Medial Regeneration Using a Biodegradable Felt as a Scaffold Preserves Integrity and Compliance of a Canine Dissected Aorta

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Background—Repair of a dissected aorta involves reattaching the media with synthetic glue and/or reinforcement with nonbiodegradable felt. Late complications specific to each aspect of this procedure have been reported. We attempted to regenerate the media by using biodegradable felt.

Methods and Results—We created a canine model of descending thoracic aortic dissection and compared 4 modes of aortic repair: biodegradable polyglycolic acid (PGA) felt in the media; PGA with basic fibroblast growth factor (bFGF) in the media; PGA with bFGF in the media plus external reinforcement with expanded polytetrafluoroethylene; and primary suture closure of the dissected lumen (control). Repaired aortic stumps were quantitatively tested for suture pull-out strength. Failure force improved 4-fold in all 3 medial reinforcement groups compared with controls. Additionally, animals were kept alive for histological examination and compliance testing 6 months after repair. Compliance of the aortic wall at the anastomotic sites was not essentially affected in the long term except in the group with concomitant external reinforcement (55.9 ± 4.5% reduction; P < 0.05). In this group, elastic fiber in the media and collagen fiber in the adventitia tended to diminish relative to the other groups. Regarding vessel density in the repaired false lumen, this external reinforced group had a significantly decreased number. Histological derangement was not observed in control or medial reinforcement groups. Basic FGF, applied with PGA in the dissected lumen, failed to yield additional modifications in this model.

Conclusions—Medial reinforcement provides sufficient augmented strength for aortic surgical repair. Medial regeneration using biodegradable felt as a scaffold preserves histological integrity and compliance in the canine dissected aorta. (Circulation. 2012;126[suppl 1]:S102–S109.)

Key Words: aorta • surgery • remodeling • elasticity

Repair of a dissected aorta involves reattachment of the media, using synthetic glue such as gelatin resorcin formalin (GRF) glue,1,2 fibrin glue,3 or BioGlue4,5 with a compression maneuver and/or external, often both external and internal, reinforcement with a nonbiodegradable Teflon felt to add sufficient biomechanical strength to anastomotic sites. The use of synthetic glue and reinforcement materials has contributed to an improved early surgical outcome. However, there have been variable reports of late complications specific to each aspect of the reparative procedure. For instance, formalin, an ingredient of GRF glue, possibly causes tissue damage1,6,7 due to its toxicity, resulting in pseudoaneurysm formation in the long term. Chronic compression of the aortic wall by nonbiodegradable material has been shown to induce tissue derangement8 and compliance mismatch9–11 between the intact host artery and the aortic segment that is involved in the anastomosis.

Joseph et al and Nakajima et al12–14 have reported the utility of medial reinforcement with fabric neomedia, with or without fibrin glue, for the repair of dissected aortic walls. However, long-term histological changes in the aortic wall have not been investigated. It is a concern that nonbiodegradable fabric in the media may cause histological derangement and compliance mismatch within the aortic wall. Compliance mismatch has been known to produce anastomotic shear stress with subsequent damage to the arterial wall.9,15

We previously reported that biodegradable felt, when applied as an external reinforcement, may play a beneficial role in preventing thinning of both the media and adventitia and reducing compliance mismatch.16,17 Therefore, we hy-
pothesized that this material inserted in a false lumen could serve as a scaffold to regenerate the media and lead to a better repair of aortic dissection.

The purpose of our study is to examine whether medial reinforcement provides augmented strength sufficient for aortic repair and to determine whether medial regeneration using a biodegradable felt preserves structural integrity and compliance of the repaired aortic wall, using a canine model with a dissected descending thoracic aorta.

**Methods**

All animals used in this study were treated in accordance with the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals. The experimental protocol and procedural protocol for animal care were also approved by the Animal Care Committee of the Graduate School of Medicine, Tohoku University.

