Histopathological Observations of a Polylactic Acid-Based Device Intended for Guided Bone/Tissue Regeneration

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ABSTRACT

Background: Barrier devices have been shown to support alveolar bone and periodontal regeneration, a procedure also known as guided bone/tissue regeneration (GBR/GTR). Popular demand and clinical convenience have raised an interest in bioresorbable barrier devices. Tissue reactions to such bioresorbable devices are, however, generally not well explored.

Purpose: The objective of this study was to evaluate short- and long-term tissue reactions following implantation of a bioresorbable polylactic acid (PLA)-based barrier device using a rat model.

Materials and Methods: Twenty-one young adult male Sprague-Dawley rats were used. The animals were divided into three groups including 15 animals receiving the PLA device and animals serving as sham surgery (five) or nonoperated (one) controls. Using aseptic techniques, the PLA device was surgically implanted in direct contact with the calvarial bone. Animals receiving the PLA device were sacrificed at 3, 5, 7, and 12 months postsurgery to provide longitudinal histopathological observations of tissue and biomaterials reactions. Control animals were sacrificed at 3 months.

Results: Animals were maintained without adverse events. Sham surgery and nonoperated control animals showed no signs of new bone formation or resorption, or signs of inflammatory reactions in adjoining soft tissues. In contrast, extensive amounts of residual biomaterial with evidence of foreign body reactions and bone resorption were observed in animals receiving the PLA device over 12 months.

Conclusions: The results suggest that the PLA device may induce bone resorbing foreign body reactions. Importantly, the PLA device does not resorb within a 12-month healing interval. These biomaterials properties may influence new bone formation and maintenance when applying the device for GBR/GTR.

KEY WORDS: bioresorbable, bone, GBR, GTR, guided bone regeneration, guided tissue regeneration, membranes, polylactic acid, rat, tissue engineering, wound healing

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The terminology guided bone/tissue regeneration (GBR/GTR) represents a tissue-engineering principle that utilizes a surgically implanted barrier device to insulate a defined wound bed to facilitate/promote migration and proliferation of desirable cell populations from adjoining tissue margins including alveolar bone and the periodontal ligament.[1–3] Preclinical histologic studies have demonstrated significant osteogenic bone formation and periodontal regeneration in defects treated in this fashion.[3–5] Clinical studies have shown relevant resolution of alveolar and periodontal defects.[6–8]

Nonresorbable and bioresorbable devices have been designed and manufactured to meet specific criteria considered important to support GBR/GTR. These

criteria include biocompatibility, cell occlusivity, space maintenance, tissue integration, and ease of use.[9] A large body of evidence is available evaluating periodontal regeneration utilizing a variety of barrier devices.[10,11] Only few studies have concerned biologic processes immediately associated with the biomaterial used, including processes associated with resorption of bioresorbable technologies.[12]

Pins, plates, rods, bolts, and screws made of polyglycolic acid or polylactic acid (PLA) used for orthopedic indications have been reported to cause adverse tissue reactions including osteolytic processes detected as late as 5 years postimplantation.[13–16] For dental applications, preclinical studies have reported formidable accumulations of multinucleated giant cells and associated resorption of newly formed and resident bone when biomaterials based on PLA technologies have been implanted into periodontal defects.[17,18] Clinicopathologic reports have described the formation of sterile abscesses following GTR treatment of gingival recession defects and following GBR utilizing PLA-based barrier devices.[19,20] The histological appearance of these human lesions was characterized as a foreign body reaction including accumulations of foamy macrophages. Over 50% of the patients examined presented with symptoms and clinical signs associated with bioresorption of the PLA-based GTR device.[19]

It is evident that effective preclinical screening must be established to evaluate biomaterials intended for use in the craniofacial skeleton to ascertain their biologic behavior and efficacy prior to clinical release. Adverse tissue reactions such as those reported earlier may not only compromise new bone formation but also the maintenance of newly formed and resident bone in the site where the biomaterial is implanted. The specific objective of this study was to evaluate local tissue reactions following implantation of a bioresorbable PLA-based barrier device intended for GTR/GBR using a rat calvarial bone model.

MATERIALS AND METHODS

Animals

Twenty-one young adult male Sprague-Dawley rats, weight approximately 175 g, were used. The animals were individually housed in plastic cages in a monitored environment (21°C; 12:12 light cycle). They had ad libitum access to drinking water and a standard laboratory rat food pellet diet. All experimental procedures were performed following a protocol approved by the local Institutional Animal Care and Use Committee.

