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SEGMENTAL NEOGENESIS OF THE DOG ESOPHAGUS UTILIZING A BIODEGRADABLE POLYMER FRAMEWORK

Marvin F. Grower, D.D.S., Ph.D.
Emery A. Russell, Jr., D.D.S., M.S.
Duane E. Cutright, D.D.S., M.S., Ph.D.

U. S. Army Institute of Dental Research, Division of Chemistry,
Fort Meade, Maryland 20755

ABSTRACT

This study evaluated the ability of biodegradable implants fabricated from polymers and co-polymers of polylactic acid (PLA) and polyglycolic acid (PGA) to induce regeneration of surgically created defects in the dog esophagus. The study utilized 12 mongrel dogs that had a 5 cm segment of the esophagus removed. Implants were fabricated by spray casting the polymers on a spinning Teflon mandril. The defects were repaired by suturing the biodegradable implants to the proximal and distal ends of the esophagus. Ten of the dogs were sacrificed from 3 days to 8 weeks after surgery while 1 of the dogs died after 3 years and 1 dog was sacrificed 4 years after graft placement. Endoscopic and histologic examination of the grafts 3 days after placement showed minimal inflammatory response and an apparent seal between the esophagus and implant at the suture lines. Two weeks after surgery a fibrous connective tissue sheath, continuous with the proximal and distal segments of the esophagus, could be seen surrounding the graft. One month after placement, the implants were partially degraded leaving a connective tissue repair continuous with the proximal and distal ends of the esophagus. The repair area was lined with epithelium and enabled the dogs to drink freely and eat semisolid foods. In conclusion, it has been shown that it is possible to fabricate a biodegradable implant which can stimulate regeneration of a hollow organ and which is compatible with long term survival. Keywords: Artificial tissue, Surgical implantation, Regeneration (Physiology), Neogenesis.

INTRODUCTION

The successful repair or replacement of hollow organs presents a continuing challenge to the surgical profession. The repair of such organs necessitated by atresia or traumatic avulsion such as experienced in penetrating wounds requires immediate and complex treatment. Often times multiple operations with secondary procedures at distant sites to bring autolgous tissue to the primary site have been required to successfully replace an avulsed segment of such organs.

The current therapy for repair and replacement of the diseased or avulsed esophagus is by the use of autografts of viscus such as the stomach [1], the colon [2], jejunal loops [3], isolated jejunal segments [4], or split-thickness skin grafts [5]. None of these procedures produce totally satisfactory results and complications of reconstructive eophageal surgery may include [6]: necrosis of the graft; infection; inadequate blood supply; difficulties in suture retention; leakage at the anastomatic sites; stenosis of the anastomosis between the esophagus and the graft; gastric stasis; reflux; and eating disorders.

This study is a report on the use of biodegradable polymers to construct esophageal implants which stimulated functional repair of avulsive defects caused by excising a segment of the dog esophagus. The polymeric materials selected to construct the biodegradable esophageal implants used in this study were polylactic and polyglycolic acid. These biodegradable polymers are aliphatic polyesters formed from the alpha-hydroxy-carbolic acids lactic acid and glycolic acid. Studies on the fate and metabolism of these materials have shown that they are eliminated primarily via respiration with less than 10% eliminated via urine and feces and with trace amounts in the tissue [7,8]. Surgical investigations utilizing various forms of the polymers and copolymers as bone fixation devices reported their disappearance via a vasofibroblastic and phagocytic response accompanied with occasional giant cells [9,10,11]. These

same authors found no evidence of toxicity, antigenicity or carcinogenicity and found the disappearance was accompanied by restoration of normal morphology in bone and marrow and accompanied by fibrous connective tissue and collagen in soft tissue sites.

The polymer polylactic acid was successfully used as a mandibular fixation device [11,12] and was used to repair blow out fractures of the orbit [13]. Peripheral nerve cuffs constructed of a copolymer of polylactic and polyglycolic acid prevented extraneous connective tissue proliferation into the anastomotic site [14] and gave similar results when used as a tendon gliding device in severely traumatized hands in primates [15].

Resorption rates of the PLA and PGA polymers and copolymers have been determined to be between 4 weeks and 16 months [8,9,16]. These times can be varied according to the size, shape, method of fibrication and the composition polymer or copolymer. In solid form pure PGA or pure PLA have disappearance times of several months duration while 50/50 ratios have disappearance times measured in weeks. When these copolymers are sprayed into fibers of 10-25 micron diameters disappearance rates can be reduced to 14 days or less.

On a conceptual basis, the use of a biodegradable polymer to fabricate a successful hollow organ graft holds promise in that, if successful, it would obviate the need for multiple operations and the concern for the vascular integrity of the graft. In addition, such structures would be readily available for immediate repair of the defect and could be custom made, depending on the defect to be repaired.

MATERIALS AND METHODS

The biodegradable polymer grafts were fabricated by dipping and spraying polymers and copolymers of polylactic and polyglycolic acid dissolved in solvent onto a spinning Teflon mandril. The materials used were as follows: (1) 1,1,1,3,3,3-Hexafluoro-2-

propanol (HFIP) (Eastman Kodak Co.), which was used to dissolve polymers of polyglycolic acid; (2) methylene chloride, analytical grade (Fisher Scientific and Baker Chemical), which was used to dissolve polymers of polylactic acid; (3) Polyglactin 910 (90% polyglycolic acid: 10% polylactic acid copolymer) (Ethicon Corp.); (4) polylactic acid (DL-PLA)(Southern Research Institute); (5) polylactic acid (60% L: 40% DL-PLA) (Ethicon Corp.); (6) 50% polylactic acid: 50% polyglycolic acid copolymer (Batelle Corp.); (7) the templates for fabrication of the esophageal grafts were Teflon cylinders. The Teflon rods used in the fabrication of Group I implants were 150 mm long x 20 mm o.d., while those for Group II were 145 mm long x 26.2 mm o.d.

