A New Model System for the Study of Wound Healing[1] (35335)

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Various models have been used to provide a simpler approach to the study of the complicated process of wound healing than that afforded by studies of reparative tissue of open wounds. Robertson (1) and others have utilized the injection of carrageenin, Boucek and Noble (2) introduced the use of polyvinyl sponge implantation, and Schilling *et al.* (3) have used implantation of stainless steel wire mesh cylinders. Various chemical and biological studies of the maturing reparative tissues have been made in all of these model systems. The stainless steel cylinder model has the obvious disadvantage that small amounts of trace minerals which have an influence on wound healing may be present. In order to avoid this problem we have recently investigated the use of a plastic model. An acrylic cylinder has been developed which appears to have promise. The present study compares the chemical composition and histological appearance of the tissue in this model with that from the stainless steel cylinder.

Methods. The stainless steel cylinders were approximately 5 cm long and 1 cm in diameter. They were formed from one sheet of No. 46 mesh, stainless steel wire, 4.3 × 6 cm. The cylinders were formed by molding the sheet over a No. 4 cork borer and joining the long edges together with a felled seam. The cross wires were removed on the 5-cm portion at each end and the remaining wires were folded into the center to make a closed cylinder. The acrylic cylinders were approximately 54 mm long and 1.1 mm o.d. They were made from acrylic tubing, 0.5-in. o.d. with 1/16-in. walls. The walls were turned down on a lathe until they were 1/32 in thick. Approximately 20 holes, 0.040-in. diameter, were bored around the cylinders in staggered rows approximately 1.5 mm apart The ends of the cylinders were closed with a Silastic rubber stopper with a 1/16-in. hole in the center making the overall length approximately 6 cm. The two cylinders are shown in Fig. 1.

Under aseptic conditions, the cylinders were implanted subcutaneously on the backs of rats. The cylinders were left in one series of animals for 2 weeks and in another series for 4 weeks. After this time, the rats were anesthetized and the cylinders were removed The fluid was aspirated using a syringe with a 20-gauge needle. The stainless steel cylinders were opened by a longitudinal cu through the cylinder. The tissue was rinsed with tap water; a cross section was placed in 10% buffered formalin for histological study the remainder of the cylinder with adherin tissue was freeze-dried. After drying, the tissue on the inside of the cylinder was stripped from the wire. The acrylic cylinders were opened by removing the rubber end plugs The tissue was then readily removed from the cylinders with a small spatula and opened by a longitudinal cut. The tissue was rinsed with tap water; a cross section was placed in formalin for histological study; the remainder was freeze-dried. The dried tissues were weighed, then ground in a Wiley Mill to pass through a 40 mesh screen.

Hexose was determined by the trytopha method and hexosamine by a modification o the Elson Morgan reaction (4). Uronic aci was determined by the Bitter and Mui method (5) after hydrolysis with Dowex 5 (4). Protein was determind by the proce dure of Lowry *et al.* (6). Hydroxyprolin

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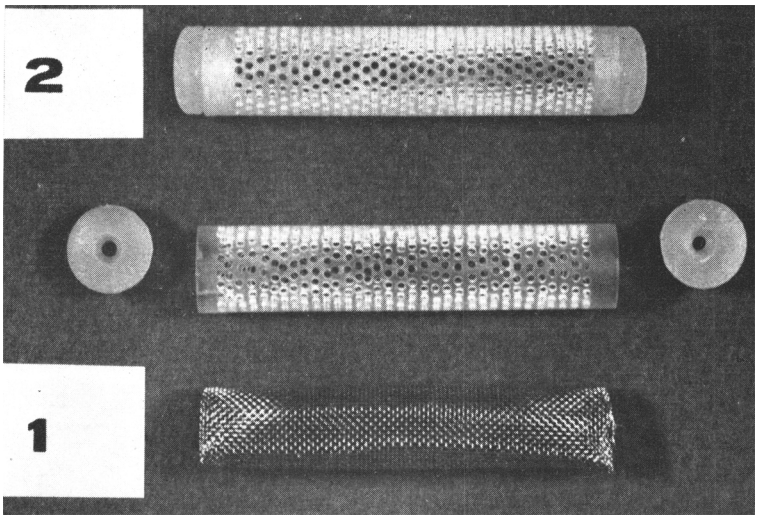

FIG. 1. Photograph of the model cylinders: (1) stainless steel model; (2) acrylic model. A complete acrylic cylinder is at the top; a cylinder with the silastic rubber stoppers removed is in the center.

was determined by using a Beckman amino acid analyzer. Partial isolation of the tissue mucopolysaccharides was made by incubation with Pronase. Following this digestion, the material was dialyzed and the resultant nondialyzible solution was concentrated by freeze-drying. Electrophoresis was carried out on cellulose acetate using the Microzone electrophoresis system with a pyridine acetic acid water buffer (1:10:89,v:v:v:), pH 3.5.

The tissue samples taken for histological examination were kept in formalin for 24 hr; the tissues from the stainless steel were then stripped from the wire mesh. Histological sections from both models were then prepared using hematoxylin and eosin, periodic acid–Schiff, trichromic Gallego and fibrin-Ledrum staining.

