Cellular Responses of Bioabsorbable Polymeric Material and Guglielmi Detachable Coil in Experimental Aneurysms

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Background and Purpose—Acceleration of healing mechanisms is a promising approach to improve current limitations of endovascular aneurysm therapy with the use of platinum coils. We evaluated a new endovascular therapeutic, bioabsorbable polymeric material (BPM), which may promote cellular reaction in the aneurysms.

Methods—Four different concentrations of lactide/glycolic acid copolymer [poly(D,L-lactic-co-glycolic acid)] (PLGA), 85/15, 75/25, 65/35, and 50/50, were used as BPMs. Sixteen experimental aneurysms were created in 8 swine. Eight-millimeter-long spiral-shaped BPMs were surgically implanted in the aneurysms without tight packing (n=3 for each BPM). Guglielmi detachable coils (GDCs) were used as control (n=4). The animals were killed 14 days after embolization, and angiographic, histological, and immunohistochemical analyses were performed.

Results—Despite loose packing of aneurysms with BPMs, faster BPMs such as 50/50 or 65/35 PLGA demonstrated more mature collagen formation and fibrosis in the sac and neck of the aneurysm. One aneurysm treated with 65/35 PLGA, 1 treated with 75/25 PLGA, and all 3 treated with 85/15 PLGA showed a neck remnant on angiography. There was a linear relationship between collagen levels and polymer degradation properties (r=-0.9513).

Conclusions—This preliminary animal study indicates that acceleration of aneurysm healing with the use of BPM is feasible. This concept can be applied to decrease and perhaps prevent aneurysmal recanalization after endovascular treatment of cerebral aneurysms. (Stroke. 2002;33:1120-1128.)

Key Words: animal models • endovascular therapy • intracranial aneurysm • polymers • tissue engineering

Current therapeutic management of cerebral aneurysms includes endovascular embolization as an alternative to standard surgical clipping. The Guglielmi detachable coil has become the gold standard for the endovascular occlusion of ruptured or unruptured intracranial aneurysms worldwide.1–9 The most important limitation of this system is the possibility of aneurysm recanalization, particularly in wide-necked or large/giant aneurysms.2, 7 Platinum GDCs produce a mild biological response when delivered into an aneurysm. Experimental and clinical reports of aneurysms embolized with GDCs have demonstrated a limited cellular reaction induced by platinum coils.10–24 The presence of an unorganized clot within the aneurysm does not provide strong anatomic support for GDCs in the early phases of treatment. Controlled biological cellular response to the presence of intra-aneurysmal embolic material is a promising method that may improve acute and long-term morphological outcomes in aneurysms embolized with this type of embolic materials. Several investigators have studied the concepts of utilization of “bioactive” embolic materials.18, 19, 25–32 However, no proven bioactive devices have been used in clinical practice for the treatment of cerebral aneurysms.

Bioabsorbable polymeric materials (BPMs) have been used as biocompatible agents such as sutures, implants, and recently as drug delivery vehicles or scaffolds for tissue engineering.33–40 BPMs can stimulate cells to regenerate tissues and act as scaffolds for cell transplantation both in vitro and in vivo. This cellular reaction is necessary to promote scar formation in the aneurysm and can be controlled by the composition of the polymers. Lactide/glycolic acid copolymer [poly(β-lactic-co-glycolic acid)] (PLGA) is widely used in the field of tissue engineering and can be used as a potential embolic material. We evaluated 4 different ratios of commercially available PLGA in this study. They included 85/15 PLGA, 75/25 PLGA, 65/35 PLGA, and 50/50 PLGA. In general, a faster degradable BPM elicits a stronger cellular reaction.

It has been demonstrated that 50/50 PLGA has the fastest degradation time, and 85/15 PLGA shows the slowest bioabsorption time.41 Brain aneurysms can be considered defects of the normal arterial wall, and tissue-engineering technology could be used to repair them.