The esophageal implants were fabricated by spraying the polymer solutions with a #152 dental atomizer (DeVilbiss Corp.) using 20-30 psi of nitrogen as a propellant at a distance of 10-18 inches from a rotating Teflon rod in a fume hood with an air flow of 150 CFM as outlined in Table I.

Sterilization of the esophageal implants was done using ethylene oxide for six hours at 55C to 60C followed by aeration for eight hours.

Table II summaries the physical properties of the Series I and II esophageal implants produced. The fabrication of the Series II grafts was similar to that outlined in Table I except that more applications of polymer were needed due to their larger size.

An AMRAY-1000 Scanning Electron Microscope was used to examine the surface of the esophageal implants. The specimens were sputter-coated with a layer of gold and then examined at an acceleration voltage of 20-30 kV.

The surgical procedures used in this study were done on twelve mongrel dogs under oroendotracheal nitrous oxide, oxygen, and fluothane general anesthesia. By a combination of sharp and blunt dissection the cervical esophagus was exposed, mobolized, and retracted from beneath the trachea. A segment of esophagus measuring approximately five centimeters was removed by sharp dissection

TABLE I

Preparation of Series I Esophageal Grafts

1. Dipped 21 mm o.d. x 150 mm length Teflon mandril in DL-PLA soln-(10g/100ml methlene chloride) and allowed to air dry for 15 min.

2. One end of the coated rod was placed horizontally in the chuck of a slow speed motor, and the other end was put in a glass supporting tube which allowed for rotation of the rod. The motor was then set to turn at a low speed.

3. Sprayed rotating rod with 110ml polymer soln (9g DL-PLA + 1.6g 60%L: 40% DL-PLA/110ml methylene chloride.

4. Placed 9 Polyglactin 910 rings on the PLA-coated mandril. (Polyglactin 910 rings were prepared by spraying Polyglactin 910 dissolved in HFIP (8g/100ml) on a 22.5mm o.d. Teflon mandril.) The rings were 5mm in width and were placed 10mm from each other in groups of 3 to make segments of 45mm each.

5. Sprayed rod with 10ml 60% L:40% DL-PLA. Soln (8g/100ml methylene chloride).

6. Dipped rod in 50% PLA/PGA soln (8g/100ml methylene chlorine).

7. Sprayed rod with 40ml DL-PLA soln (10g/100ml methylene chloride).

8. Dipped rod in 50% PLA-PGA soln (8g/100ml methylene chloride and let air dry.

9. Sprayed rod with 55ml DL-PLA soln (8g/100ml methylene chloride) and placed in sealed jar which was placed in desiccator overnight.

10. Sprayed rod with 72ml polymer soln (3.6g DL-PLA + 2.88g 60% L:40% DL-PLA in 72ml methylene chloride)

11. Polymer coated rod was air dried in hood for 2 hours.

12. Polymer coated rod was lyophilized for 48 hours to remove residual methylene chloride, solvent.

13. After lyophilization the polymer implant was cut into 3 sections, removed from the Teflon mandril, and stored in a sealed desiccator prior to sterilization and use.

TABLE II

Physical Characterization of Biodegradable Esophageal Grafts (a)

o.d. mm (b)	Wall Thickness mm (c)	Length mm	Wt g
	GROUP I		
24.9 ± 0.1	1.6 ± 0.05	44.7 ± 2.0	3.8 ± 0.2
	GROUP II		
32.8 ± 0.4	3.4 ± 0.16	42.8 ± 1.1	7.4 ± 0.3

a. Mean ± standard error of 6 implants.
b. Outside diameter of samples was measured around the Polyglactin reinforcing rings. Internal diameter of Group I implants was 20 mm; Group II implants was 26.2 mm.
c. Wall thickness of polymer between Polyglactin 910 reinforcing rings.

after placement of umbilical tape slip ties to control secretions from the proximal and distal ends of the transectioned esophagus. The PLA graft was then anastomosed to the distal end of the esophagus by use of interrupted 000 Dexon sutures (Ethicon Corp.). The proximal end of the esophagus was then anastomosed to the PLA graft in such a manner as to prevent the esophagus from being twisted upon itself. The umbilical tape was removed from the esophagus and the anastomosis inspected for leakage. The surgical wound was closed in the usual layered manner and the suture line sprayed with Topazone (Easton Veterinary Labs).

The animals were given Bicillin 1.2 million units intramuscularly for three days post-operatively followed by Keflex, 250 mg three times a day for a period of one week. To prevent dehydration each animal received twice daily subcutaneous feedings of 750 cc of 5% dextrose and water for three days following surgery. On the fourth post-operative day the dogs were fed a liquid diet of canned

dog food emulsified in water and fortified with fat (Pig Kalorie Supplement, Haver-Lockhart Labs).

One dog was sacrificed at 3 and 9 days post surgery and two dogs at 14, 21, 30 and 56 days with an overdose of barbiturates. Two of the animals were retained for long-term study.

The dog with a series I graft survived for 3 years and the other animal implanted with a series II graft was sacrificed 4 years after graft placement.

