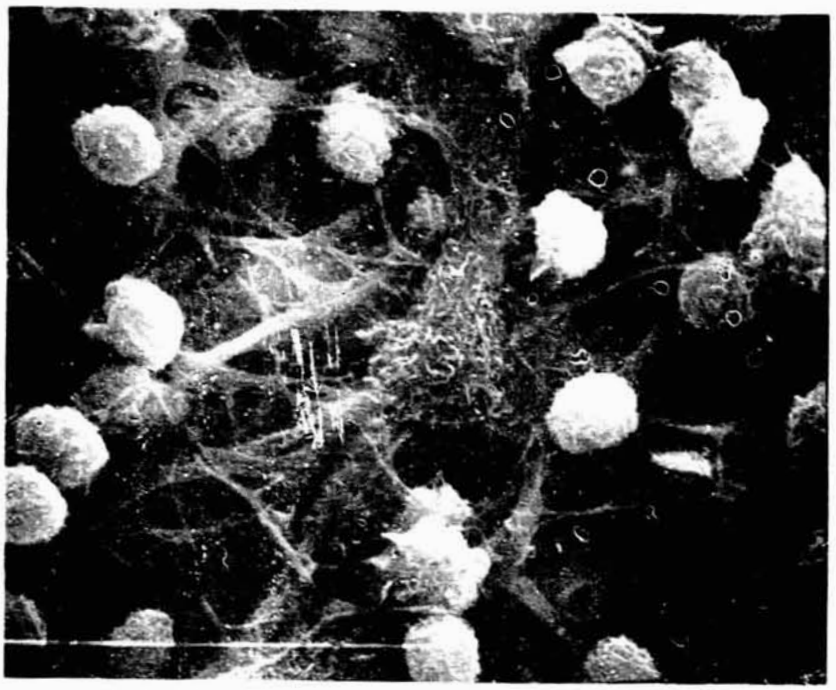

Figure 33. - PTFE pits, 7-days. Extensive celluiar contact and pseudopod formation shown.

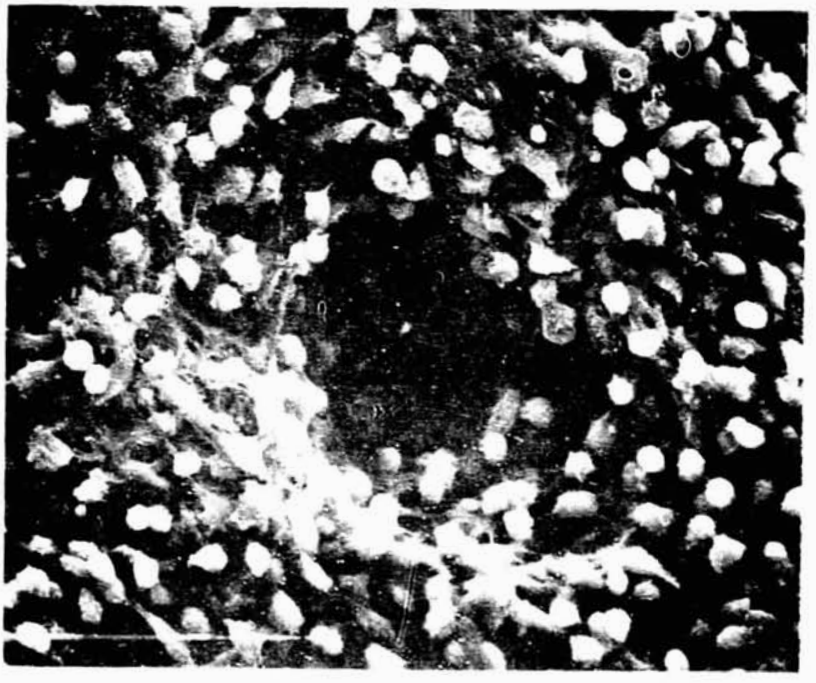

Figure 34. - PTFE pits, 7-days. Cells are aligned circularly around a pit.

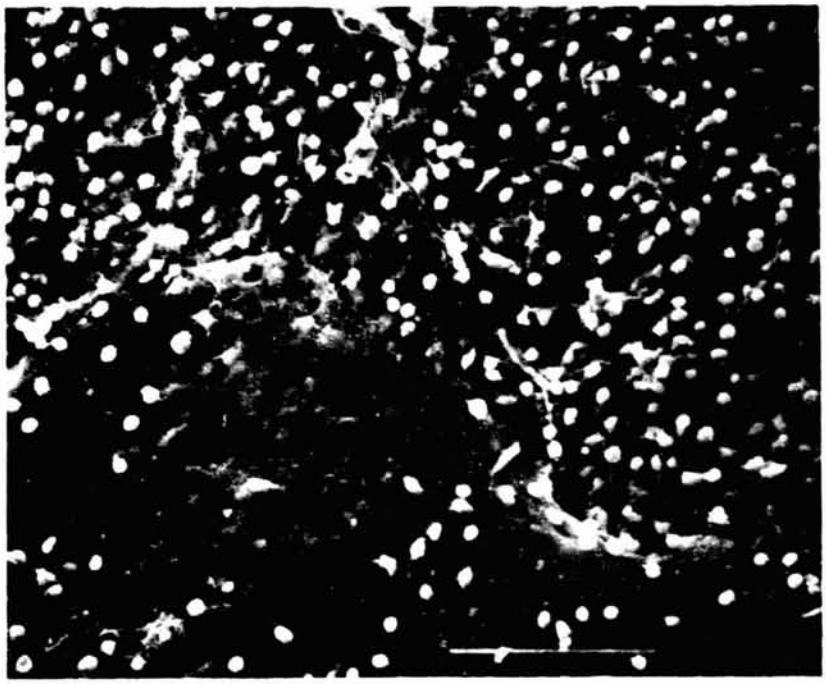

Figure 35. - PTFE pits, 7-days. The lower left appears similar to the natural texture with the matted appearance and cell flattening.

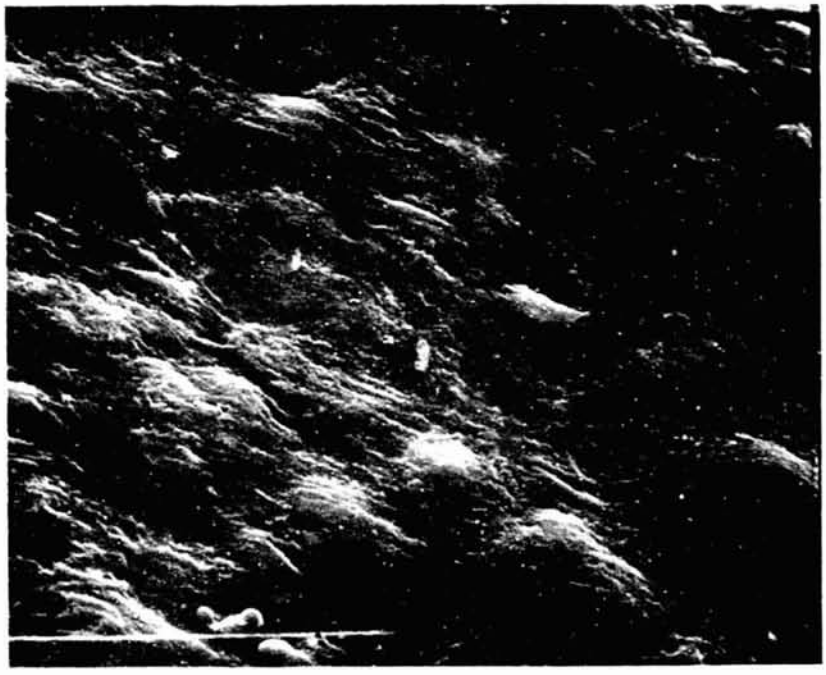

Figure 36. - PTFE pits, 14-days. Nuclear raised areas are not as large as controi mesothelium areas. However, no further cell incorporation was observed to be taking place at the surface.

Figure 37. - PEU ANN, 6-hour. This region is more typical of the cell spreading though atypical of cell numbers.