We hypothesized that BPM can be used as a bioactive, bioabsorbable embolic material for the treatment of cerebral aneurysms.
aneurysms. Endovascular embolic implants made from BPM can be used as temporary scaffolds for tissue regeneration that may induce a complete and permanent occlusion of an aneurysm with mature connective tissue. Recently, we developed a prototype of BPM/platinum hybrid coil as a new generation of embolic coils.42

In this study we evaluated the treatment feasibility of BPM in experimental aneurysms with different strength of BMs and analyzed anatomic, angiographic, histological, and immunohistochemical findings.

Materials and Methods
Eight-millimeter-long, 5-mm-diameter, spiral-shaped BMs (85/15, 75/25, 65/35, and 50/50 PLGA) ribbons were molded from polymer and surgically implanted in experimental aneurysms created in swine (Figure 1). No radiopaque materials such as platinum or tantalum were added to the BPM ribbons in this study. Standard GDCs were used as control to evaluate qualitative and quantitative differences in intra-aneurysmal inflammatory responses. Aneurysms and parent arteries were histologically evaluated with particular attention to the cellular organization of the clot, development of collagen and fibrosis, and neointimal proliferation. Untoward stenosis or occlusion in the parent artery was also evaluated.

Creation of Lateral Aneurysm Model
All animal experiments were conducted in accordance with policies set by the University of California at Los Angeles Chancellor’s Animal Research Committee and National Institutes of Health guidelines. Eight Yorkshire swine were used in this study. The animals were 3 to 4 months old, weighed 30 to 40 kg, were of mixed sex, and were maintained on a standard laboratory diet. After an overnight fast, each swine was premedicated with intramuscular injection of 20 mg/kg of ketamine and 2 mg/kg of xylazine. General anesthesia was maintained with mechanical ventilation and inhalation of 0.5% to 1.5% halothane after endotracheal intubation.

Experimental lateral aneurysms were constructed microsurgically in both common carotid arteries. Details of aneurysm creation were

![Figure 1. Appearance of BPM ribbon (5 mm in diameter and 8 mm in length).](image1)

Figure 2. A, Angiogram of experimental aneurysm created on the common carotid artery of swine (preembolization of BPM ribbon). Note vascular clip placement on the distal end of venous graft (aneurysm dome). B, Immediately after embolization of the aneurysm with 50/50 PLGA (faster-degradation BPM). Note contrast filling of the aneurysm dome. C, Immediately after embolization of the aneurysm with 85/15 PLGA (slower-degradation BPM). Note contrast filling of the aneurysm dome. D, Fourteen days after embolization of 50/50 PLGA, showing complete occlusion of the aneurysm with parent artery stenosis. Arrowheads indicate location of the embolized aneurysm. E, Fourteen days after embolization of 85/15 PLGA, showing remnant of the aneurysm (arrow) without parent artery stenosis.
described previously. On the BPM side, a temporary clip was placed at the end of the venous graft. This temporary clip was not removed until diagnostic angiography was performed. When BPM was surgically implanted into the aneurysm, 2 vascular clamps were used temporarily. The removal of clamps from the parent artery resulted in filling of the aneurysm and flow resumption in the common carotid artery. The swine received 0.9 to 1.2 units of penicillin G intramuscularly. Via the transfemoral route, a 6F catheter was positioned in the common carotid artery, and 6 mL of nonionic contrast medium was injected to evaluate size and shape of the created aneurysms (Figure 2A).

Emboliolation of Experimental Aneurysms With Polymer Coils and GDCs

Overall, 16 experimental aneurysms were constructed in 8 swine. Twelve aneurysms were embolized with 4 different BPM ribbons (n=3 each), and 4 aneurysms were embolized with standard GDCs. A bolus of 3000 U of heparin was injected intra-arterially to prevent thrombosis during the procedure.

Surgical Embolization With BPM

After diagnostic angiography was performed, the 2 small vascular clamps were placed again at each end of the isolated common carotid artery segment, and the temporary vascular clamp that had been placed at the end of the venous graft was removed to insert BPM ribbon. The aneurysm (venous graft) and isolated common carotid artery segment were then irrigated with saline solution. Subsequently, an 8-mm-long, 5-mm-diameter BPM ribbon was placed in the aneurysm from its opened end. After BPM ribbon placement, the open end of the aneurysm was closed with 3-0 silk suture ligation, and postembolization angiogram was then performed in all cases.