At sacrifice the graft sites were immediately removed in a cervical block to include the surrounding tissue and at least 2 cm of normal esophagus at either end of the graft. Excess tissue was trimmed off the specimens and they were placed in buffered 10% formalin. After fixation, the graft sites were grossed serially into 5 to 8 mm transverse segments and photographed. Tissue sections were then prepared at 6 microns thickness and stained with hematoxylin and eosin for histologic analysis.

RESULTS

Implant Construction

Spraying of the polymer solutions produced fibers of 3-25 microns in diameter and 2-10 cm in length which oriented themselves in a circular manner as they attached to each other around the rotating Teflon mandril. The spraying of methylene chloride solutions of the amorphous structured DL-PLA produced shorter and finer fibers of polymer while the presence of the more crystalline L-PLA in the solutions produced longer and larger diameter fibers.

An example of the polymeric grafts, which were produced by utilizing the procedures outlined in Table I, is shown in Figure 1. The fabricated grafts were rigid and showed very little tendency to flex. The inner surface of the graft was smooth due to its being composed of a solid film of PLA produced when the Teflon template was dipped into the PLA solution. Figure 2 shows the scanning

electron microscopic view of the smooth inner lumen as well as the outer portion of the graft. The inner portions of the graft was more solid in nature than the peripheria due to filling up of inter-fibrillar networks by the 50% PLA/PGA dips used in fabrication of the core portion of the implant. This solid core tended to provide a certain rigidity to the implant. The outer half of the implant was composed of a circular network of PLA fibers 3-25 microns in diameter which could be observed as a series of laminations around the inner core as seen in Figures 1 and 2.

The fibrillar outer coating of the graft allowed for the rapid infiltration of fibrin and fibrovascular tissue into the implant which resulted in a water-tight seal. These implants exhibited initial resistance to flex and collapse; however, the consistency of the wall material was flexible enough to allow the needle from a 3-0 Dexon suture (Ethicon Corp.) to be placed completely through its wall. The sutures placed through the graft wall were retained in position and the wall of the graft did not show any tendency to tear after placement of the sutures. Comparison of the physical properties of Group I implants and Group II implants (Table II) shows that the Group II implants had an inner diameter 6.2 mm greater than Group I and a wall thickness at least two times as thick. These larger implants were constructed to provide more resistance to lumen collapse which was noted in some two-week specimens, and to produce a larger diameter esophageal replacement which would be more resistant to esophageal stricture during the repair phase of healing.

Ethylene oxide sterilization of the implants caused an average 4% decrease in length and a 6% decrease in diameter from the dimensions listed in Table II. This dimensional shrinkage was also accompanied by a slight increase in flex resistance.

Clinical Findings

The post-operative periods progressed uneventfully. There was

very little swelling at the surgical site. Endoscopic examination of the grafts in situ at three days and eight days showed an unobstructured esophageal graft which was in continuity with the rest of the esophagus. Oral administration of slurried canned dog food was thus begun on day four after initial surgery. By the fourth week after surgery, the dogs showed some difficulty in oral feeding and endoscopic examination showed contraction of the repair tissues present in the graft site. Dilation with a series of metal bougies starting at a French #29 (9.5 mm) and ending at a French #45 (14.8 mm) was begun at this time and the graft sites were dilated biweekly until two weeks prior to sacrifice in the two 56-day dogs.

The dog which survived 3 years after graft placement had received a series I implant. It was dilated biweekly until six months post surgery. Some esophageal constriction was seen at this time, but was readily relieved by bougienage therapy and a size #45 French dilator freely passed the length of the esophagus after dilation. This dog was then dilated monthly for the next three months and then one more time two months later. At this time bougienage therapy was terminated. At 18 months post surgery, barium X-rays taken of the esophagus showed that an area of stricture was present in the esophagus in the area of the implant. However the dog ate well and showed an increase in body weight from 45 pounds prior to surgery to 48 pounds at that time. Three years after the initial surgery, the dog died. The necropsy report stated that the cause of death may have been gastric torsion.

The second long-term survival animal had been implanted with a series II graft. This dog was not dilated until 40 days after surgery and then was dilated monthly for two months up to a size #45 French dilator, after which time it had received no further dilations although the endoscope used to examine the esophagus had a diameter equivalent to a #35 French dilator. Monthly endoscopic examinations done over the next 9 months showed that no esophageal constrictions were present and that the epithelium of the graft area was continuous with that of the original esophagus.

At 18 months post surgery, endoscopy was again done. The endoscope passed easily through the esophagus; the lining of the lumen appeared to be completely epithelialized and only a slight area of constriction was noted. The dog ate well and after an initial weight loss post surgery, the dog eventually regained his preoperative weight of 40 pounds.

The animal was sacrificed 4 years after placement of the implant due to transfer of the principal investigators.

Gross and Histologic Findings

The gross morphology of the esophageal grafts after three days of implantation is shown in Figure 3. Gross examination of the implant site did not show any evidence of leakage around the anastomosis between the polymer tube and the esophageal tissue. All the original sutures were present and they showed only minimal inflammatory reaction around them. The implant did not show any evidence of collapse or loss of structural integrity at this time.

Histologic examination of the original connective tissue wall of the esophagus and the graft itself as seen in Figure 4 showed a layer of fibroblastic tissue demonstrating hemorrhage with early vascular proliferation. At the interface between the graft and the connective tissue there was evidence of platelet and fibrin accumulation within the interstices of the graft, but little evidence of organization. The inflammatory response to the graft and sutures was minimal at this time. Although there was no proliferation of the early fibroblastic tissue into the interstices of the graft, at 3 days there was a definite increase in mitotic activity in the surrounding connective tissues. This was evidenced by an increased number of fibroblasts and small vascular channels proliferating around the edges of the graft.