Results. Average weights of the tissue produced in the two models are given in Table I together with a summary of the chemical composition of the tissues. The amount of tissue produced is less in the acrylic model than in the stainless steel. Similar protein contents were found. The total ash in the acrylic model was lower than that found in the stainless steel model. Hexose and uronic acid contents were similar; however, the hexosamine contents of the acrylic tissue were higher. The hydroxyproline content of the tissue from the acrylic model was definitely lower than that from the stainless steel cylinders.

Histological examination revealed no significant difference between the tissue in the two models. In the 2-week samples, the tissue was typical granulation tissue formed by histiocytes, fibroblasts, collagen fibers, small vascular channels, and scattered white cells. There were isolated areas of microhemorrhage and necrosis; these areas were somewhat more common in the acrylic model. In the 4-week samples, there were fewer areas of

TABLE I. Composition of Tissue from Stainless Steel and Acrylic Wound Healing Models.

	Stainless steel		Acrylic	
(Weeks):	2	4	2	4
Total dry wt	137.09	272.36	54.66	112.69
Total dry wt[a]	137	272	54	112
Protein	62.2	61.2	66.5	61.4
Ash	10.29	10.36	9.45	6.92
Hexose	3.16	3.10	3.54	2.69
Hexosamine	0.36	0.35	0.62	0.50
Uronic acid	0.09	0.11	0.11	0.15
Hydroxyproline	2.71	4.88	1.66	3.31

[a] Average weight (mg) is exposed based on the results of 8 acrylic and 3 stainless steel cylinders. All other values are percentages of the dry weight.

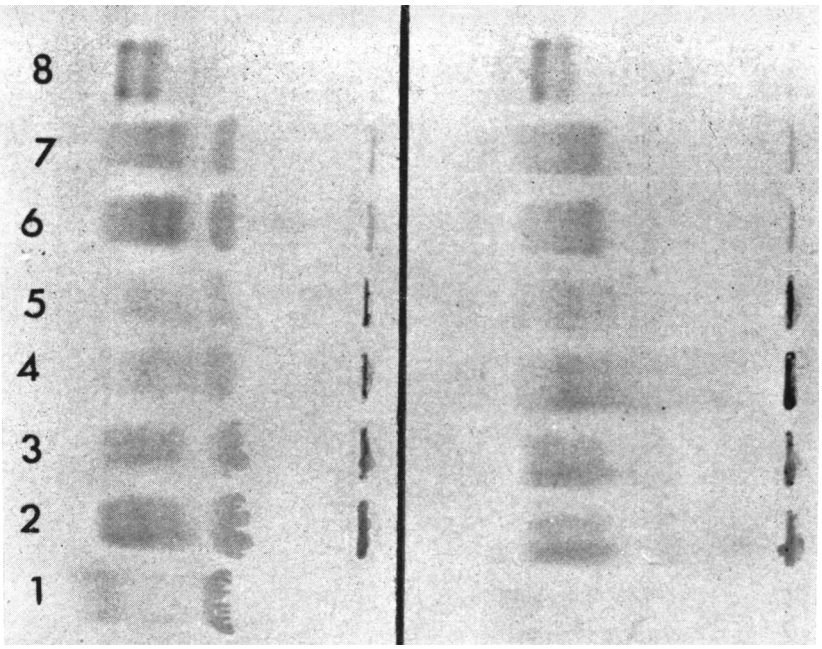

FIG. 2. Cellulose acetate patterns of mucopolysaccharides from wound healing models: Pattern to the right is stained with alcian blue; that to the left with toluidine blue. (1) a standard containing hyaluronic acid (band to the right) and heparin (band to the left). (8) a mixture of chondroitin sulfate B (to the right) and the chondroitin sulfate A (to the left). (2) 2-week acrylic sample; (3) 4-week acrylic sample; (4 and 5) 4-week stainless steel samples; (6 and 7) 2-week stainless steel samples.

hemorrhage and necrosis and more collagen fibers.

Typical electrophoretic patterns are shown in Fig. 2. The mobilities indicate the presence of both hyaluronic acid and chondroitin sulfate. Relatively more hyaluronic acid is found in the acrylic model tissue at both 2 and 4 weeks. The amount of hyaluronic acid in the stainless steel tissue at 4 weeks is very small. Upon staining with toluidine blue, only the faster moving fractions stains metachromatically, thus confirming the presence of chondroitin sulfate.

Discussion. Histologically, the tissues from the two models appear to be similar. Except for hydroxyproline, the gross chemical analyses are also similar. The lower hydroxyproline content and the higher hyaluronic acid content of the tissue from the acrylic tissue at comparable times indicate that the connective tissue in the acrylic model is less mature than comparable tissue from the stainless steel model. The reason for this is not clear at this time. It is possible that the stainless steel model may provide trace minerals which influence connective tissue formation. However, sample assays indicate that the zinc levels are not significantly different in the two models. A distinct advantage of the model is that the tissue can be more readily removed from it than from the stainless steel wire mesh.

Summary. An acrylic cylinder model for the study of wound healing is described and compared with the stainless steel wire cylinder model. Histologically, the tissue from the two models are similar; however, less tissue is produced in the same time in the new model. The hyaluronic acid content is higher relative to the chondroitin sulfate in the acrylic cylinder tissue at a comparable time; in the same tissues, the hydroxyproline is lower in the acrylic tissue. It is concluded that the connective tissue in the acrylic model does not mature as rapidly as does tissue in the stainless steel model.

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designing and fabricating the acrylic cylinders is gratefully acknowledged.

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