Experimental Protocol

The animals were divided into three groups including 15 animals receiving the PLA device (ATRISORB® FreeFlow™ GTR Barrier, Atrix Laboratories, Inc., Fort Collins, CO, USA) following the manufacturer's instructions, and animals serving as sham surgery (five) or nonoperated (one) controls. Animals receiving the PLA device were sacrificed at 3, 5, 7, and 12 months postsurgery to provide longitudinal observations of healing. Control animals were sacrificed at 3 months (Table 1).

Surgery Procedures

The animals were anesthetized using isoflurane inhalation anesthesia (E-Z Anesthesia, Euthanex Corp., Palmer, PA, USA; 4–5% induction; 2–3% maintenance). Buprenorphine HCl, 0.02 to 0.03 mg/kg, was administered for pain control. The animal's head was shaved, washed with disinfectant, and stabilized by a nose cone apparatus (Euthanex Corp.). An incision was made from the nasofrontal area to the external occipital

TABLE 1 Distribution of Treatments Among Animals and Healing Intervals

Treatment	Healing Interval (months)	Number of Animals
PLA device	3	5
PLA device	5	4
PLA device	7	3
PLA device	12	3
Sham surgery control	3	5
Nonoperated control	—	1

PLA = polylactic acid.

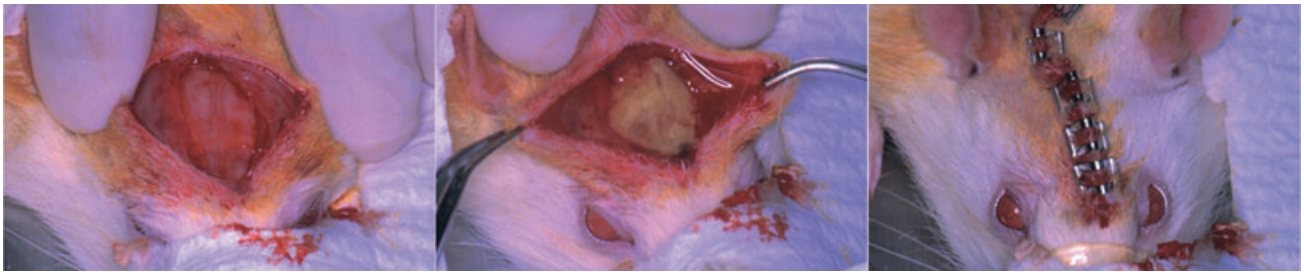

Figure 1 Calvarial bone site before (*left*) and after (*center*) implantation of the polylactic acid device, and wound closure using autoclips.

protuberance along the mid-sagittal suture. The skin and underlying tissues including the temporal muscle were reflected bilaterally to expose the calvarial bone (Figure 1). The PLA device was applied in direct contact with bone. The size of the device was standardized using a surgical template. The skin and underlying tissues were then readapted to cover the exposed calvaria and PLA device. The wound margins were closed using autoclips (Autoclip® Wound Closing System, Stoelting Co., Wood Dale, IL, USA). A bacitracin–neomycin–polymyxin antibiotic ointment (Vetropolycin® Ophthalmic Ointment, Pharmaderm, Melville, NY, USA) was applied to the animal's eyes. The animals were maintained on a warming matt throughout the procedure and until anesthesia recovery when they were returned to their cages. Sham surgery control animals received the same surgical procedure with the exception for implantation of the PLA device.

Postsurgery Procedures

The animals received buprenorphine HCl (0.02 to 0.03 mg/kg) 8 to 12 hours postsurgery to alleviate any discomfort. They were monitored daily until the date of euthanasia for signs of infection or deterioration. Animals were sacrificed using CO_2 inhalation. The cranial bone was removed in total, rinsed in water, and placed into 10% buffered formalin in separate labeled vials for the histological processing.

Histological Procedures and Analysis

The calvarial block specimens were demineralized in ethylenediaminetetraacetic acid, dehydrated with ascending concentrations of ethyl alcohol, cleared in xylene, and infiltrated with paraffin. Transverse 7 μm serial sections were cut from the center of each PLA device using a microtome (RM2155, Leica Microsystems GmbH, Nussloch, Germany). Sections mounted on glass were stained with hematoxylin and eosin.

An experienced oral pathologist (GAP), masked to the specific experimental procedures, evaluated the tissue samples using a light microscope (CX-40, Olympus America, Inc., Melville, NY, USA) equipped with 4×, 10×, and 40× objectives. Presence of residual biomaterials, associated inflammatory reactions, and associated bone and soft tissue reactions were evaluated using five central step-serial sections from each biopsy block.

