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TECHNICAL REPORT 6608

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APR 1966

U. S. ARMY MEDICAL BIOMECHANICAL RESEARCH LABORATORY
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ABSTRACT

High molecular weight polymer from lactic acid can be made from the cyclic lactide intermediate, suitable for casting films or spinning fibers. The films are quite permeable to water vapor and can soften in presence of water.

Histological studies indicate that the polylactic acid is non-toxic, non-tissue reactive, and biodegradable, as evidenced further by the study of degradation of C^{14} tagged polymer in vivo.

The degradation studies also point out that the polymer or its degradation products are not retained in any of the vital organs of the animals. The polymer implant, however, degrades slowly in vivo, losing 12-14% in three months.

This study indicates polylactic acid to be a very suitable material for sutures, vascular grafts, and other surgical implants.

INTRODUCTION

Lactic acid in its racemic or optically active form can undergo acid catalyzed homopolymerization to yield a polymer of lower molecular weight, which is not suitable for plastic or fibers. (1) However, the cyclic diester, the lactide (2) of lactic acid, can polymerize by anionic ring opening addition mechanism under the influence of catalysts to a high polymer, which can be cast into strong films or spun into fibers (3), (4), (5), (6) comparable to the industrial polyesters like dacron. This property, and in addition, the possibility of this polyester undergoing hydrolytic de-esterification finally to the lactic acid, which is a normal intermediate in the lactic acid cycle of the carbohydrate metabolism, make this a highly interesting product, as a synthetic surgical repair material possibly for vascular grafts and sutures. Moreover, the material is expected to elicit no immunological response due to the absence of peptide linkages. No literature references, however, are available regarding the use of polylactic acid in this field. Therefore, an investigation of the synthesis, characterization, histological evaluation of tissue reactivity, and the in vivo degradation of the tagged polylactic acid was undertaken, the results of which are reported in the present paper.

EXPERIMENTAL

DL-Lactic acid was obtained commercially in the form of 85% aqueous solution, and L (+) lactic acid was obtained from Miles Chemical Company, Elkhart, Indiana, as a 40% aqueous solution and also as a crystalline solid, for the preparation of lactide, an intermediate in the polymer synthesis. The lactide of L (+) or DL-Lactic acid was prepared in accordance with the following procedure based on that of Moser. (2)

Lactide: A solution containing 500 g of lactic acid or 500 g of pure lactic acid were mixed with 10 g of zinc oxide and subjected to distillation for six hours at 140° C pot temperature, and a 760 mm pressure in the beginning and gradually decreasing to about 25 mm. At this stage, about 90% of the water content and the water of formation of lactide was removed; then, the temperature of the bath was increased to 250° C and the lactide was distilled at 1 mm pressure for about 8 hours. The yields were of the order of 70% of the theoretical. The lactide so obtained was recrystallized first from ethyl acetate and then from methyl ethyl ketone, to get highly pure crystals suitable for polymerization. The M. P. of the DL lactide and the L (+) lactide was 125° and 96° C, respectively.

Poly Lactic Acid: The lactide was placed in a polymerization tube or a round bottom one-necked flask, and the air and residual solvents were removed by gradually applying vacuum and heat until the lactide melted under high vacuum. The flask was sealed under vacuum and placed in the oven at $170^{\circ} \text{C} \pm$ for 6 to 8 hours, when the polymer formation was completed. The catalysts used for polymerization were SnCl_4 , ZnCl_2 , PbO , SnO , ZnO , and tetraphenyl tin in the amounts ranging from 0.001 to 0.01% on the lactide. From the point of view of nontoxicity and ease of handling, tetraphenyl tin was preferred in the larger scale preparations.

The intrinsic viscosity measurements were made in a Ubbelohde viscometer at $25^{\circ} \pm 0.01$ in benzene and chloroform. The data on different samples are presented in Table I. The optical rotations and the dispersions were measured on a Bendix Spectropolarimeter; the moisture permeabilities were measured by using Payne permeability cups. The physical properties of the polymers are listed in Table I.

Polylactic acid tagged in the alpha carbon atom with C^{14} was prepared in the same manner as before by first preparing the lactide from the lactic acid L (+) diluted with C^{14} DL-lactic acid and further polymerizing the lactide. The interference of the optical isomer in the process is expected to be negligible because of the very small amount of the C^{14} acid used. The 100 mg polymer gave the radioactivity of 5.05×10^5 dpm, in the scintillation counter.

