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HISTOLOGIC EVALUATION OF A PULYLACTIC ACID CONFLUENT SHEET IN THE TREATMENT OF OSSEOUS DEFECTS

BY

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Acknowledgement: This project was supported, in part, by a grant from the United States Navy, Office of Naval Research, Grant No. N00014-90-J-4087.

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NWW 6/25/92

INTRODUCTION

Polylactic and polyglycolic acids (alpha polyamides) or combinations thereof are biodegradable materials commonly used in suture materials and surgical meshes for the temporary support of abdominal organs. The materials are well known in the clinical literature and have been in common usage without risk for over two decades (1-5). The polyamides are rich in polyester linkages that are degraded in aqueous environments by hydrolysis (5-7). Various investigators have shown that the pure form of polyglycolic acid (PGA) degrades over a period of 5 months whereas pure polylactic acid (PLA) takes about 6.5 months (7, 8). Copolymers of PLA/PGA have been synthesized that degrade within a few weeks to several months depending upon the ratios employed and the specific sequencing of the chemical units (7, 9). Further, the alpha polyamides show no adverse host tissue reactions when implanted in numerous animal models (10-15). As the materials are biodegradable, the rate of which can be controlled by changing the material density, we are suggesting that it may be employed as a matrix for osseous grafting, for the occlusion of large bony defects, for soft tissue contour defects, and also as a bone plating system. All of these applications are based upon the assumption that normal host fibrous and/or osseous tissues will replace the PLA and PGA materials as they undergo biologic degradation. The potential of PLA and PGA or their combination for use in osseous grafting and bone stabilization systems as not been extensively investigated. Indeed, if our assumptions prove correct, pure PLA, PGA or their combination could accomplish all the functions of present day surgical titanium but with the distinct advantage of not requiring a secondary surgery for removal of fixation plates.

Thus, the purpose of this study is to determine the fate of a pure PLA when used as a continuous sheet within osseous defects or when placed on both sides of continuity defects in the manner of a bone plate. Further, the type and rate of host tissue replacement was determined as well as the percent fill of the defect with new bone.

MATERIALS AND METHODS

Animal Model: Twenty-five adult male New Zealand albino rabbits between the ages of 9 and 12 months and weighing approximately 3000 gm were obtained from a licensed commercial vendor through the Laboratory Animal Care (LAC) facility of the University of Missouri-Kansas City. All surgical procedures, post-surgery care, and animal sacrifice were performed in the LAC facility. Animals were caged individually in a standard manner and fed Purina Rabbit Chow plus water *ad libitum.* All animals were allowed one week of acclimation to their new environment before initiating the experimental protocol.

Anesthesia: Upon completion of the acclimation period, animals were prepared for the surgical implantation of the PLA confluent sheets. Food and water were withheld for a twelve hour period prior to surgery. Anesthesia was obtained using the following regimen:

(1) Preanesthetic intramuscular (IM) administration of Atropine using a dosage of 0.06-0.08 mg/kg.

(2) Anesthetic induction using IM administration of Xylazine 5 mg/kg plus Ketamine hydrochloride 30-45 mg/kg.

(3) Anesthetic maintenance was accomplished by using IM Ketamine as required.

Anesthesia was confirmed by loss of reflex when pinched on the abdomen and/or loss of eye-blinking reflex. Subsequent to anesthesia, cranial hair was shaved and removed by vacuum. The resulting surgical field was then swabbed with Betadine surgical scrub solution.

Surgical and Implantation Procedures: Two full-thickness semilunar flaps were raised using sharp incision and blunt dissection. The anterior border of the ears formed the base of each flap. Using a surgical steel trephine dental-implant bur in a slow-speed high-torque dental handpiece, three osseous defects, approximately 4 mm in diameter, were created in the calvaria equidistant from the mid-sagital suture and from each other. Defects penetrated the complete thickness of calvarial bone but did not violate the integrity of the dura mater. A new sterile bur was used for each animal. Further, during osseous penetration, the field was continuously irrigated with sterile saline containing penicillin (100 units/ml), streptomycin (100 μg/ml), gentamicin (50 μg/ml), and Fungizone (2.5 μg/ml).