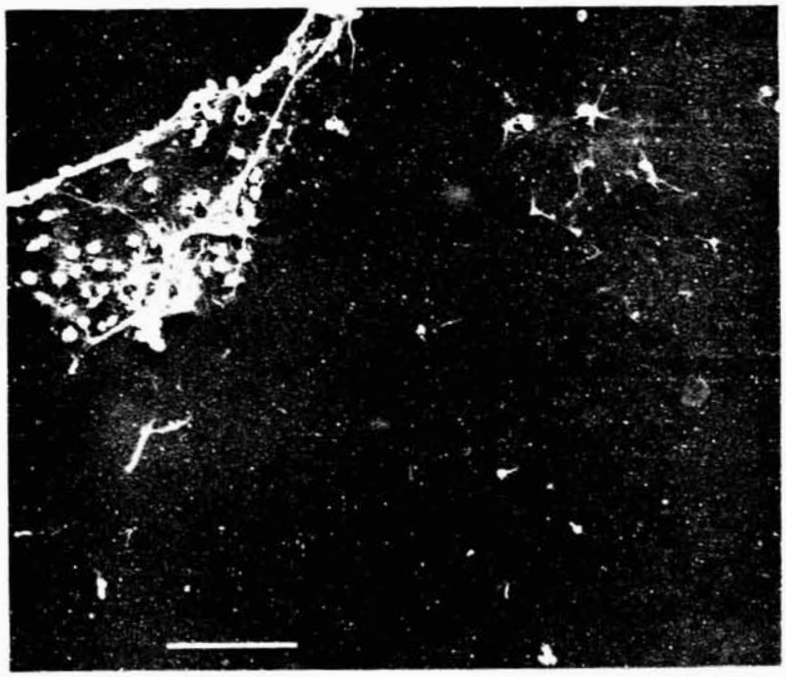

Figure 38. - PEU ANN, 24-hours. There is increased spreading; however, cell numbers continue to be low when compared to PTFE NT.

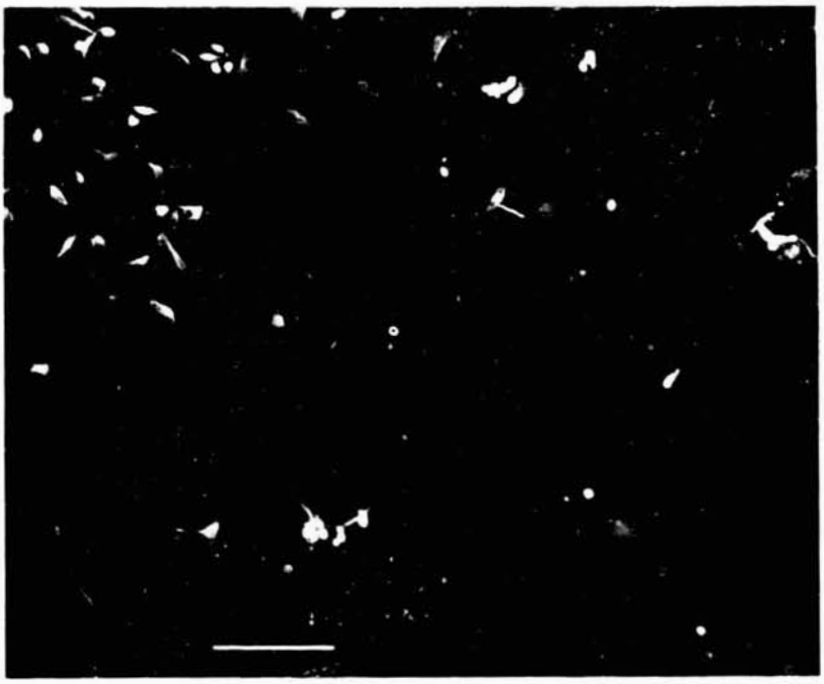

Figure 39. - PEU FAD, 24-hours. There is increased spreading; as compared to the 6-hour sample, however, cell numbers continue to be low.

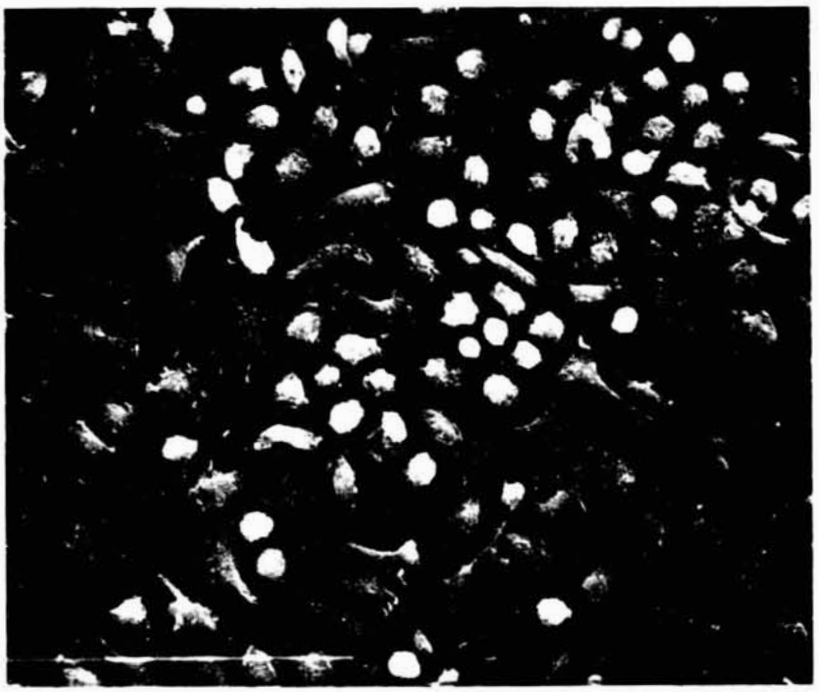

Figure 40. - PEU FAD, 24-hours. A region of high cellularity near the edge of the sample. Notice regular spacing of cells.

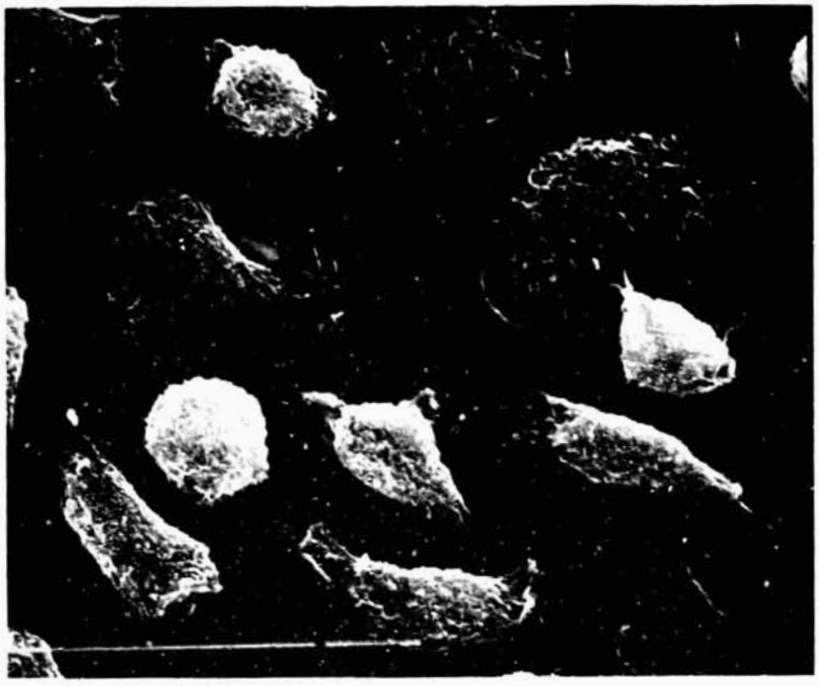

Figure 41. - Higher magnification of figure 40. Notice the spreading cell in upper right. It represents a flattened macrophage.

Figure 42. - PEU ANN, 14-day. Note amorphous coating and more uniform cell coverage.