The gross section of the 9-day surgery sample shown in Figure 5 demonstrates that the interface between the connective tissue and the graft showed a maturation of the fibrous connective tissue and

ingrowth of the vasofibroblastic tissue into the interstices of the graft material. The thickness of the connective tissue wall, measured from the outer surface of the tracheal cartilage rings, was 2 mm. The trachael wall did not show any evidence of erosion due to presence of the graft. Figure 5 also shows that the thinner walled implants of Group I (Table I) exhibited a tendency to close down; however, a sufficient opening for maintenance of the nutritional requirements of the dog was still present. Histologically, there could be seen a beginning orientation of the connective tissue fibers in a circular manner around the graft site (Figure 6). There was very little tendency to form giant cells surrounding the polymer fibers. The interface between the graft and the original connective tissue showed an ingrowth of 1.5 mm of fibrous connective tissue into the interstices of the graft with minimal inflammation present. At the margins, or at the interface between the graft and the esophageal epithelium, there was a proliferation of new epithelium at least 3 mm down over the graft site.

Examination of the 14-day samples revealed a vasofibrobastic connective tissue wall of approximately 3 mm in thickness which surrounded the esophageal implant. In certain areas of the connective tissue graft interface there was multinucleated giant cell formation where the polymer had been incorporated into the tissue and this extended back about 1.5 mm from the apparent edge of the graft itself, indicating that the vasofibroblastic ingrowth had penetrated at least 1.5 to 2 mm into the graft. In addition, there was minimal evidence of collagen organization into both circular and longitudinal bands in the implant area as well as epithelial migration down the edges of the draft itself as seen in Figure 7.

Gross examination of the 30-day samples, as seen in Figure 8, showed a vasofibroblastic wall of about 5 mm (measured from the outer tracheal ring) which had formed both as a result of tissue growth around the implant and growth into the implant itself of about 2.5 mm. Most of the inner core of the implant had become

delaminated from the outer sections which had induced the peripherial fibroblastic response and, or which, has become invaded by repair tissue. Histologically, the advancing front of granulation tissue into the graft was characterized by occasional multinucleated giant cells which were phagocytosing the polymer after it had been hydrolyzed. The collagen fibers present in the new esophageal wall showed a variable orientation and there was variable epithelial migration up to 5 mm from the anastomosis along the inner aspect of the graft site.

By eight weeks the graft resorption and elimination was almost complete and a collagenous tube with a wall thickness up to 5 mm measured from the tracheal rings was present (Figure 9). In addition, it was evident that the esophageal implants had not affected or caused any erosion of the trachea. The lumen of the esophagus was open although some contraction of the repair tissue was evident as indicated by the narrowing of the esophageal lumen. There was no macroscopic evidence of graft material. Microscopic examination, however, showed some evidence of residual polymer between the collagen fibers as seen in Figure 10. The Dexon sutures on the other hand appeared to have become completely degraded. Only minimal inflammation and slight edema were present at the graft site and the collagen showed a variable orientation with a small amount of circular orientation. There were islands and sheets of epithelium growing over the lumenal surface of the repair tissue in the area of the original graft, although some areas were still not completely epithelialized at eight weeks.

Necropsy of the animal, which died after 3 years of graft placement (series I), showed that the area of the esophagus anterior to the graft site showed some dilation while the esophagus distal to the graft site (gastric end) was normal in appearance. The graft site itself showed constriction and shrinkage to about 1.5 cm from its original 4 cm length.

Histologic analysis was not done due to the advanced postmortem degeneration of the tissues. Gross and histologic

analysis of the 4 year dog was not done due to transfer of the principal investigators.

DISCUSSION

This study demonstrated the feasibility of using a biodegradable hollow organ implant fabricated from polylactic acid and polyglycolic acid polymers to serve as a template for regeneration of an excised segment of the dog esophagus. The inner core provided for rigidity of the implant as well as a seal which prevented egress of stomach fluids into the surrounding tissues. The outer covering of PLA fibers provided for strength and allowed for a vasofibroblastic ingrowth into the graft as well as promoting growth of a collagenous sheath of tissue around the peripheria of the graft. The fabrication of 100% PLA into 3-25 micron fibers in the outer covering, as was done in this study, allowed degradation of some polymer in as little as 14 to 21 days by hydrolysis of tissue fluids and cellular action; although some polymer incorporated into the repair tissue appeared to be retained for up to 8 weeks (Figure 10). This resorption time is less then the time required for solid PLA plates as plates are partially resorbed in six weeks and total resorption may taks six months [11,12].

The decrease in size and increased resistence to flex which occurred on ethylene oxide sterilization may have been due to an increase in crystallinity of the polymer resulting from the heat used in the sterilization process as suggested by a recent study by Gindoe and Gupta [17].

The laminar construction of the grafts apparently allowed for partial removal of the graft by a delamination of the inner core at 3-4 weeks post-surgery. This delamination appeared to be an essential requirement for the success of these grafts since the Polyglactin supporting rings in the implants only provided limited resistence to graft collapse as was seen in some early specimens (Figure 6). The use of more rigid cast or machine formed solid

rings of PLA or PGA as an inner support would probably prevent such graft collapse while allowing for their degradation in stitu or in the stomach if delamination of the graft occurred.