Complete removal of all residual osseous fragments was insured by irrigation and simultaneous suctioning. This step was particularly important as osseous grindings left in defects may act as autogenous grafts and bias the experimental evaluation.

The implant material used in this investigation consisted of a pure poly (L-lactide) *. One cranial defect in each animal was assigned by random selection and packed with a single 1 mm thick x 4 mm diameter plug of the PLA implant material (Group #1). A second defect, again selected by random assignment, was treated by placement of 1 mm thick x 4 mm diameter confluent sheets of the implant material on either side of the osseous cavity, leaving the defect unfilled (Group #2). This latter procedure required the implant material to be placed in direct contact with the osseous surface, thereby being interposed between bone and dura mater and/or bone and scalp connective tissues. The remaining defect was left untreated and serve as the control (Group #3). Scalp flaps were repositioned and held in place with polyglycolic 4-0 sutures. The scalp overlying implant and control sites was tattooed to aid identification of surgical areas for biopsy at the time of sacrifice.

Post-Surgical Care: As it was reasonable to expect some post-surgical pain or distress, a regimen of 1-5 mg/kg of Diazepam administered IM was used on an "as needed basis". All animals were checked daily for the first 3-4 days post-surgery for changes in appetite, physical activity and general appearance.

Sample Procurement and Analysis: Animals were randomly selected for sacrifice using Pentobarbital, 75-100 mg/kg by IV administration until cessation of respiratory and cardiac

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* Resomer L210. From Boehringer-Ingelheim Corp., Ingelheim AM Rhein, Germany

function was apparent. Five animals were sacrificed at each of the following time intervals: 3, 7, 14, 21, and 35 days.

At the specified time interval, the two implant and control surgical sites were biopsied and tissue placed in labeled bottles containing 10% buffered formalin. After fixation, specimens are demineralized in a solution of EDTA (0.003 M) and HCl acid (1.35 N) and processed for routine hematoxylin and eosin staining

Sections for light microscopic examination were cut at 5-6 microns thickness and at 400 micron intervals throughout the diameter of the implant and/or control sites. Thus, each surgical site yielded a minimum of 8-9 histologic sections for data analysis.

All microscopic sections at each time interval were evaluated for rate of resportion of and host response to the PLA implant material, i.e., presence, intensity and character of any inflammatory infiltrate; presence of foreign body giant cells, macrophages and osteoclastic resorption of host bone. Further, each section was analyzed for surface area of regenerated bone within the implant site as related to total surface area of the surgical defect. Thus, the percentage of new bone could be calculated and a mean value determined for all implant sites, thereby allowing for comparison of the difference between means for implant versus control sites and calculation of statistical significance using a single factor ANOVA with post hoc multiple pairwise comparisons applied using the Newman-Keuls Studentized Range Statistic assuming a significant omnibus F test. All histomorphometric measurements were accomplished with a commerically available computer software package (Java Video Analysis Software, Jandel Scientific, Corte Madera, CA).

RESULTS

All twenty-five animals survived the experimental protocol. Thus, the histologic evaluation and statistical analysis was based on a total of 5 specimens (40-45 histologic sections) from each treatment group at each time interval.

Histology: At 3 days post-surgery the nontreated control defects (Group #3) exhibited a moderate degree of inflammatory cell infiltrate consisting of neutrophils, lymphocytes and a relatively few macrophages. By 7 days, the inflammatory cell population was a minor feature, having all but disappeared. Initially the intrabony defect was filled with a well formed fibrin clot with evidence of an early capillary proliferation from adjacent bone marrow spaces. Organization of the clot continued at 7 days with both capillary and fibroblastic proliferation originating from adjacent marrow spaces and periosteal and dura mater surfaces. The bony surfaces comprising the proximal walls of the defects exhibited isolated areas of osteoclastic mediated resorption that were still obvious in the 7 day specimens. One or two residual bony spicules were noted within the defect in most sections.