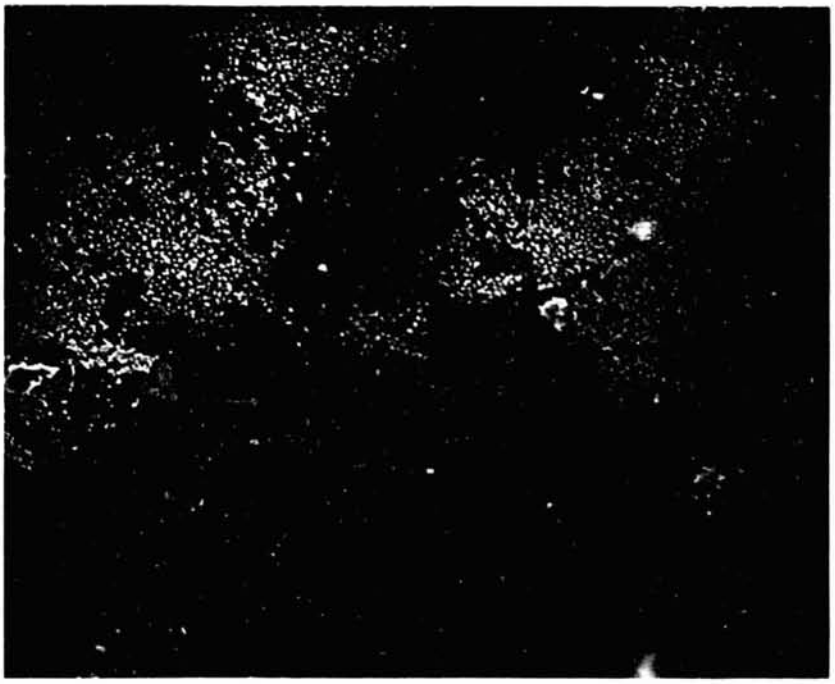

Figure 43. - PEU FAD 14-day. Variable cell coverage. Not all samples were this sparcely covered.

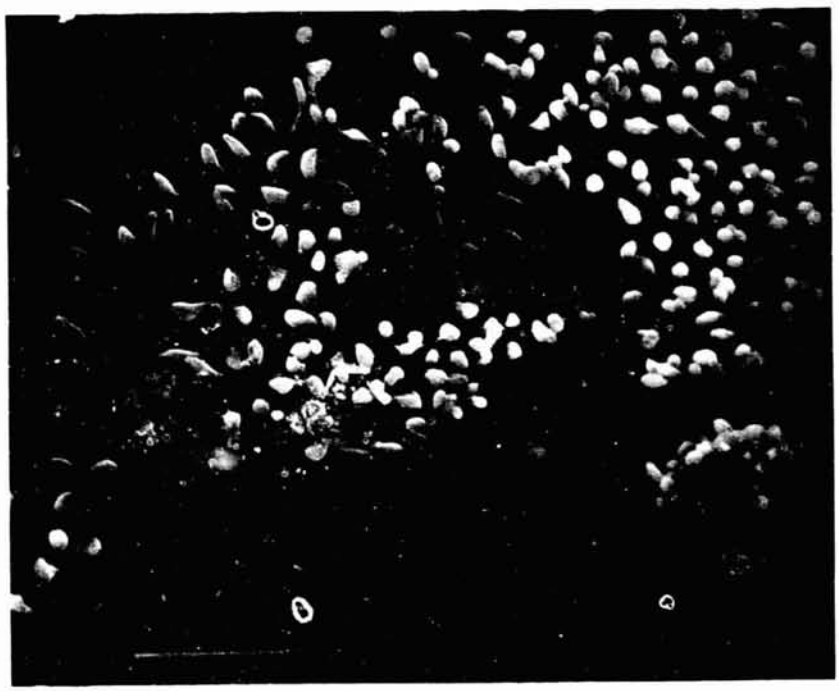

Figure 44. - PEU FAD, 14-day. Some cell clumping is beginning to occur similar to the 3-day PTFE (1/2, 1/2) sample on the smooth surface.

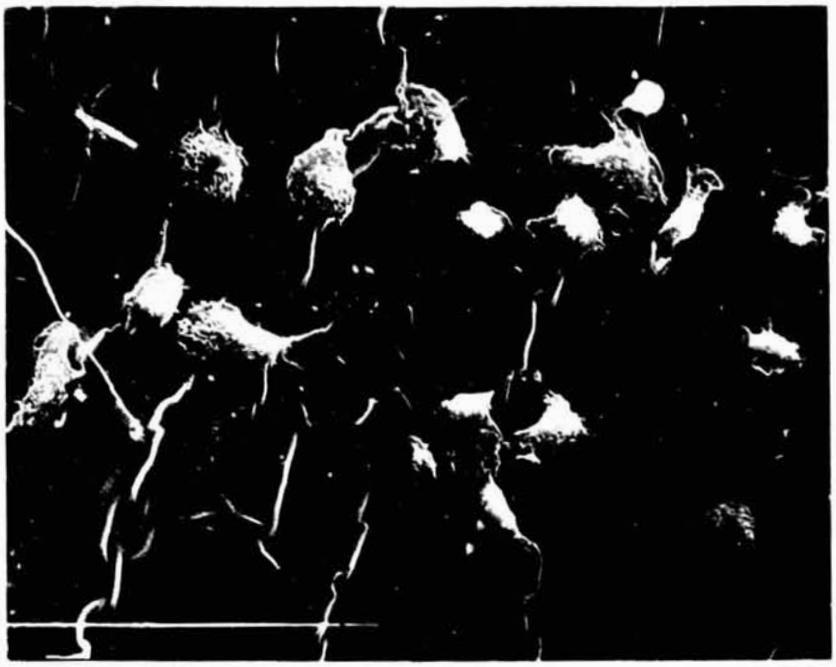

Figure 45. - HEMA, 30-minute. The wrinkles on the surface are felt to represent polymer shrinkage. Notice cell flattening and regular margins.

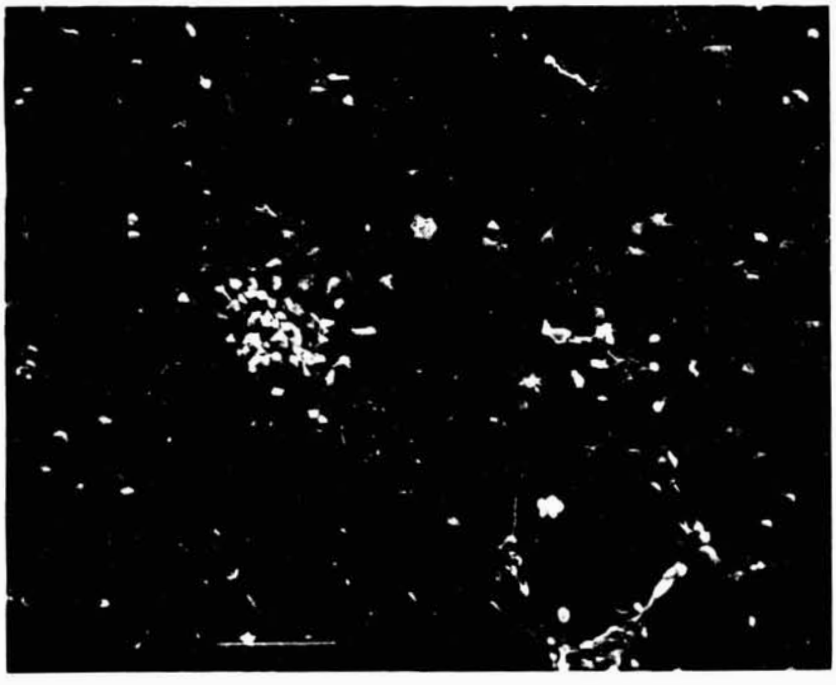

Figure 46. - HEMA, 30-minute. Regions of cell clumping were noted, but the extensive pseudopod formation as with the PTFE NT are absent.