The fibrillar portions of the polymer implants in contact with the cut ends of the esophagus as well as the connective tissue in the implant site promoted a favorable healing reaction as evidenced by the complete repair of the graft site. This repair was by a collagenous sheath connecting the two ends of the esophagus and was achieved as early as two weeks. Histologically, the final repair seen at 8 weeks was by a dense hyalinized type of connective tissue in sheath form. The connective tissue sheath allowed the animal to eat freely with minimal discomfort until the fourth week after surgery, when some contraction of the new segments was noted. This constriction was readily relieved by dilation of the esophagus with metal bougies. Similar problems may also be seen with autogenous viscus replacements [18] and the course of bougienage therapy used on the long term surviving dogs is similar to the therapeutic regimens used on human patients with constrictive esophagitis [19].

The ability of the dog receiving a series I implant (OD = 24.9mm) to survive 25 months without bougienage therapy, and that of the dog receiving a series II implant (OD = 32.8mm) to survive for at least 36 months without additional dilation therapy, suggests that a functional maturation of the connective tissue at the repair site eventually occurred. Nevertheless, the constriction noted at 18 months in the dog receiving the series I graft as well as the shrinkage and contraction noted 18 months later at necropsy suggests that further dilation therapy may have reduced this shrinkage. However, the main criteria for stimulating and maintaining a physiologic repair may be the initial size of the polymer graft. The dog which received the larger series II graft did not appear to need additional dilation therapy after the initial series in order to maintain its nutritional intake up to its sacrifice 4 years after graft placement.

These results compare favorably with results achieved using viscus grafts in which patients report difficulties in eating and slow weight gain for 12 to 18 months or longer [18].

The esophageal contraction and shrinkage noted on the necropsy of the dog receiving the series I graft may or may not have been a factor in the death of the dog due to possible gastric torsion, since gastric torsion is a condition of unknown pathogenesis which can occur in caged animals.

In conclusion, it has been shown in this study that: (1) It is possible to construct a completely biodegradable off-the-shelf graft which can replace lost segments of hollow organs. (2) Regeneration of the hollow organ occurred by a new tube of connective tissue lined by epithelium utilizing the technique of neogenesis within a biodegradable polymer-copolymer framework. (3) The replacement supported the dogs' nutritional intake requirements. (4) The replacement showed adequate strength and allowed for maintenance of esophageal diameter by bougienage therapy. (5) It did not appear to exhibit problems such as the need for multiple operations, leakage at anastomosis sites, lack of blood supply, and postoperative infections seen with other therapeutic procedures in current use. (6) Grafts made of spun biodegradable PLA and PGA co-polymers meet the general requirements for an effective and easy-to-use replacement and should be studied further.

"In conducting research described in this report, the investigators adhered to the 'Guide for the Care and Use of Laboratory Animals' as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources National Research Council."

"Commercial materials and equipment are identified in this report to specify the investigative procedure. Such identification does not imply recommendation or endorsement, or that the materials and equipment are necessarily the best available for the purpose."

"The views of the authors do not purport to reflect the views of the Department of the Army or the Department of Defense."

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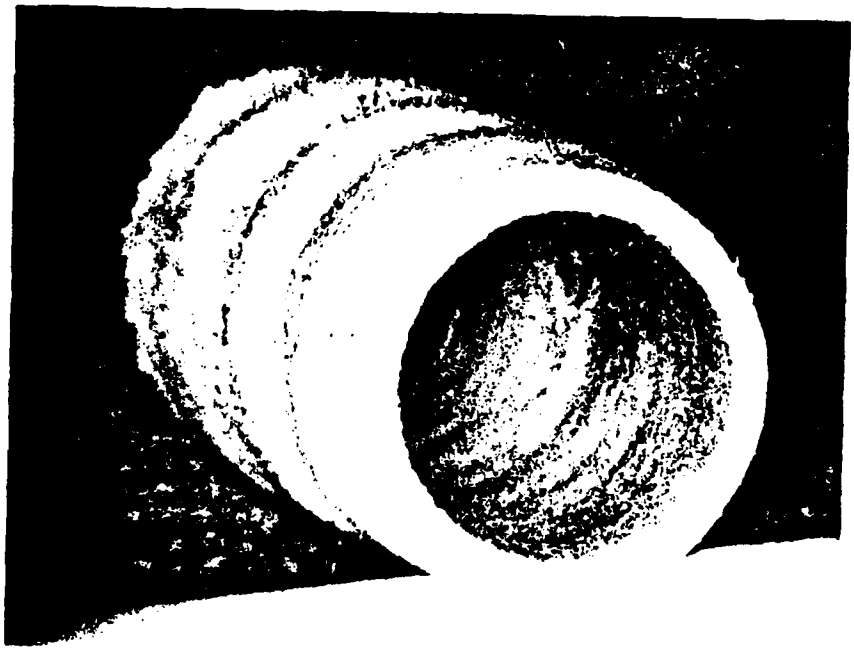

FIG 1. End view of a Group II biodegradable polymer esophageal graft. X 1.5.