Embolization With GDCs

The details of GDC embolization were described previously.

Follow-Up Angiography

Follow-up angiography was performed 14 days after embolization. With the use of standard approved procedures, the animals were euthanized after the follow-up angiography was obtained.

Histopathological Evaluation

The harvested aneurysms were fixed with 10% formaldehyde. The aneurysms embolized with GDCs were slowly infiltrated with, and embedded in, methylmethacrylate. Methylmethacrylate blocks were cut with a diamond band saw, and transverse sections were taken through the midline of the neck of the aneurysm. Sections were ground to a thickness of approximately 50 μm, polished, and surface-stained with hematoxylin and eosin. The aneurysms embolized with BPM were embedded in paraffin, serially sectioned twice at 5 μm, and stained with hematoxylin and eosin and Masson trichrome. The images of the histopathological specimens of aneurysms were digitized with the use of a high-resolution scanner (1000 dots per inch). With the use of low-magnification views, the areas of intra-aneurysmal unorganized thrombus were calculated with the aid of the program NIH Image 1.60 (Freeware; http://rsb.info.nih.gov/nih-image) to evaluate the difference of tissue organization between the GDC and BPM groups. The degrees of inflammation and fibrous tissue infiltration were graded as follows: grade 1, minimal; grade 2, mild; grade 3, moderate; grade 4, marked; and grade 5, severe.

Immunohistochemistry

Immunohistochemical staining was performed to evaluate cellular infiltration and proliferation and collagen deposits in BPM groups. The specimens embolized with GDC were not evaluated with immunohistochemistry. Localization of type I collagen was performed with an anti-bovine type I collagen polyclonal antibody raised in guinea pigs. The antibody has been shown to react with bovine, human, rat, and porcine species on Western blots and immunocytochemistry. We used biotinylated secondary antibody and detection with avidin–fluorescein isothiocyanate following the manufacturer’s recommendations (Vectastain Elite Peroxidase Kit, Vector Laboratories). Propidium iodide (a solution of 0.01% in PBS) was used as a counterstain to visualize nuclei.

Confocal Analysis and Quantification

Examination, imaging, and data collection were performed on a krypton/argon confocal microscope (MRC 1024; Biorad). Images were scanned with the use of ×10 and ×40 objectives at 1-μm

Figure 3. A, Macroscopic appearance of aneurysm orifices 14 days after embolization, with GDCs. Thin neointima covered the neck of the aneurysm. B, Macroscopic appearance of aneurysm orifices 14 days after embolization, with 50/50 PLGA. Note thick fibrous neointima coverage over the orifice of the aneurysm despite loose packing. C, Macroscopic appearance of aneurysm orifices 14 days after embolization, with 85/15 PLGA. Note incomplete neck coverage and small dimple at the neck.
thickness and at 3% confocal power. Quantification of collagen was performed on 10 independent 250-µm² areas per specimen. Fluorophore intensity was obtained on each image with the use of ImagePro3.0 software. To ascertain whether pixel number correlated with actual collagen levels, known amounts of collagen were coated onto coverslips and blocked with bovine serum albumin. Immunocytochemistry and quantification were performed in formats identical to those used with the specimens. A linear relationship was found between coated collagen levels and pixel number for 5 logs (0.1 ng to 1 mg/mL). None of the samples exceeded the nonlinear range. In addition to collagen levels, quantification of cellularity was obtained as function of propidium iodide staining. For this purpose, normalization was performed with the use of a standard curve that correlated pixel intensity with actual cell number. Degree of collagen organization was assessed by direct examination of the specimens. Two independent specimens per group were evaluated.

Statistical Analysis
Analysis of the percentage of unorganized thrombus was reported as a mean±SD when repeated measures were done. Assuming normal distributions, data were analyzed by 1-way ANOVA, followed by Newman-Keuls test for multiple comparisons between groups. Results were considered significant at \( P<0.05 \).

Angiographic Findings
All embolization procedures were performed without technical complications. In the GDC group, all aneurysms were tightly packed with GDCs. In the BPM group, BPM ribbons were loosely deposited in the aneurysmal sac. Immediate postembolization angiography demonstrated complete aneurysm obliteration in all cases of the GDC group, while the BPM group showed >50% to 80% contrast filling in their aneurysms (Figure 2B and 2C).