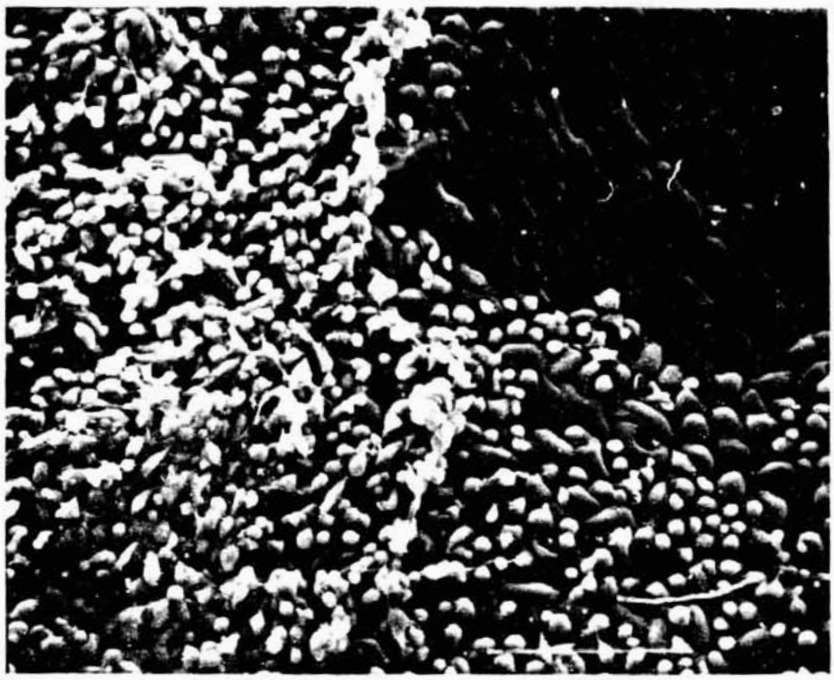

Figure 47. - HEMA, 7-days. Three regions are represented here. A confluent, apparently quiescent region is in the upper right. The lower right is a slightly more cellular region not as quiescent as the upper right but less distinct in individual cell forms as compared with the upper left. Finally the upper left region appears to be actively incorporating cells. The last two regions are magnified in figures 48 and 49.

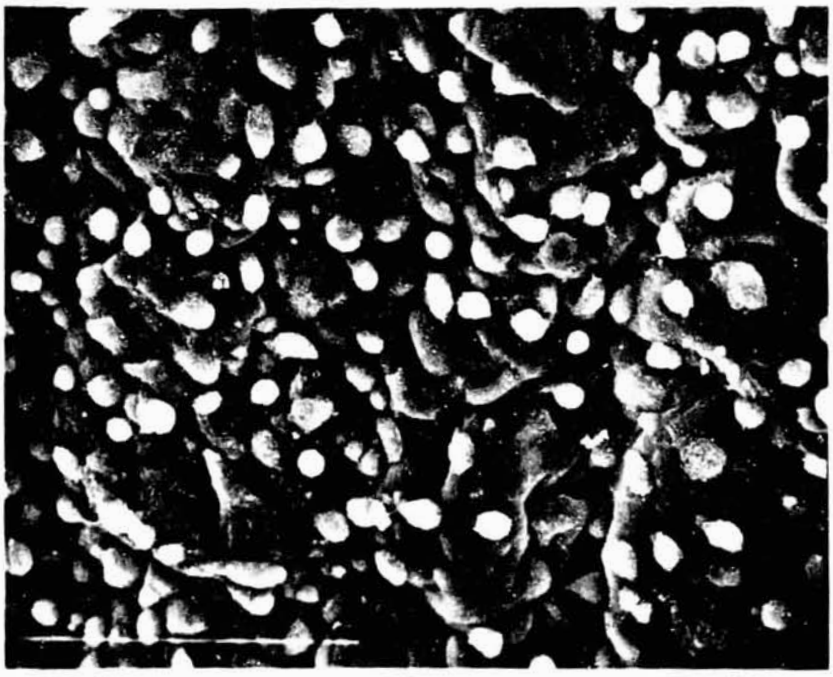

Figure 48. - Lower right area of figure 47. Note fusion of cells, clumping and loss of filipodia.

Figure 49. - Left side of figure 47 displaying continued active cell fusion and incorporation.

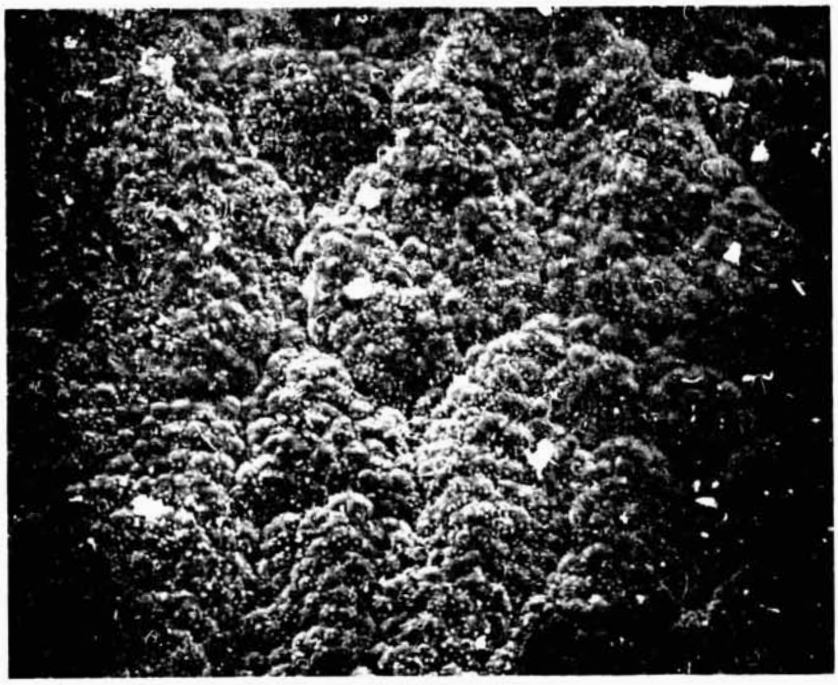

Figure 50. - HEMA, 14-days. Confluent coverage by cells.

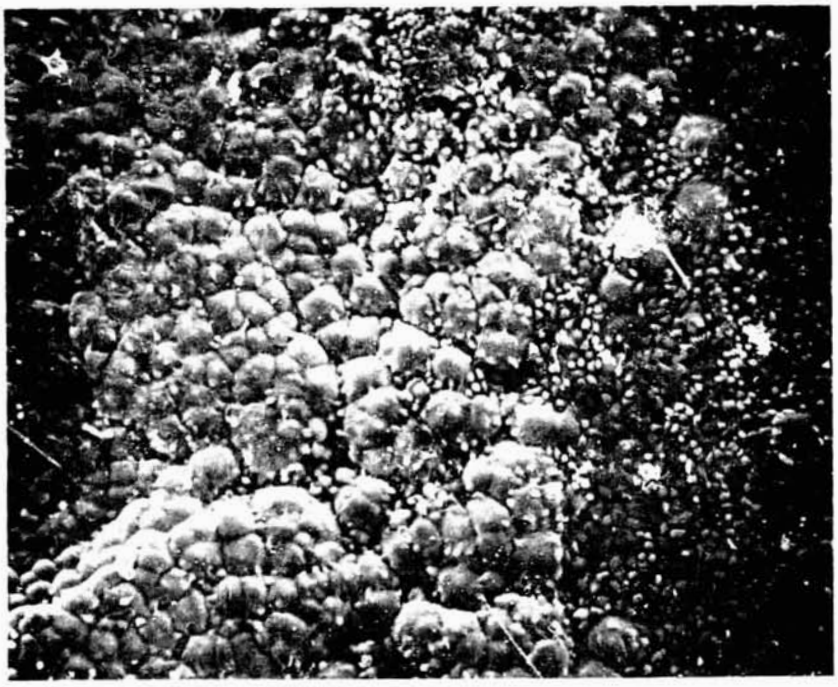

Figure 51. - HEMA, 14-days. Higher magnification of figure 50 at the margin of confluent cell forms.