The bony defects of 14 day post-surgery control specimens were filled with a dense fibrous connective tissue whose individual fibers exhibited a parallel orientation to one another and to the bony surfaces of the cranium. Interspersed within the connective tissue were randomly distributed areas of active intramembranous bone formation appearing to be trabecular in nature. In contrast, to 3 and 7 day specimens, there was no evidence of osteoclastic activity and the new bone formation made it difficult to identify the walls of the original defect.

By 21 and certainly at 35 days post-surgery, the control defects were nearly filled with new trabecular bone and exhibited a well organized endosteum. Further, the periosteum appeared completely regenerated and represented a confluent and intact layer covering the original defect.

The specimens in which the PLA implant material was positioned over the superior and inferior bony surfaces of the defect, thereby isolating the defect space from surrounding connective tissues (Group #2), exhibited no apparent differences when compared to controls at the corresponding 3 and 7 days post-surgery time intervals. However, specimens from 14, 21, and 35 days post-surgery appeared to lag behind controls with respect to the density of newly forming trabecular bone. Further, the periosteum regenerated as a confluent layer of fibrous connective tissue covering the superior aspect of the implant material positioned on the cranial surface. In all other respects, Group #2 and Group #3 specimens from the longer time intervals appeared very similar.

Regardless of time interval, those intrabony defects in which a plug of the PLA implant material had been placed (Group #1) exhibited only a slight inflammatory reaction which appeared localized to peripheral tissue areas. As the implant material occupied the entire defect, there was little opportunity for capillary or fibroblastic proliferation into the defect space. Further, there was no evidence of a foreign body or giant cell reaction. Osteoclastic resorption of the intrabony defect walls was less active than that seen in controls specimens at corresponding time intervals.

The 14, 21, and 35 day specimens all featured an intact layer of periosteum covering the cranial surface of the implant plug. None of the specimens from the longer time intervals showed evidence of a foreign body reaction or resorption of the implant plug or adjacent bone.

Table 1 presents the results of the computerized vidio analysis of the surface area of newly formed bone within the defect as a percentage of the total surface area of the defect space. The mean percentage and standard divaiations were derived from measurements of 40-45 microscopic sections from each experimental group at each time interval.

In 12 of 45 microscopic sections from the 7 days post-surgery specimens there were bony spicules evident in Groups #2 and #3 which accounted for a mean percentage of bone formation of 0.50% ± 0.41 and 0.70% ± 0.62 respectively. However, the spicules did not exhibit histologic features characteristic of newly forming bone but rather appeared to be residual from the surgery. Consequently, a statistical evaluation of these specimens was not performed.

Analysis of the data revealed a statistically significant increase ($p = < .001$) in mean percentages of bone regeneration at

14 days post-surgery in Group #2 (29.00% ± 8.50) and Group #3 (34.10% ± 12.96) when compared to Group #1 (0%). The difference in bony regeneration in Groups #2 and #3 was also significant ($p = < .05$). These same comparisons were also statistically significant at the 21 days post-surgery interval ($p = < .001$) when comparing Group #2 (78.30% ± 11.36) and Group #3 (84.20% ± 8.95) to Group #1 (0.13% ± 0.08). A comparison of the difference between the mean percentage bone increase in the control Group #3 versus that of Group #2 revealed a statistical significance at the $p = < .01$ level.

Analysis of the 35 days post-surgery data revealed statistically significant differences in the mean percentage increase of bone ($p = \leq .001$) in Group #2 (89.60% ± 10.66) and Group #3 (94.30 ± 8.32) when each was compared to Group #1 (0.44% ± 0.23). The increase in mean percentage of bone in Group #3 when compared to that of Group #2 was significant at the $p = < .05$ level.

CONCLUSIONS

When compared to control defects (Group #3), the placement of confluent sheets of polylactic acid in close apposition to the superior and inferior bony surfaces of cranial continuity defects (Group #2) resulted in delayed healing responses at 14, 21 & 35 days post-surgery. Based on a comparison of the mean percent surface area of regenerated bone, the delay in healing approximated one week. Given the advantages of avoiding a secondary surgery to remove surgical bone plates, a one week delay in healing becomes clinically insignificant.