The films of the polymer were cast on Teflon sheets by means of doctor blades from the solutions in benzene, chloroform, dioxane, or butyl acetate. The water vapor transmission (9) of the films was 53.2 g per sq. meter at 75°F at 4 mils film thickness at a pressure head of 24 mm hg. The polymer could be spun in continuous fibers by extrusion of the dioxane solution into water or chloroform solution into methanol. The tensile strength of the fibers obtained by spinning dioxane solution was 2300 psi.

Histological Evaluation of the Polylactic Acid Implants: Polylactic acid powder was sterilized at $60-70^{\circ} \text{C}$ for 36 hours and then implanted subcutaneously in the ventral wall of twelve guinea pigs at 20 mg each.*

* The "Principles of Laboratory Animal Care," as promulgated by the National Society for Medical Research, were observed during this project.

The guinea pigs were divided into three groups and sacrificed at intervals of one, two, and four weeks with suitable controls. In another study, the polylactic acid film was cut into discs of 1.5 cm diameter, sterilized with 70% absolute ethanol, washed with sterile saline solution and implanted on each side of the abdominal wall of six guinea pigs. The animals were sacrificed at intervals of two, four, and six weeks.

At the time of sacrifice, the tissues from the implant sites were fixed in cold buffered formalin. The histological sections of 5 μ thicknesses were cut and stained with hematoxylin and eosin and for connective tissue stain (Movat). The figures 1 to 5 are the microphotographs of the histological sections showing the details of tissue reactivity of the polymer implants.

In Vivo Degradation: The implant chambers were prepared by firmly adhering together two millipore filter membranes on both sides of a polyethylene ring by a special M. E. cement, along with 100 mg of polylactic acid implant material inside the chamber. These chambers were implanted in either side of the midline subcutaneously in 22 rats. In 15 rats the polymer was labeled with C^{14} in the asymmetric carbon, and 7 rats were implanted with nonradioactive polylactic acid for control.

The seven rats (5 with tagged polymer and 2 with untagged polymer) were placed in metabolic cages and urine and feces were collected at 4-day intervals for analysis of radioactivity. At the end of 3 months, the rats were sacrificed and the radioactivity was measured in the liver, kidney, lung, heart, brain, spleen, muscles, pouch around the chamber and the contents of the chamber. (10)

The remaining 15 rats (10 with tagged polymer and 5 untagged polymer) were placed in conventional cages and three rats (2 with untagged polymer and 1 with tagged polymer) were sacrificed at 2 hours, 7 days, 14 days, 1 month, and 2 months, after implantation and the analysis of the vital organs and chamber contents for radioactivity were carried out.

Urine, feces, and tissue were prepared and analyzed for radioactivity exactly as described previously. (10) However, the chamber contents were removed and placed in a glass tube containing 6 ml of hyamine which was kept in a water bath at 60° C for 3 hours. An aliquot of 3 ml was then mixed with 10 ml of scintillation fluid for counting. The results of the degradation study are presented in Table II.

RESULTS AND DISCUSSION

The commercially available racemic DL-lactic acid (85% in water) has negligible optical activity. The L (+) lactic acid which is a normal product of muscle contraction in animals, shows the specific rotation of $+12^\circ$. However, it has been shown and later confirmed that the acid itself has negative rotation and its observed positive rotation is due to its existence in the epoxide form in equilibrium with itself, in aqueous solutions (6), (7). Its dimeric lactide has $[\alpha]_D^{25} = -300^\circ$. On heating, the acid partially converts to lactide and a polymer with elimination of water, and on further distillation, under high vacuum with a catalyst like ZnO, the polymer slowly converts to the lactide which distills over. Heating lactic acid alone or in presence of small quantities of weakly basic catalysts like ZnO, or PbO, at about 250°C under high vacuum, does not appreciably racemize the optically active acid. (2) It is observed that L (+) lactic acid yields a lactide having $[\alpha]_D^{25} = +300$, when it is heated under vacuum in removing water and decomposing the polymer to lactide under the conditions of the experiments.

The lactide polymerizes in the presence of a catalyst-like tetraphenyl tin at approximately 170°C to a high polymer in 6-12 hours as described before. Its intrinsic viscosity ranging from 0.5 to 2.0, a measure of molecular weight can be controlled by changing the amount of catalyst used. The kinetics and mechanism of this addition-polymerization will be the subject matter of a different paper. However, 0.005% of tetraphenyl tin on the lactide in the method described yielded a material of $[\eta] = 1.5$, which is very suitable for casting films and spinning fibers from its solution in dioxane or chloroform.