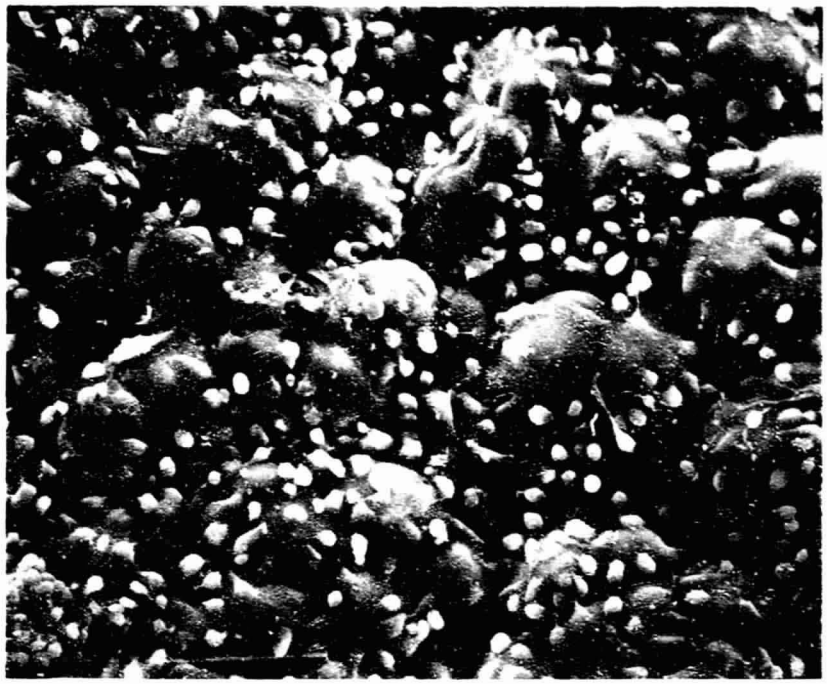

Figure 52. - HEMA, 14-days. The longer cell forms appear to be in continued contact with the smaller cell forms.

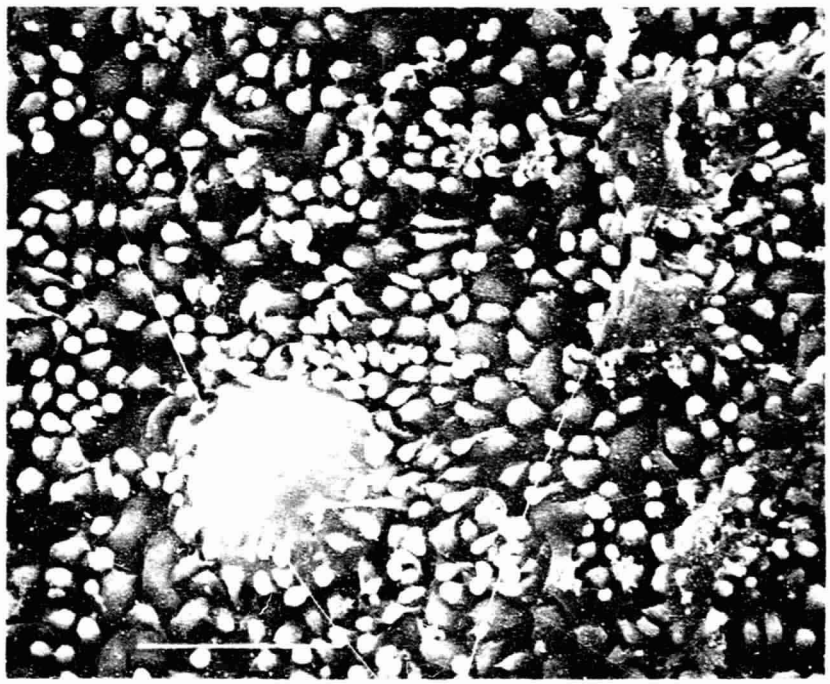

Figure 53. - HEMA, 14-days. This photo displays the dimensional size and morphology of the large cell form as well as the cells in contact with the polymer surface. The intermediate cell size is felt to be a macrophage.

Figure 54. - Control mesothelial lining removed from stomach. The cell's nucleus is approximately 15 u in diameter. 1000X.

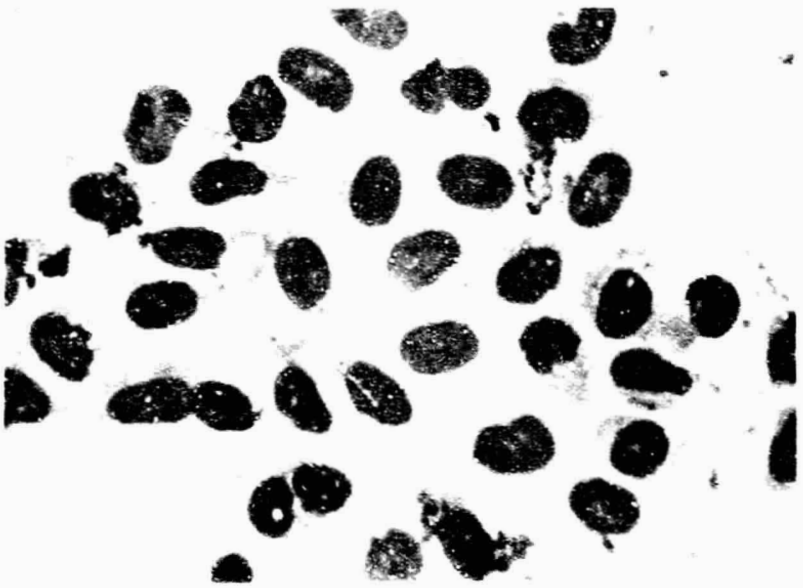

Figure 55. - Control mesothelium on glass surface smear made from abdominal cells. 1000X.

Figure 56. - PEU FAD, 30-minutes. Macrophage cell and intermediate cell form "I". 1000X.

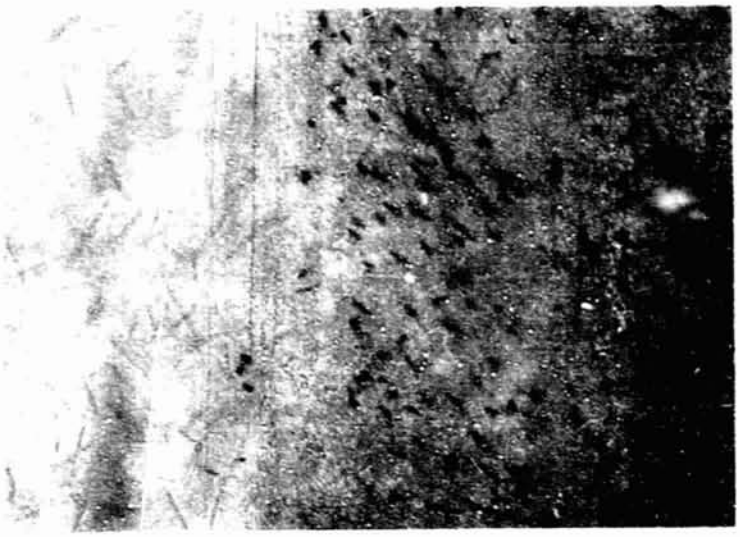

Figure 57. - PTFE (1/2,1/2), 30-minutes. The textured surface has a greater cellular affinity. 250X.