Angiograms performed 14 days after embolization showed a complete aneurysm occlusion in all 4 aneurysms treated with GDCs. There was no evidence of aneurysm recanalization. The aneurysms treated with BPMs demonstrated various degrees of occlusion. All aneurysms treated with 50/50 PLGA (n = 3), 2 treated with 65/35 PLGA, and 2 treated with 75/25 PLGA demonstrated complete occlusion despite their loose packing (Figure 2D). Five of 7 of these completely embolized aneurysms showed mild to moderate parent artery stenosis: 2 of the aneurysms treated with 50/50 PLGA, 1 of the aneurysms treated with 65/35 PLGA, and 2 of the aneurysms treated with 75/25 PLGA.

One aneurysm treated with 65/35 PLGA, 1 treated with 75/25 PLGA, and all 3 treated with 85/15 PLGA showed neck remnants on angiography (Figure 2E). No parent artery stenosis was observed in the aneurysms treated with 85/15 PLGA.

Macroscopic Findings
Three of 4 of aneurysms treated with GDC showed complete neck coverage with thin neointima. GDC loops were seen through the neointima (Figure 3A). In 1 aneurysm a small opening was visible at level of the neck of the aneurysm.

All aneurysms treated with 50/50 PLGA, 2 of the aneurysms treated with 65/35 PLGA, and 2 of the aneurysms treated with 75/25 PLGA showed complete neck coverage with thick white neointima (Figure 3B). One of the aneurysms treated with 65/35 PLGA and 1 of the aneurysms treated with 75/25 PLGA showed small slit-shaped neck remnant with near complete neointimal coverage. All aneurysms treated with 85/15 PLGA showed small dimple-shaped neck remnant, with the rest of aneurysm neck covered with thick neointima (Figure 3C).

Histopathological Findings
Figure 4 shows the average amount of unorganized thrombus in each group. Despite the loose coil placement in the aneurysm sac, aneurysms treated with 50/50 PLGA (Figure 5A) and 65/35 PLGA showed a smaller amount of unorganized thrombus than the aneurysms treated with GDCs (Figure 5B). Both 75/25 PLGA– and 85/15 PLGA–treated aneurysms (Figure 5C) showed a higher percentage of unorganized thrombus than the GDC group. Statistical analysis demonstrated that only differences between aneurysms treated with 50/50 PLGA versus 85/15 PLGA and 65/35 PLGA versus 85/15 PLGA were significant.

Guglielmi Detachable Coils
Histopathological findings of GDCs were similar to those from a previous study. 

Figure 4. Rate of unorganized thrombus in GDC group and BPM groups. Mean±SE values are shown for GDC group (25±18.9%), 50/50 PLGA (21.7±7.6%), 65/35 PLGA (19.33±12%), 75/25 PLGA (39.3±31.9%), and 85/15 PLGA (40.7±12.4%). Statistical analysis demonstrated that only 50/50 versus 85/15 and 65/35 versus 85/15 PLGA were significant (\( P<0.05 \)).
Figure 5. A, Low-magnification light microscopy view of a 50/50 PLGA–treated aneurysm 14 days after embolization (Masson trichrome; magnification ×3.1). Note the excessive organized connective tissue across the aneurysm neck despite small amount of polymer (star mark) in the sac. Intra-aneurysmal unorganized clot is present near the sac. B, Low-magnification light microscopy view of an aneurysm embolized with GDCs 14 days after embolization (hematoxylin and eosin; magnification ×3.1). Intra-aneurysmal unorganized clot is present in the center of the sac. C, Low-magnification light microscopy view of 85/15 PLGA–treated aneurysm 14 days after embolization (Masson trichrome; magnification ×3.1). Note neck remnant and presence of intra-aneurysmal unorganized clot in
multifocal inflammation with scantly foreign body reactive cells and loose connective tissue surrounding the GDCs was present (grade 1 to 2) (Figure 5D). The wall of the aneurysm was moderately organized, with minimal to mild inflammation (grade 1 to 2).