RESULTS

Clinical Observations

All animals tolerated the surgical procedures without clinical signs of adverse reactions.

Histopathological Observations

Sham Surgery and Nonoperated Controls. The calvarial bone between the auricular areas included an external and internal cortical plate separated by marrow spaces (Figure 2). The calvarial bone was divided in two segments by the mid-sagittal suture. There were no notable differences between sham surgery and nonoperated controls. These animals showed no signs of new bone formation or resorption. The adjoining soft tissues did not exhibit signs of inflammatory reactions.

PLA Devices at 3 Months. The 3-month specimens exhibited a substantial amount of residual biomaterial (data not shown). The PLA device was surrounded in part by a fibrous capsule that included periosteum with occasional foreign-body-type multinucleated giant cells, some macrophages, and lymphocytes. The device displayed limited apparent effect on the adjoining calvarial bone. The cortical bone facing the biomaterial showed occasional scalloped areas indicative of resorption. Lateral to the biomaterial, there was evidence of new bone formation, possibly because of a tenting effect of the flap allowing for new bone formation.

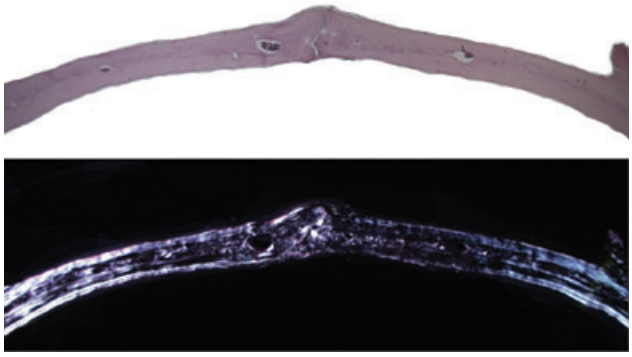

Figure 2 Photomicrograph of the calvarial bone between the auricular areas represented by an external and internal cortical plate separated by a marrow space in animals receiving sham surgery, 3 months postsurgery. The mid-sagittal suture divides the calvarial bone into two segments. No signs of new or recent bone formation or resorption are evident (hematoxylin and eosin). The lower photomicrograph shows the section using polarized light microscopy. There were no appreciable differences between sham surgery and nonoperated controls.

PLA Devices at 5 Months. There was no apparent change in volume and tissue invasion of the PLA implant at the 5-month compared to the 3-month observation interval (Figure 3). The 5-month specimens exhibited evidence of foreign body reactions. The fibrous capsule surrounding the PLA device appeared thicker as compared to the 3-month observations. Fibrous "septae" were observed penetrating or traversing the PLA device. Pronounced resorption of the external calvaria plate represented a remarkable observation for this healing interval. This was particularly evident using polarized light microscopy. Lateral to the biomaterial, more extensive bone formation was observed compared to the 3-month observations.

PLA Devices at 7 Months. The bulk of the PLA device still appeared intact at 7 months postsurgery (Figure 4). The biopsy specimens exhibited evidence of foreign body reactions, including features not significantly different from that observed at previous observation intervals. Two of three animals showed extensive bone metabolic activity involving the external calvarial plate extending into the PLA device. Dystrophic calcification was noted in areas around the periphery of the biomaterial and at some fibrous septae traversing the device. Irregular bone projections protruding from the cortical plate into the PLA device were observed. This bone, in turn, included calcified globules and areas of dense collagen deposition resembling amorphous osteoid, some of which also calcified. This observation provided a sense of progressive "ossification" within the biomaterial, that is, dense collagen deposits and dystrophic calcification leading to calcification and eventual remodeling to resemble bone. However, osteoblasts and osteoclasts were notably inconspicuous in this process; the ossification did not resemble that of newly forming bone as occurs in the jaws. The ossification within the PLA device beginning largely at the periphery of the device appeared intimately associated with the foreign body reactions; calcifications occurring at random and the small globular nonfunctional bone-like material. Notably, the ossification was localized; whereas some aspects of the PLA device were associated with extensive bone metabolic activity, others appeared quiescent.