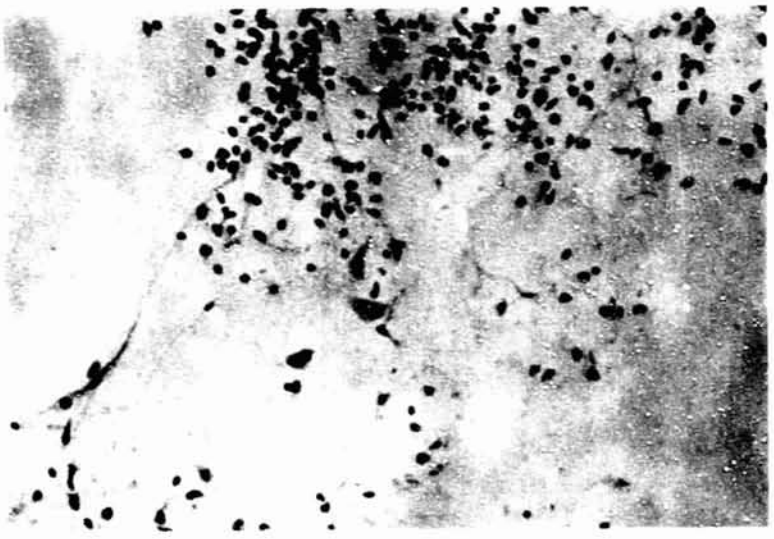

Figure 58a. - PTFE (1/2,1/2), 6-hours, untextured surface. Several macrophages are in the center of the field. Most other cells are lymphocytes.

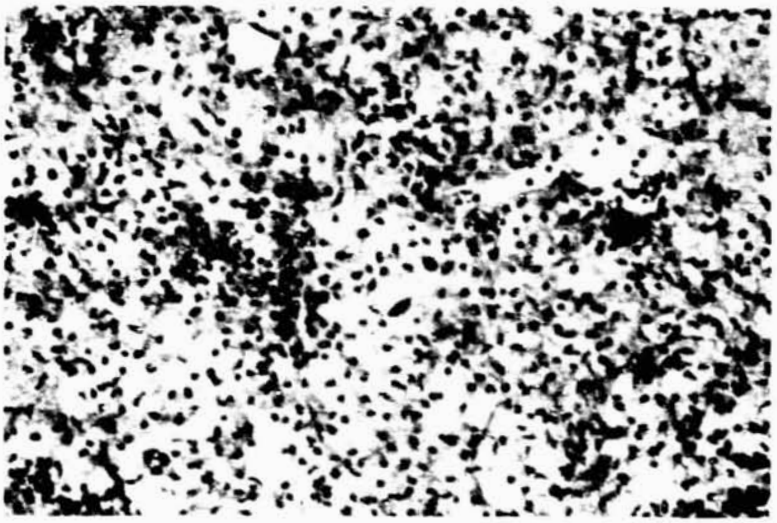

Figure 58b. - PTFE (1/2,1/2), 6-hours, natural texture surface. Notice cell clumping already taking place.

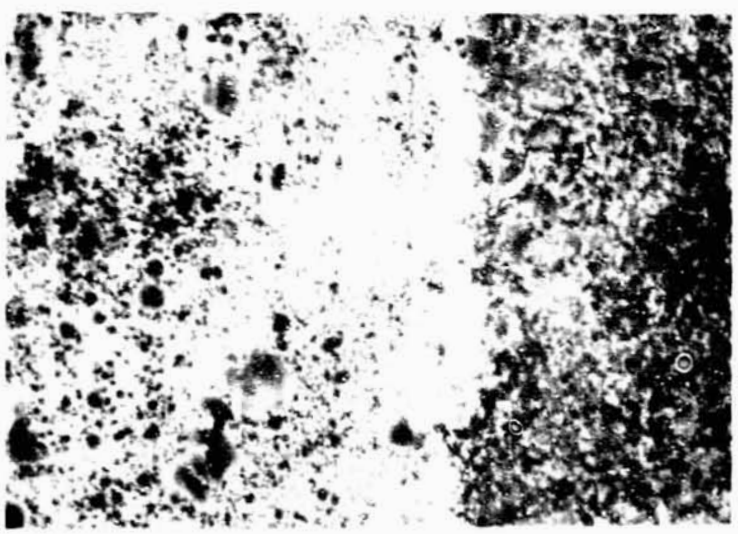

Figure 59. - PTFE (1/2,1/2), 72-hours. This figure demonstrates the formation of MNGC's on the control surface. The texture surface is density populated with vaculated cells. 100X.

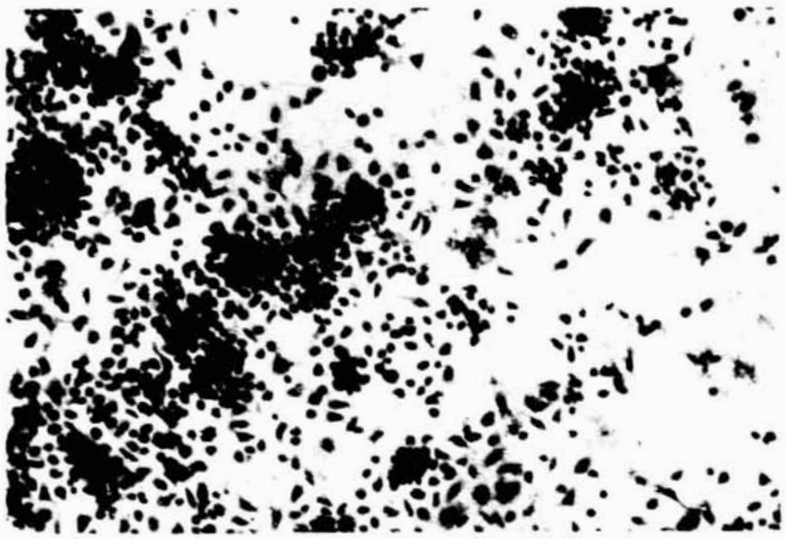

Figure 60. - PTFE (1/2,1/2), 3-days, smooth surface. Cell clustering taking place on the control surface. 250X.

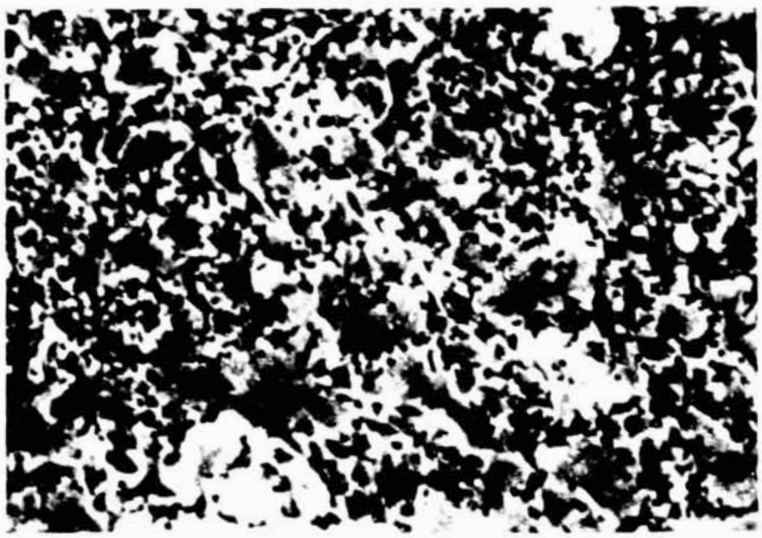

Figure 61. - PTFE NT, 72-hours. MNGC's on the textured surface displaying irregular margins and vacuolization. 250X.

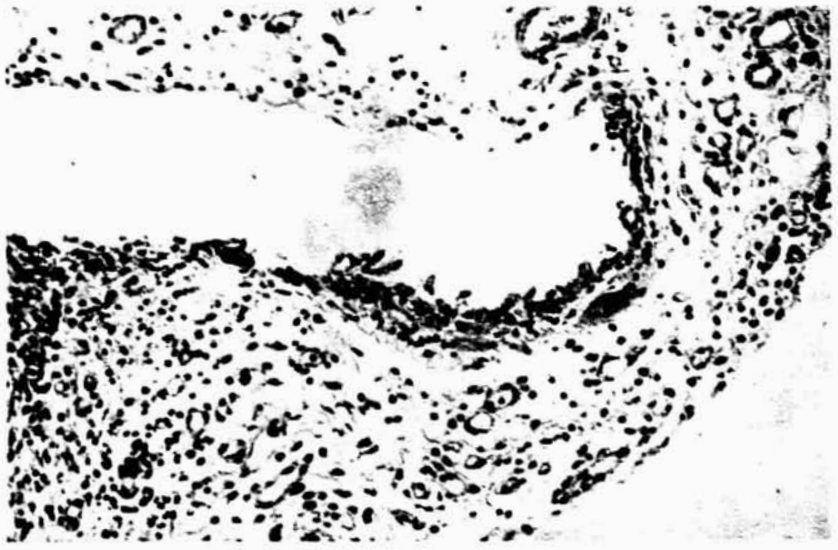

Figure 62. - PTFE smooth control, 14-days. Edge effect displayed to the right and capsule response at the top. 250X.