50/50 PLGA

There was extensive thrombus organization, and the majority of the aneurysmal sac was filled with BPM incorporated into immature to mature fibrous tissue with foreign body cells (Figure 5E). Unorganized thrombus occupied 21.7 ± 7.6% of the sac. The neck of the aneurysm was covered with a complete layer of maturing neointimal hyperplasia covered by neointimal. BPMs were engulfed in mild to marked inflammatory infiltration (grade 2 to 4). These histological findings were particularly notable in the area near the neck. Multifocal infiltrates of hemosiderin-laden macrophages were scattered throughout the wall, and several mild foci of degeneration and mineralization of the wall were present.

65/35 PLGA

Unorganized thrombus occupied 19.33 ± 12% of the sac, and the rest was occupied with mature fibrous tissue. Scattered foci of mineralization and inflammation were present throughout the fibrous tissue matrix of the sac. There was moderate to marked foreign body inflammatory reaction covering most of the surface of BPMs (grade 3 to 4). Extensive remodeling of the aneurysmal wall with marked adventitial fibrosis was seen. Neointimal and neointimal coverage of the neck of the aneurysms was complete in 1 specimen, and 2 aneurysms were incompletely covered with neointimal hyperplasia.

75/25 PLGA

A lower percentage of tissue organization was observed than that seen with GDCs in the aneurysmal sac. Unorganized thrombus occupied 39.3 ± 31.9% of the sac. There was mild to moderate foreign body inflammatory reaction around the BPMs (grade 2 to 3). Areas of matrix mineralization and associated foreign body inflammation were scattered throughout the aneurysmal sac. The aneurysmal wall was moderately well organized, with scattered foci of mild inflammation. There was marked fibrosis of the adventitia with foci of inflammation. Endothelial and neointimal coverage at the neck of the aneurysms was complete in all cases.

85/15 PLGA

Unorganized thrombus occupied 40.7 ± 12.4% of the sac. There was mild to moderate foreign body inflammatory reaction around the BPMs (grade 3). There were multifocal, moderate foci of mineralization of the fibrous tissue of the sac. Partial neointimal coverage at the neck of the aneurysms was observed in all cases. There was extensive reorganization of the wall of the aneurysms with decreased fibrosis of adventitia.

Immunohistochemistry

A linear relationship was found between collagen deposition levels and polymer composition (r = −0.9513) (Figure 6). Faster-degradation BPM (50/50 PLGA and 65/35 PLGA) demonstrated more mature collagen formation (green) and less cellularity (orange) surrounding the polymer (Figure 7A). Slower-degradation BPM (75/25 PLGA and 85/15 PLGA) showed higher cellularity and a smaller amount of collagen deposition surrounding the polymer (Figure 7B). The necks of aneurysms treated with faster-degradation BPM also demonstrated more collagen-rich tissue (Figure 7C) than the necks of aneurysms treated with slower-degradation BPM (Figure 7D).

Discussion

The most noticeable limitation of endovascular occlusion of an intracranial aneurysm with GDCs is the potential risk of aneurysm recanalization. This anatomic limitation occurs more frequently in small aneurysms with wide necks or in large or giant aneurysms. Tamatani et al demonstrated no endothelial cell proliferation on the platinum coil surface in vitro. Biological modification of the surface of the GDC is a factor that may improve anatomic outcomes if acceleration of cellular reaction and organization of the intra-aneurysmal clot is achieved. Platinum coils elicit mild and delayed biological response when deployed into an aneurysm. Surface modification of the GDC with the use of a bioactive material is a promising approach to enhance cellular reaction on the GDC surface, and many investigators have performed intensive research on this topic.

Tissue Engineering and BPM

Basic scientific and clinical research on the medical utilization of BPMs has increased dramatically during the past decade. These bioabsorbable polymers do not elicit a permanent chronic foreign body reaction because the human body gradually absorbs them. Some have recently been found to be capable of regenerating tissues through the interaction of their biodegradation with immunologic cells such as macrophages, fibroblasts, and osteoblasts.