When compared to control defects (Group #3), the placement of polylactic acid plugs within the bony defect (Group #1) severely retarded the healing response at all time intervals. Based on a comparison of the mean percent surface area of regenerated bone, the delay in healing approximated 7-8 weeks. However, as many of the plugs showed little evidence of resorption by day 35 (termination point of study), the delay in healing response is likely to be considerably longer. Thus, it would appear that planned or inadvertent inclusion of polylactic acid materials within bony defects should be avoided.

Table 1. Mean Percent Surface Area of Regenerated Bone

	Defects Filled With Implant Plug (Group #1)		Implant Material Positioned Over Superior & Inferior Surfaces of Defect (Group #2)		Controls (Group #3)	
	Mean %	S.D.	Mean %	S.D.	Mean %	S.D.
3 Days	0		0		0	
7 Days	0		0.50	± 0.41	0.70	± 0.62
14 Days	0		29.00	± 8.50	34.10	± 12.96
21 Days	0.13	± 0.08	78.30	± 11.36	84.20	± 8.95
35 Days	0.44	± 0.23	89.60	± 10.66	94.30	± 8.32

Statistical Significance:

Group #1 vs. Group #2: $p = < .001$ at 14, 21, and 35 days.
Group #1 vs. Group #3: $p = < .001$ at 14, 21, and 35 days.
Group #2 vs. Group #3: $p = < .01$ at 21 days; and
$p = < .05$ at 14 and 35 days.

REFERENCES

1. Bostman O, Makela EA, Tormala P, et al.: Transphyseal fracture fixation using biodegradable pins. J Bone Jt Surg (Br), 71B:706, 1989.

2. Hirvensal E: Fracture fixation with biodegradable rods. 41 cases of severe ankle fractures. Acta Orthop Scnada, 60:601, 1989.

3. Bostman O, Hirvensalo E, Vainionpaa S, et al.: Degradable polyglycolide rods for the internal fixation of displaced bimalleolar fractures. Int Orthop, 14:1, 1990.

4. Cutright DE, Hunsck EE: Tissue reaction to biodegradable polylactide acid suture. Oral Surg, 31:134, 1971.

5. Kulkurni RK, Pani KC, Neuman C, et al.: Polylactic acid for surgical implants. Arch Surg, 93:839, 1966.

6. Parsons JR: Restorable materials and composites. New concepts in orthopedic biomaterials. Orthopedics, 8:907, 1985.

7. Hollinger JO, Battistone GC: Biodegradable bone repair materials: Polymers and ceramics. Clin Orthop, 207:290, 1986.

8. Miller RA, Brady JM, Cutright DE: Degradation rates of resorbable implants PLA/PGA. J Biomed Mater Res, 11:711, 1977.

9. Olson RJ, Roberts DL, Osborn DB: A comparative study of polylactic acid, gel foam in surgical healing of extraction sites. Oral Surg, 53:276, 1982.

10. Makela EA, Bainionpaa S, Vihtonek K, et al.: The effect of a penetrating biodegradable implant on the epiphyseal plate: An experimental study on growing rabbits with special regard to polyglacin 910. J Pediatr Orthop, 7:415, 1987.

11. Bos RRM, Rozema FR, Boering G, et al.: Bone plate and screws of bioabsorbable poly-L-lactide. An animal pilot study. J Oral Maxillofac Surg (Br), 27:467, 1989.

12. Rozema FR, Bos RRM, Pennings A, et al.: Poly-L-lactide implants in repair of defects of the orbital floor. An animal study. J Oral Maxillofac Surg, 48:1305, 1990.

13. Bos RRM, Rozema FR, Boering G, et al.: Bio-absorbable plates and screws for the fixation of mandible fractures. A study in six dogs. Internat J Oral Maxillofac Surg, 18:365, 1989.

14. Suuronen R: Comparison of absorbable self-reinforced poly-L-lactide screws and metallic screws in the fixation of mandibular condyle osteotomies. J Oral Maxillofac Surg, 49:989, 1991.

15. Hollinger J, Schmitz JP: Restoration of bone discontinunities in dogs using biodegradable implants. J Oral Maxillofac Surg, 45:594, 1987.