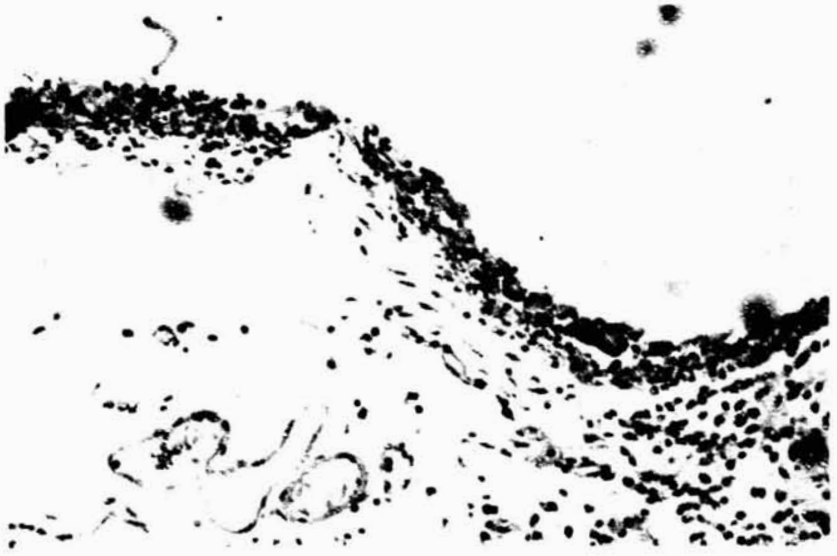

Figure 63. - PTFE smooth control, 14-day. Edge effect shown with the formation of MNGC's. 250X.

Figure 64. - PTFE smooth control, 14-day. MNGC's at the edge. Notice the reduced nuclear staining once the macrophage fuses. 1000X.

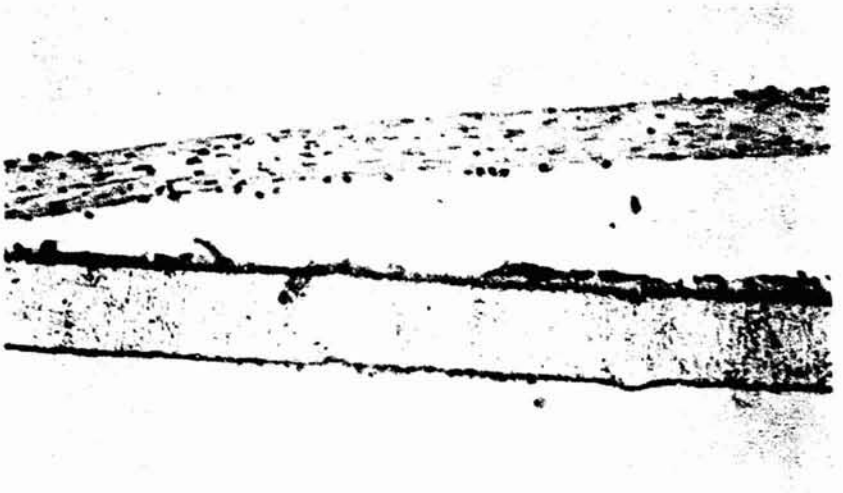

Figure 65. - PTFE NT, 7-days. MNGC's at the interface with fibrous capsule. The capsule is moderately cellular with large round cells attached to the capsule at the interface of the capsule and implant. 250X.

Figure 66. - PTFE NT, 14-days. The cytoplasm appears to be penetrating between the textured cones. 1000X.

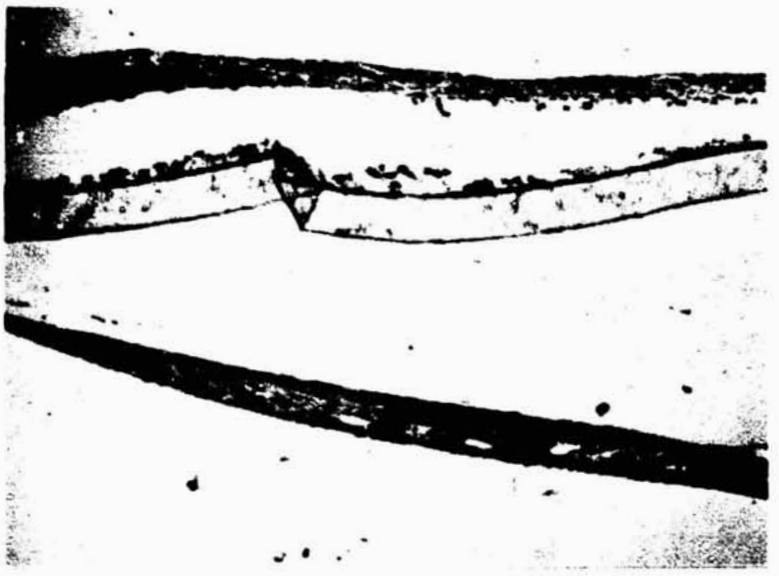

Figure 67. - PTFE NT, 14-days. Both sides of the capsule are shown. At 14 days there appears to be little difference between the N.T. and the control. 100X.

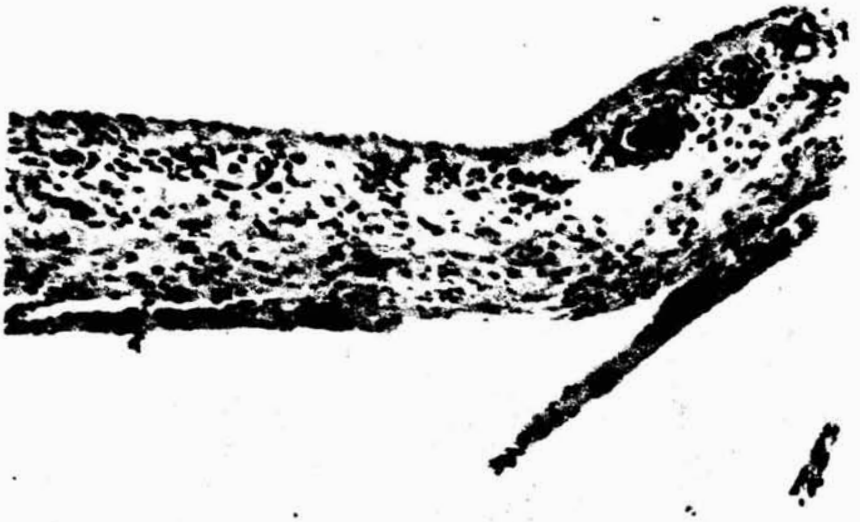

Figure 68. - PTFE NT, 14-days. This capsule and MNGC response is similar in appearance to the subcutaneous response. 250X.

Figure 69. - PTFE NT, 14-days. The interface is intact with a closely adherent capsule. The capsule is highly cellular with fibroblasts appearing as the flattened cell form. 1000X.