Ideally, cell scaffolds for tissue engineering should meet several design criteria: (1) the surface should permit cell adhesion and growth; (2) neither the polymer nor its degradation products should provoke untoward overinflammation or toxicity; (3) the material should be reproducibly processible into 3-dimensional structures; (4) the polymer should reabsorb once it has served its purpose of providing a template for the regenerating tissue because foreign materials carry a potential risk of chronic inflammation; and (5) the scaffold degradation rate should be adjustable to match the rate of tissue regeneration by the cell type of interest. PLGA is a synthetic polymer that meets all of these criteria.
Endovascular embolic implants made from bioabsorbable materials could be used as temporary scaffold for tissue regeneration that could induce a complete and permanent occlusion of an aneurysm with mature connective tissue. The aneurysm might not be necessarily tightly packed with embolic material because of active acceleration of unorganized thrombus to scar formation. Retraction of this highly organized connective tissue could also decrease the mass effect observed in large or giant aneurysms.

We used 4 different ratios of PLGA copolymer to evaluate this hypothesis. The magnitude of this inflammatory reaction and the time of bioabsorption can be controlled by changing the ratio of copolymers. Copolymers of glycolide with L-lactide have been developed for device and drug delivery applications. It has been demonstrated that 50/50 PLGA shows the fastest degradation time (approximately 2 to 4 weeks for 50% weight loss) and the strongest inflammatory reaction, while 85/15 PLGA shows the slowest degradation time (approximately 3 to 4 months for 50% weight loss) and the weakest inflammatory reaction; 65/35 PLGA and 75/25 PLGA are intermediate.

In this feasibility study, all copolymers with the exception of 85/15 PLGA polymer ribbons elicited nearly complete or complete obliteration of the aneurysm after being surgically and loosely deposited into the aneurysm. The sacs of the aneurysms were filled with more mature and organized thrombus when compared with aneurysms tightly packed with GDCs. The necks of the aneurysms were also covered with thicker neointimal hyperplasia. Immunohistochemical staining of histological specimens demonstrated accelerated and more intense collagen deposits and spindle cells in aneurysms embolized with copolymers with faster degradation time. Copolymers with slower degradation time produced a more prolonged cellular response with more delayed collagen deposition. At day 14, fast bioabsorbable materials (50/50 PLGA and 65/35 PLGA) already developed mature fibrous tissue with dominant collagen deposits that engulfed and infiltrated the copolymer. Slow-degradation materials (75/25 PLGA and 85/15 PLGA) still showed an active cellular phase with fewer collagen deposits.

These histological and immunohistochemical findings strongly suggest that the deposit of copolymers with a faster bioabsorption time in experimental aneurysms in swine accelerates thrombus organization and transformation into scar tissue compared with standard GDCs in the same animal model.

Five of 7 completely occluded aneurysms treated with BPM demonstrated mild to moderate parent artery stenosis. The stenosis did not cause significant reduction of blood flow, but it showed that the inflammatory reaction produced by BPM in aneurysms may overpass the limits of the aneurysm neck and produce untoward stenosis or occlusion of the parent artery. This response can be critically important in small-caliber arteries such as the middle cerebral or anterior communicating arteries. Further research on the control of this inflammatory reaction is necessary to find the appropriate combination of copolymers that will elicit controlled scar tissue formation that will be confined to the aneurysm. The addition of selected cytokines and growth factors to BPM also warrants further investigation.

**Limitation of the Study**

We acknowledge that the total number of aneurysms in this preliminary study is small and that the long-term anatomic effects of BPM in this biological scenario were not evaluated. Only the BPM groups were evaluated immunohistochemically. GDC histological specimens could not be evaluated with antibodies because they were prepared with methylmethacrylate. This embedding procedure was not compatible with epitope exposure. An alternative solution regarding GDC specimen staining will be necessary for further evaluation.

**Conclusions**

This preliminary animal study shows that the hypothesis of acceleration of aneurysm wound healing in aneurysms with the use of bioactive BPM is feasible. Several challenges, such as the regulation of the healing process by appropriate selection of BPMs, with or without cytokines and growth factors, and the evaluation of the long-term local and systemic anatomic and biological effects, need to be evaluated in...
vi tro and in the animal laboratory. This concept can also be applied to improve the anatomic outcome of the current endovascular treatment of cerebral aneurysms.

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