The optical Rotatory dispersion of the polymer from the L (+) lactic acid shows a large negative trough at 275 m μ in dioxane as well as in methylene chloride, whereas the same trough is observed at 242 m μ in case of the lactide. The specific rotations shown in Table I indicate that there is some unavoidable racemization involved in the preparation of the polymer from the lactide, because there is variation in specific rotation at 578 m μ from sample to sample. It was found by applying the Moffitt equation to the data, that polymers were mainly random coils in solution and that the data presented does not obey Drude equation. (11)

Histological Studies: The gross evaluation of the results of the polylactic acid implants in 18 guinea pigs for 6 weeks showed no inflammatory reaction on the skin, although the powder or the films could be palpated in the implanted areas. The inflammatory response during the first week was very mild in that the reactive zone was limited to only a thin layer of polymorphonuclear leukocytes, occasional lymphocytes and a few eosinophiles. At the end of this period, some edema of the tissue by the early formation of giant cells of the foreign body reaction was seen. At the end of two weeks, the powder enmeshed in the connective tissues (Fig. 1) was seen to elicit marked fibroblastic activity. The gradual ingrowth of the tissue fibers in and around the powder was seen after four weeks with formation of a firm sheet of connective tissues similar to surgical scar tissue, whereas the original birefringency of the polymer faded away. Strikingly, there were no indications of inflammatory reaction from the implants made, thus giving evidence of inertness and tissue receptivity.

Polylactic acid films gave evidence of change in the physical state. From original thin and transparent form, they changed to opaque and swollen state until the end of four weeks. In sequence with the change in the films, there was corresponding change in the pocket wall. Although there was a fine collagen fiber layer formed at the end of two weeks, the inflammatory response was entirely absent (Fig. 2). There was active fibroblastic proliferation at the end of four weeks, along with the appearance of some vascular channels (Fig. 3). At the end of six weeks, the wall of the cavity showed localized renewed proliferation of the fibroblasts. This might be due to the swelling of the film or formation of the roughened surface due to degradative erosion.

The In Vivo Degradation: In the study of the C^{14} tagged polylactic acid implants, no significant radioactivity was recovered in the feces or urine during the three-month period; at sacrifice, no radioactivity was found in any of the vital organs mentioned before. This indicates that the polymer is degraded and eliminated possibly through the CO_2 in the respiration. The total results of the analysis of radioactivity in the chambers and the contents at each sacrifice shows that there is slow degradation of the polymer. The radioactivity of the implants decreases from the original 0.902×10^6 dpm at the end of 3 months, amounting to 12-14% loss of the tagged polymer. This result shows the evidence of biodegradability of (L+) polylactic acid, although slow. When considered in conjunction with the inertness exhibited in the histological studies, this result is understandable.

It is possible that the DL polymer which would be expected to be less crystalline than the L (+) polylactic acid may show a higher rate of biodegradation in vivo. Such studies are underway.

CONCLUSION

The poly L (+) lactic acid of high molecular weight can be spun into fibers, cast into films, and used as a coating composition.

It is found to be non-toxic and non-tissue reactive, degrading slowly when implanted in guinea pigs and rats. The degraded polylactic acid is entirely metabolized possibly through the respiratory system without any accumulation in the vital organs. This shows that the polymer can be a very useful synthetic material for surgical implants.

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TABLE I
PHYSICAL PROPERTIES OF POLYLACTIC ACID

Sample No.	Intrinsic Viscosity in Benzene 100 ml/g	25 at 578 mu		25 at 280 mu		Frequency of Max. in 25.
		Dioxane	Ch ₂ Cl ₂	Dioxane	Ch ₂ Cl ₂	
Lactide	--	-176.7	-283.4	-2453.2	-2753.0	242 mu
22 A	0.50	-153.0	-190.1	- 669.2	- 648.8	275 mu
22 B	1.18	--	-146.7	--	- 660.2	"
22 C	1.05	-144.3	-190.5	- 649.5	- 709.0	"
19	0.95	--	-120.8	--	- 635.3	"
20	0.70	--	-146.2	--	- 626.9	"

The possible Cotton effect in the Rotary Dispersion Curves could not be experimentally obtained due to UV cut off at about 235 mμ under the conditions of the experiments.

TABLE II

In Vivo Degradation of Polylactic Acid Implants
(Results of Scintillation Counting)

Time	DPM	% of Implant Left
2 Hours	1, 048, 960	100. 0
7 Days	938, 884	89. 5
14 Days	961, 288	91. 6
1 Month	985, 036	93. 9
2 Months	878, 068	83. 7
3 Months	902, 408	86. 0



Figure 1

The surrounding polylactic acid powder, showing foreign body type of reaction with marked fibroblastic activity and many phagocytic giant cells. Fourteen days after subcutaneous implantation in guinea pig. Hematoxylin and Eosin (H&E X 35).