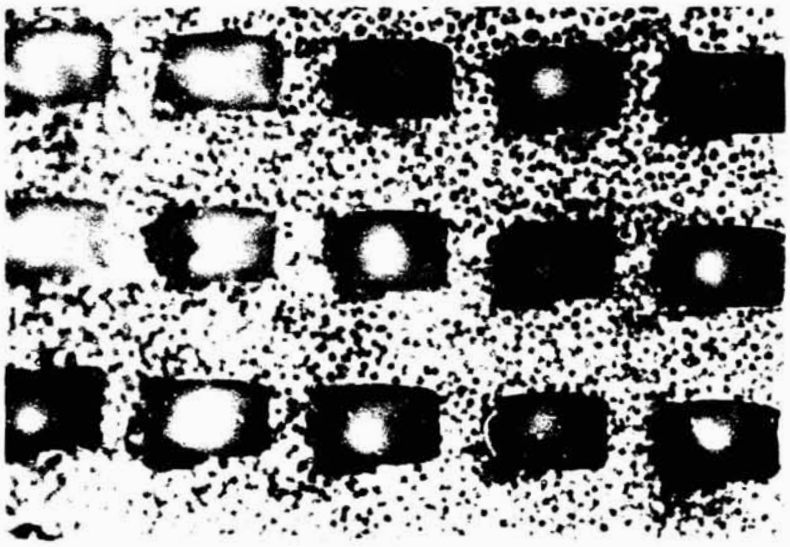

Figure 70. - PTFE pits, 6-hours. Moderate cell attachment over the smooth surface with enhanced adherence in upper right corners of pits presumably where the natural texture extended onto the surface. 250X.

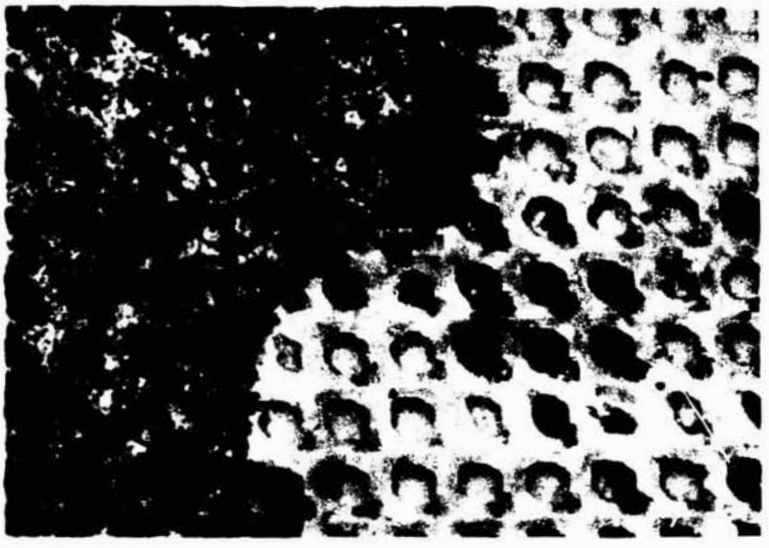

Figure 71. - PTFE pits, 3-days. A sheet of cells adhering to pitted surface has been partially peeled off. 100X.

Figure 72. - PTFE pits, 3-days. Texturing of pits near the edges produced a regular array of cells at the surface. 250X.

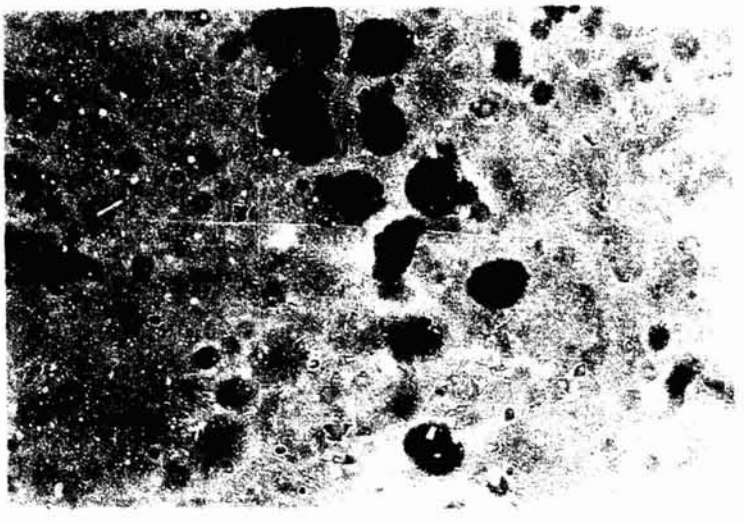

Figure 73. - PEU-ANN, 30-minute. Cells (primarily macrophages) have not had a chance to spread onto the surface. 1000X.

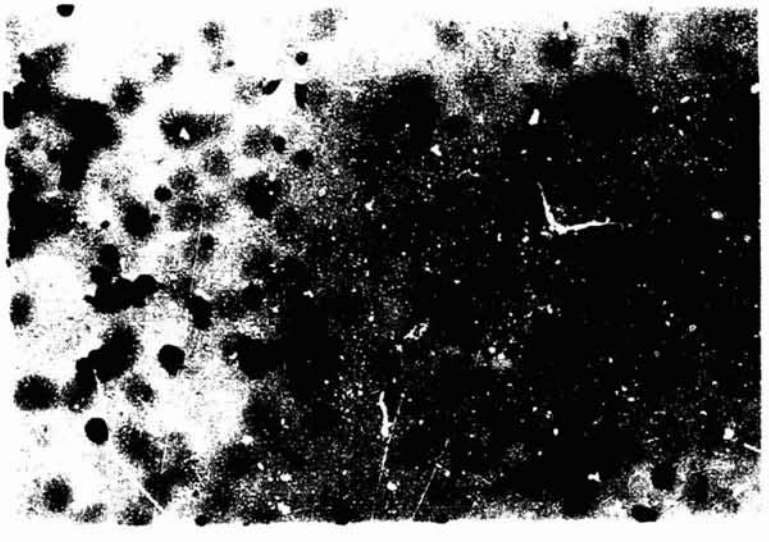

Figure 74. - PEU-FAD, 30-minute. These cells at lower magnification are similar to those on the PEU-ANN sample at 30 minute. 250X.

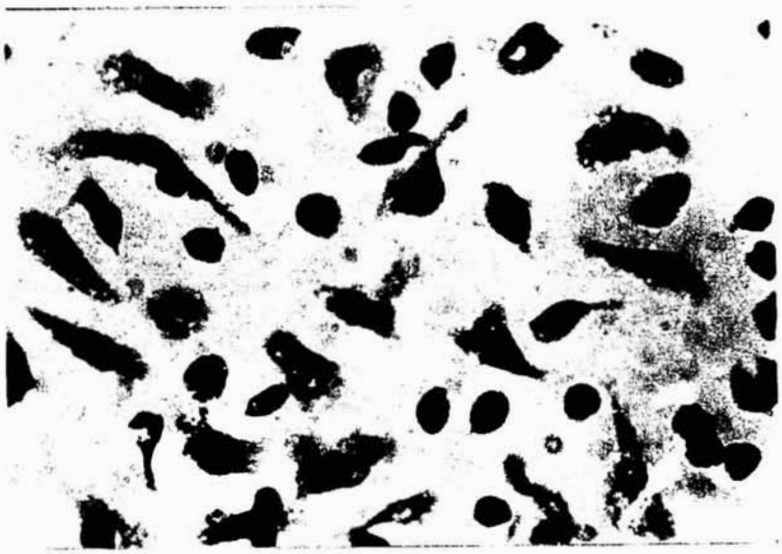

Figure 75. - PEU-ANN, 24-hours. Cells are now flattened out. The dark staining cells with minimal cytoplasm are lymphocytes; the others are macrophages. 1000X.

Figure 76. - PEU-FAD, 24-hours. Cells appear similar to the cells on the PEU-ANN sample. 1000X.

Figure 77. - PEU-FAD, 14-days. Fibrous capsule on PEU-FAD is extremely thin and relatively acellular. 250X.

Figure 78. - PEU-FAD, 14-days. This photograph shows the loose fibrous capsule with pale fibroblasts and other monocytic cells most likely macrophages. 1000X.

Figure 79. - HEMA, 7-days. Large 30 u cell forms at the surface of the capsule. It is highly cellular with loose connective tissue. 1000X.

Figure 80. - HEMA, 14-days. This capsule is more representative of the HEMA response. Several large cells appear to the right. These are felt to be mesothelial cells. The capsule is thin with many flattened cells. 1000X.

Figure 81. - HEMA, 14 - days. These cells lined the surface of the HEMA polymer. No capsule was present. The cells are approximately 30 - 40u in length. 1000x