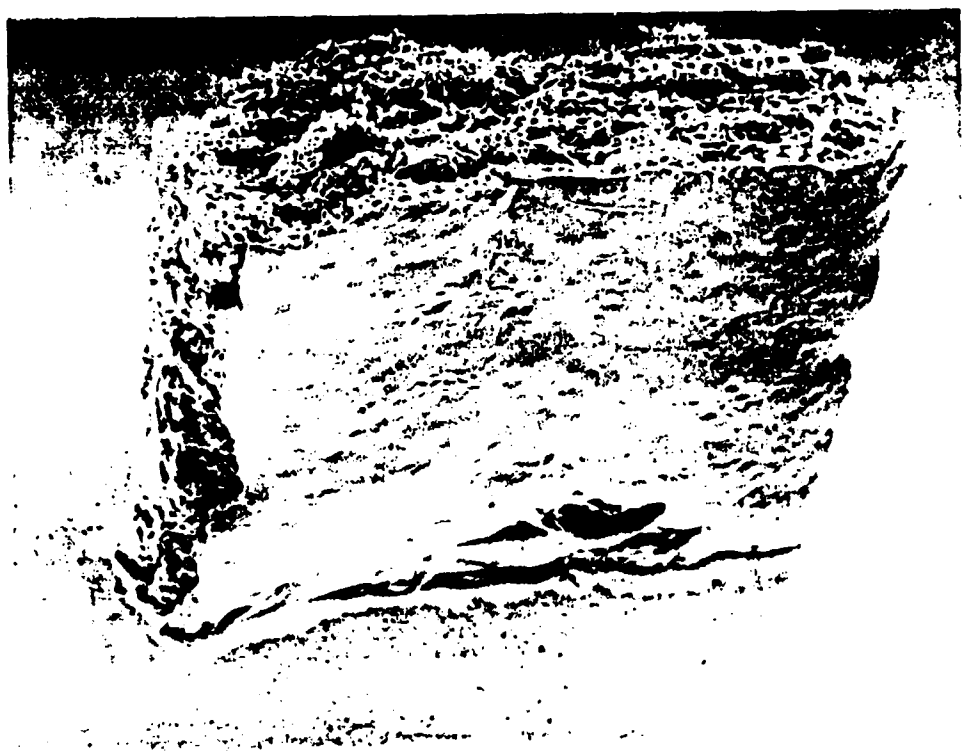

FIG 2. SEM view of a section of esophageal implant showing smooth inner core and outer fibrillar matrix. X 42.

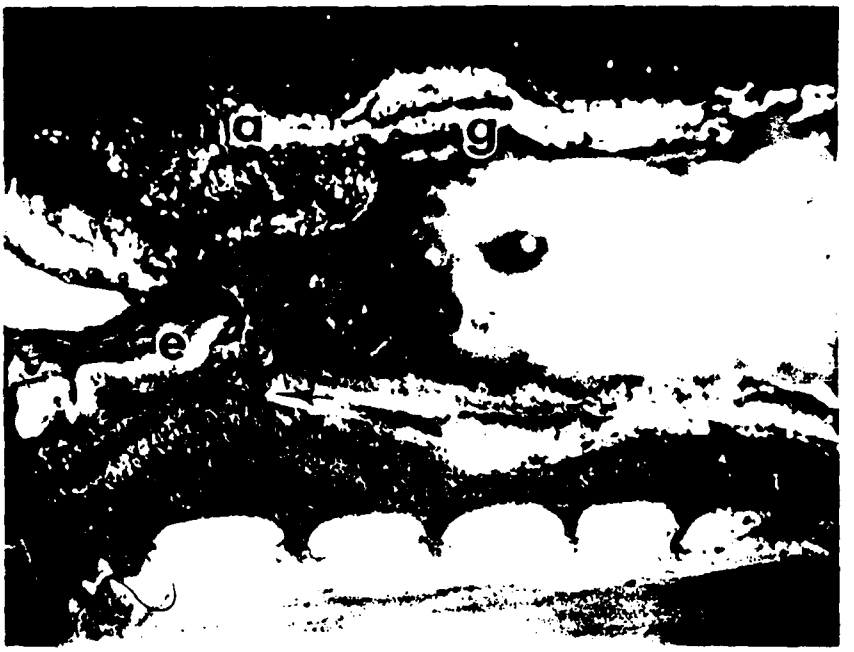

FIG 3. Three-day gross specimen of the anterior anastomosis of the esophagus with the polymer graft (longitudinal section). (a) anastomosis site; (e) anterior esophagus; (g) main body of implant. The adherence of tissue to graft and fibrin penetration into graft is shown by the arrow.