PLA Devices at 12 Months. The 12-month specimens still exhibited residual biomaterial including lingering foreign body reactions (Figure 5). The PLA device appeared surrounded in part by a fibrous capsule that included periosteum with occasional foreign-body-type multinucleated giant cells, some macrophages, and

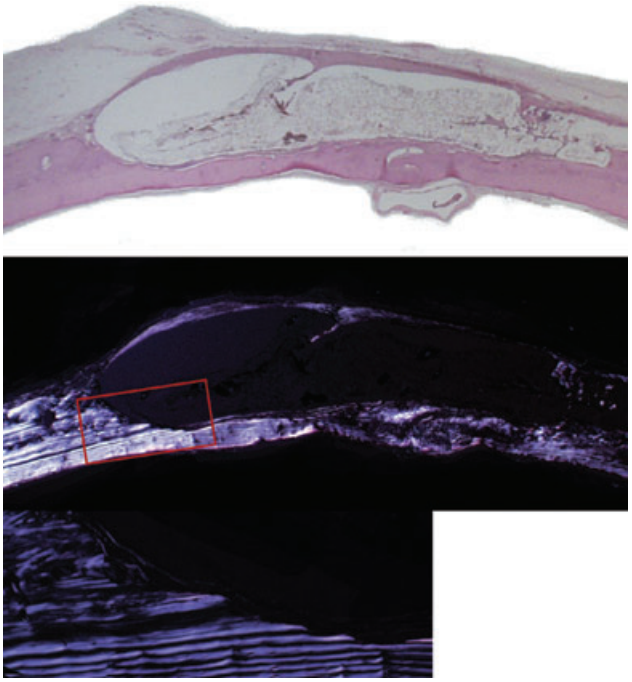

Figure 3 Photomicrograph of the calvarial bone at 5 months following implantation of the polylactic acid biomaterial. A substantial amount of biomaterial appears encapsulated in connective tissue. There is a limited inflammatory reaction located to tissue strands invading the biomaterial. Resorption of the calvarial plates can be observed (hematoxylin and eosin). This is particularly evident using polarized light microscopy (lower photomicrographs). Insert shown in high magnification.

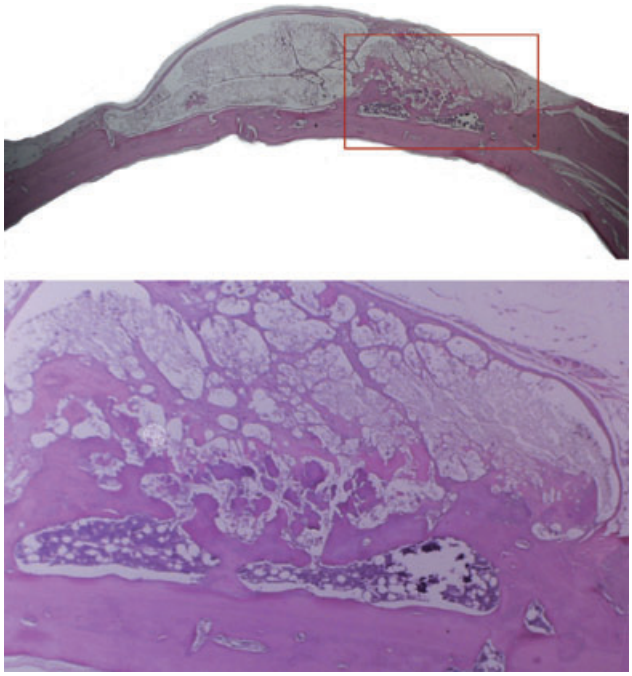

Figure 4 Photomicrograph of the calvarial bone at 7 months following implantation of the polylactic acid (PLA) biomaterial. The bulk of the PLA biomaterial appears intact. Aggressive resorption of the external calvarial plate extending into medullary space is present. Connective tissue invading the biomaterial does not exhibit signs of extensive inflammatory reactions. Nonetheless, the inflammatory response appears more intense including multinucleated cells in areas of calvarial bone resorption. Notably, the resorptive activity is localized, whereas some aspects of the PLA implant are associated with extensive resorption, other areas appear quiescent. The biomaterial does not appear encapsulated in areas of calvarial bone resorption (hematoxylin and eosin).

lymphocytes. Remarkably, the fibrous capsule appeared thicker at 12 months as compared to earlier observation intervals.

DISCUSSION

The objective of this study was to evaluate short- and long-term tissue and biomaterials reactions following implantation of a PLA-based barrier device for GBR/GTR in a rat calvarial model. Young adult male Sprague-Dawley rats were surgically implanted with the device, or served as sham surgery or nonoperated controls. Animals receiving the PLA device were sacrificed at 3, 5, 7, and 12 months postsurgery to provide longitudinal observations of tissue and biomaterials reactions. Control animals, sacrificed at 3 months, showed no signs of new bone formation or resorption, or signs of inflammatory reactions in the adjoining tissues. In contrast, extensive amounts of residual biomaterial with evidence of foreign body reactions and bone resorption were observed for animals receiving the PLA device over the 12-month healing interval. These biomaterials properties may have a detrimental influence on bone formation and maintenance when using the PLA device for GBR/GTR.