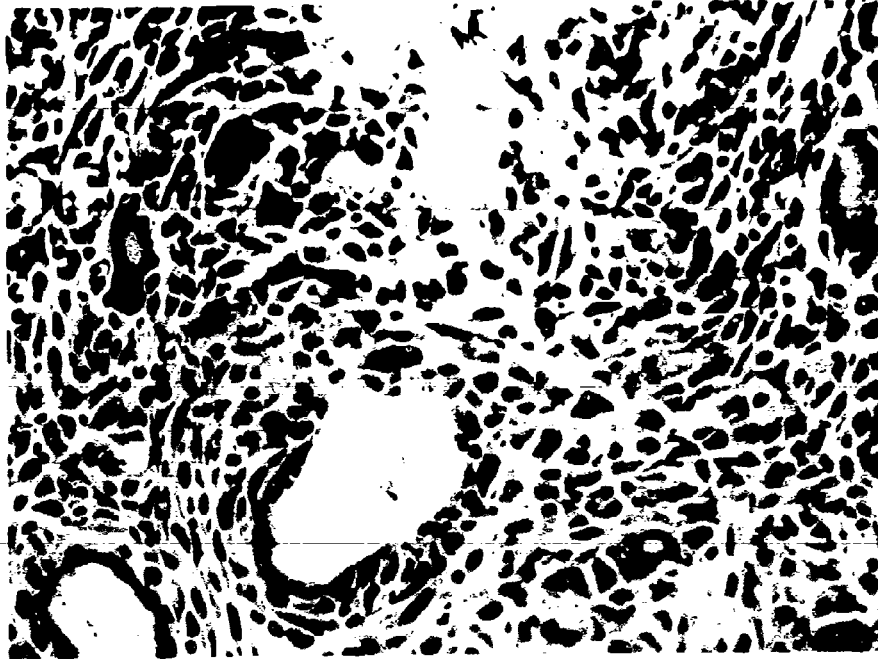


Figure 2

Higher magnification of the area marked in Fig. 1. Note the absence of acute inflammatory response. Occasional plasma cells, eosinophiles, and few lymphocytes are seen. The foreign body type of giant cells are seen around the implanted poly-lactic acid powder. There is considerable fibroblastic activity. (H&E X 210)



Figure 3

Pocket formed around imbedded poly-lactic acid film at two weeks, showing a thin wall of collagen fibers in guinea pig. The film is present at the bottom of the section. (H&E X 32)



Figure 4

Thin fibrous walled pocket formed around the polylactic acid film at the end of 4 weeks in guinea pig. Pronounced vascular channels are present. The film was removed before sectioning. (H&E X 228)

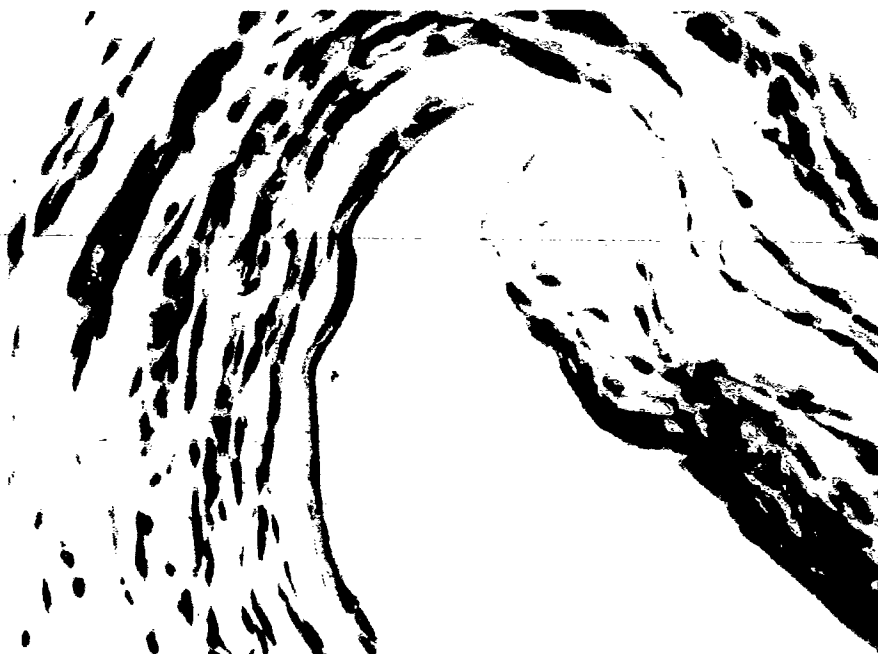


Figure 5

Connective tissue pocket surrounding the polylactic acid film six weeks after imbedding in guinea pig, showing a proliferation of fibroblasts. The film was removed before sectioning. (H&E X 228)

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