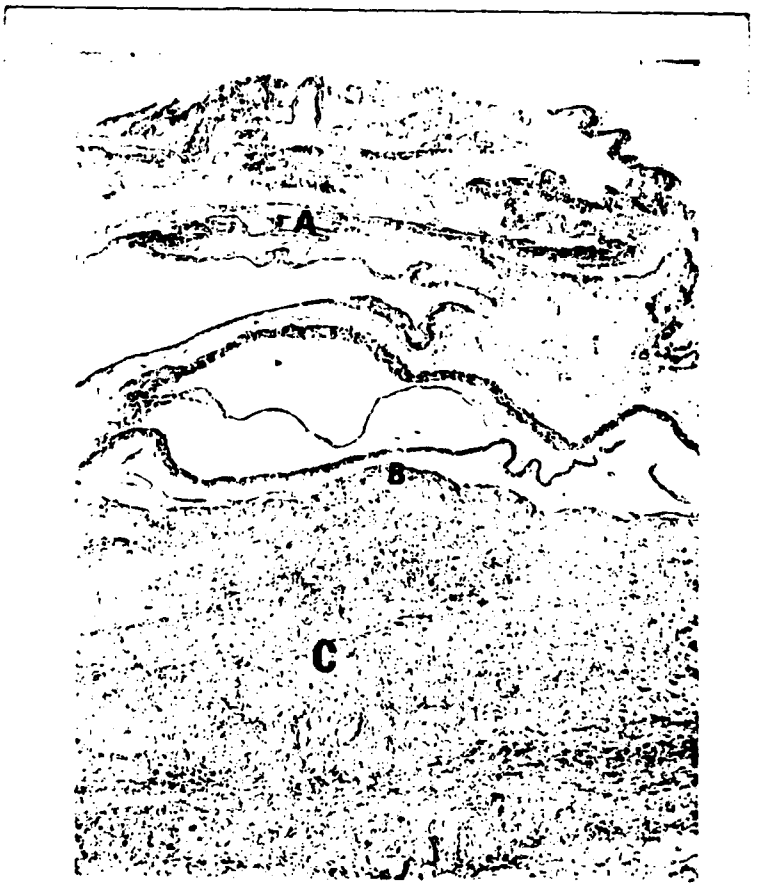

FIG 4. Histologic view of graft host interface at 3 days. X 40. The graft (A) is infiltrated with fibrin and cellular elements. The interface between the graft and connective tissue margin (B) is predominantly fibrin. The deeper tissues (C) show a reparative response.

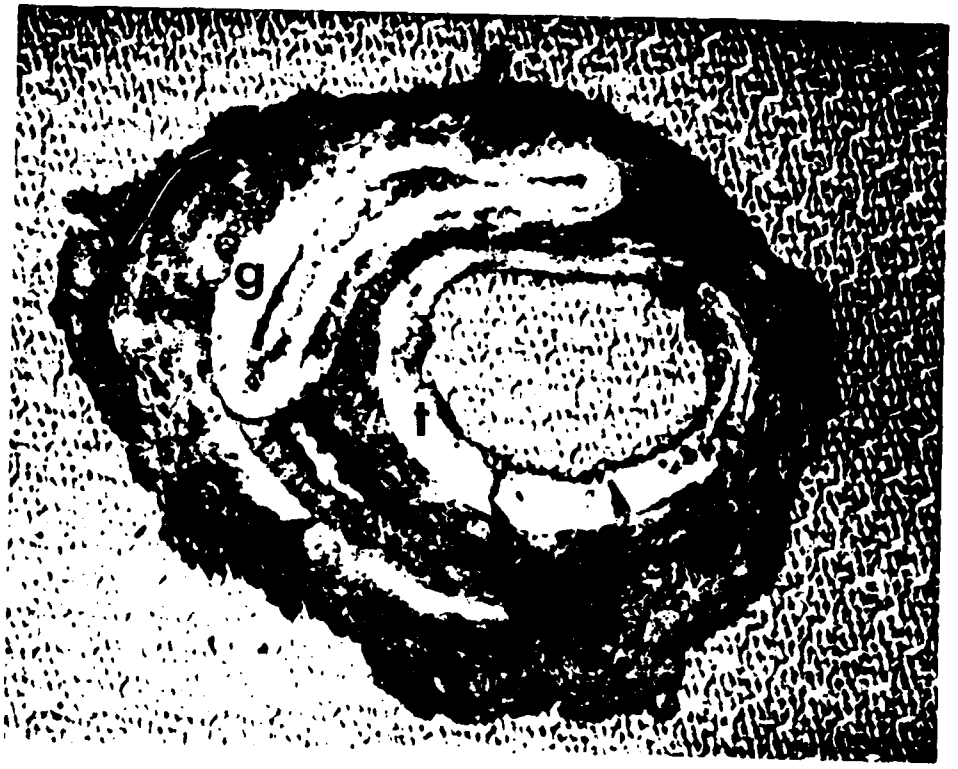

FIG 5. Gross section of graft and trachea at 9 days. The esophagus shows tissue ingrowth and beginning obliteration of graft host interface. (g) is the polymer graft; (t) is the trachea below the esophagus.

FIG 6. Histologic section of the reparative response at 9 days. X 110. The orientation of the reparative tissue fibers (f) follows the orientation of the original copolymer fibers (p).

FIG 7. Histologic section of epithelialization of esophageal lumen at 14 days. X 60. The epithelium (e) has migrated onto the reparative tissue (r) of the new lumen (l) from the original anastomotic site (a).