In this study, the greater part of the PLA device remained intact over the 12-month healing interval. Even though signs of bioresorption were present, they were confined to the periphery of the device. Furthermore, the resorption process involved only limited aspects of the device. Thus, it remains to be established when complete resorption of this biomaterial may be accomplished using this or similar discriminating animal models. A concern for a biomaterial intended for GBR/GTR lingering in the tissues is evident from the observations presented by Tatakis and Trombelli[19] showing PLA biomaterials-associated sterile abscess formation in patients over 12 months postsurgery, reactions associated with accumulation of foamy macrophages, and osteolytic processes.[13–18] This is of particular concern because observations from experimental models suggest that regeneration of large alveolar and periodontal defects may be accomplished within 2 months postsurgery.[21,22]

One critical factor for the outcome of GBR/GTR is space provision. Several reports have observed a direct positive relationship between space provision and the

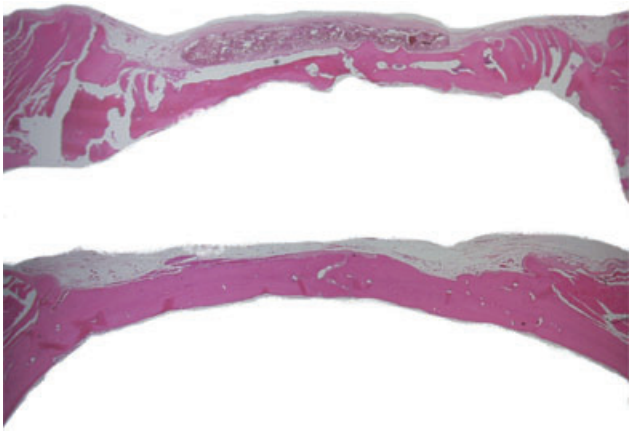

Figure 5 Photomicrograph of the calvarial bone at 12 months following implantation of the polylactic acid (PLA) biomaterial. The PLA implant exhibits a dense inflammatory infiltrate including clusters of multinucleated cells. The external calvarial cortical plate is resorbed, in part. The fibrous capsule appears thicker at 12 months compared to earlier observation intervals. The lower photomicrograph shows a site adjacent to the PLA implant. Observe the intact calvarial bone without inflammatory reactions or resorption (hematoxylin and eosin).

amount of newly formed bone in several experimental settings.[23–25] Thus, an ideal biomaterial intended for GBR/GTR should maintain functionality (space provision) for a defined period of time without interfering with the development of the regenerate to resorb shortly thereafter without detrimentally influencing wound maturation. Although the PLA device was not evaluated for GBR/GTR in the present study, it is lacking structural integrity to provide adequate space provision for regeneration of alveolar bone and periodontal tissues.

The safety and effectiveness of a biodegradable GBR/GTR device may not only be regulated by its chemical and physical components but also by its degradation/end products. These must not negatively influence the net effect of the regenerative process.[12] In this study, evidence of dystrophic calcification was present at the periphery of the biomaterial at the 7- and 12-month healing interval. Irregular bone projections protruding from the cortical plate into the PLA device were observed. This bone included calcified globules and areas of dense collagen deposition resembling amorphous osteoid, some of which calcified. However, osteoblasts and osteoclasts were notably inconspicuous; the ossification did not resemble that of newly forming reactive bone. It is unknown how these tissue reactions may influence a regenerative protocol.

Evidence of foreign body reactions was noted at all healing intervals. Multinucleated giant cells, macrophages, and lymphocytes could be observed in the different specimens at different observation periods. Moreover, resorption of the resident calvarial bone was persistently present in the proximity of the inflammatory lesions. It may be speculated that degradation products of the PLA device may adversely affect a regenerative process as well as newly formed bone much like it affected the resident calvarial bone in this experimental model. Moreover, it remains unknown whether continued and potentially accelerated bioresorption of the PLA device might still advance bone resorption.

In summary, this study points to biomaterials and associated tissue reactions that may negatively influence regenerative processes. Devices based on biomaterials associated with such untoward reactions may not unreserved be used for regenerative procedures including periodontal tissues and alveolar bone.

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