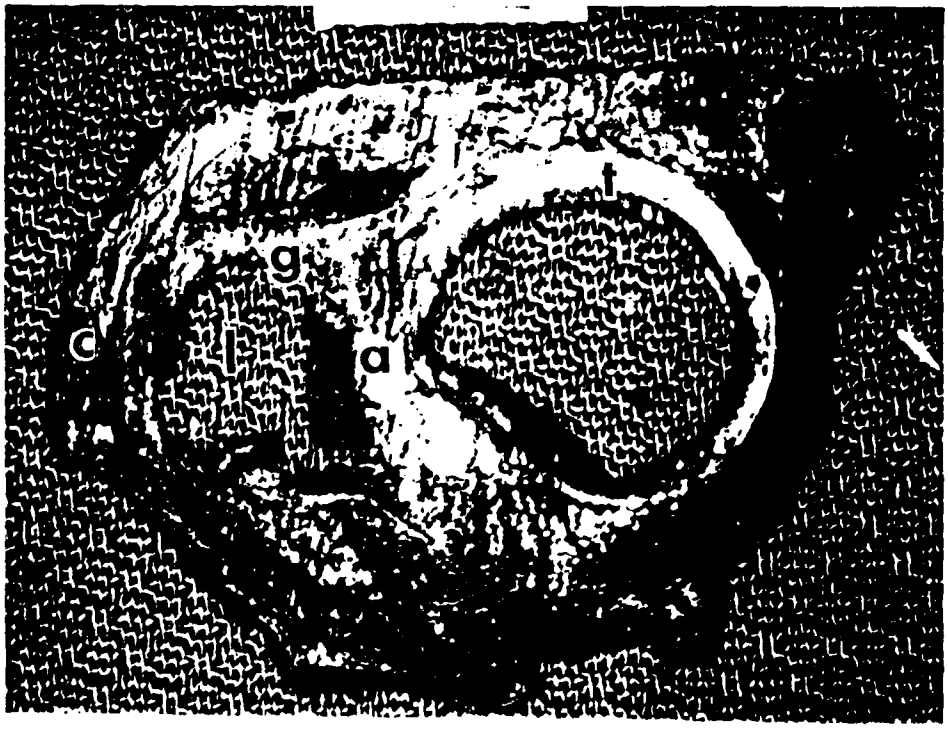

FIG 8. Gross section at 30 days (tangential view) showing tissue infiltration into graft (g); graft anastomotic site (a); and sutures (c). The lumen of the esophagus is shown by (l) and the treachea by (t).

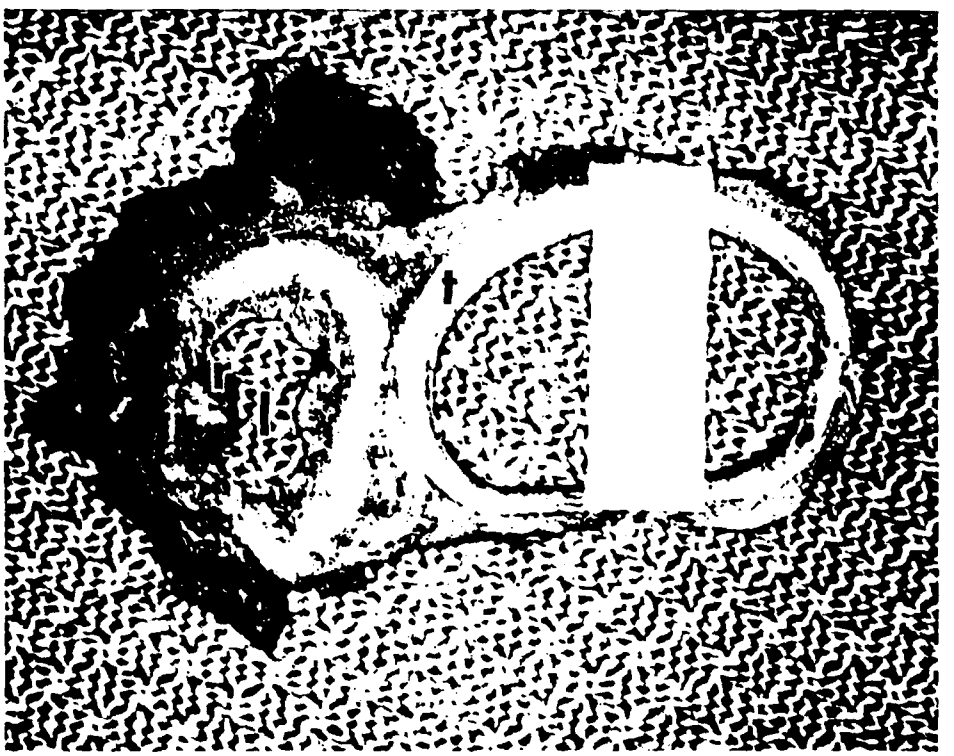

FIG 9. Gross section of 8-week esophageal implant (cross section): (r) regenerated esophageal wall; (l) lumen of esophagus; (t) trachea.

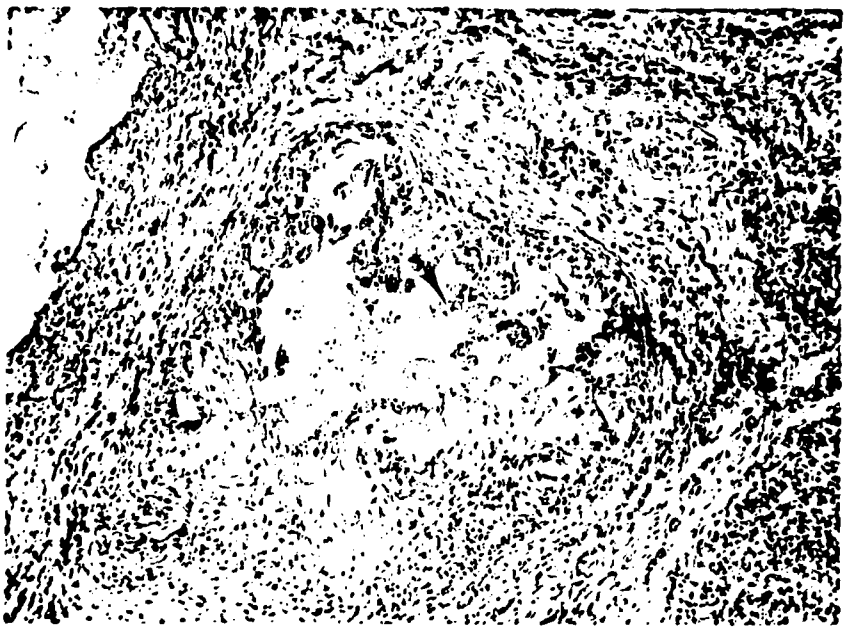

FIG 10. Histologic section at 8 weeks of regenerated esophageal wall which shows an area of final resorption of the copolymer. X 110. Phagocytes (arrow) can be seen phagocytosing the polymer in its final soft state.