**Ex Vivo Tensile Test of Suture Pull-Out at First Break on the Repaired Aortic Dissection**

**Methods Used to Create and Repair Aortic Dissection**

The experimental model of aortic dissection was created using 10 beagles weighing between 9 and 12.4 kg (mean body weight, 10.8 kg), from which 20 dissected aortic samples were made. These were divided into 4 groups according to the reinforcing material used, with 5 samples in each. Under the general anesthesia, the descending thoracic aorta was exposed through a left lateral thoracotomy incision. Aortic specimens were extracted from the descending thoracic aorta and trimmed to 8-mm-long tubular segments. An 8×8-mm false lumen was then carefully made in the layer of the media with a sharp-pointed knife and was extended by the use of 3 types of raspatories (Nagashima Medical Instruments Co, Ltd, Tokyo, Japan). Care was taken not to disrupt the intima. We used 4 different modes of aortic repair: (1) control, primary suture closure of the dissected lumen; (2) AFm, gelatin-coated absorbable polyglycolic acid (PGA) felt in the media; (3) AFm+bF, PGA with bFGF in the media; and (4) AFm+bF/E, PGA with bFGF in the media plus external reinforcement with an expanded polytetrafluoroethylene (ePTFE), as illustrated in Figure 1A. The materials used in this experiment included PGA felt (Neoveil, Gunze Co, Kyoto, Japan), 500 μm; ePTFE (Gore-Tex EPTFE PATCH II, Japan Gore-Tex Inc, Tokyo, Japan), 110 μm in thickness; and bFGF (Fibrest, 10 mg/mL, Kaken Pharmaceutical Co, Tokyo, Japan).

**Biomechanical Testing**

A longitudinal incision was made on the tubular segments at the opposite side from the dissection to create a rectangular aortic strip measuring approximately 24 mm in length and 8 mm in width. A 4-cm loop stitch of 5–0 polypropylene was placed on the aortic specimen with a 2-mm bite width. Fixing the specimen to the jaw of the tester, an ex vivo tensile test of suture pull-out strength was performed on the repaired aortic dissection (Figure 2A). In addition to the above-mentioned reparative procedures in the 4 groups, we created additional aortic samples of dissection repaired with clinically used materials including external PTFE, intramural GFR glue (Cardial, Technopole, Sainte-Etienne, France), Bio glue (CryoLife Inc, Kennesaw, GA), and fibrin glue (Bolheal, The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan). An intact aortic sample without a dissection was also served for the above-mentioned reparative procedures in the 4 groups mentioned above. An interposition with an 8-mm-diameter, 3-cm-long prosthetic vascular graft (Gelweave, Vascutek Ltd, Scotland, United Kingdom) was carried out with a 5–0 polypropylene over-and-over running suture after transection of the aorta. The animals were allowed to recover from the anesthesia and the procedures for the subsequent 6 months.

**Preparation of the Gelatin-Coated PGA Felt Strips**

The details of the method have been described elsewhere.16–18

**Preparation of the Gelatin-Coated PGA Felt Strips Containing bFGF**

We elected to apply bFGF concomitant with a biodegradable felt in our novel approach to the repair of aortic dissection, in an attempt to establish a temporary bypass, the descending thoracic aorta was cross-clamped at the second and sixth thoracic vertebral levels. A 10-mm transverse incision was made on the anterior aspect of the midportion of the descending aorta. A false lumen was then created in the layer of the media in the distal aorta (Figure 1B) with a sharp-pointed knife to begin with, which was extended to make it 10×20 mm in size by the use of 3 types of raspatories described above. Again, care was taken not to disrupt the intima. For the reparative procedure, each animal was randomly assigned to 1 of the 4 groups mentioned above. An interposition with an 8-mm-diameter, 3-cm-long prosthetic vascular graft (Gelweave, Vascutek Ltd, Scotland, United Kingdom) was maintained by the inhalation of halothane. After systemic heparinization (100 U/kg) and establishment of a temporary bypass, the descending thoracic aorta was resected at the second and sixth thoracic vertebral levels. A 10-mm transverse incision was made on the aorta, and the false lumen was then created in the layer of the media in the distal aorta (Figure 1B) with a sharp-pointed knife to begin with, which was extended to make it 10×20 mm in size by the use of 3 types of raspatories described above. Again, care was taken not to disrupt the intima. For the reparative procedure, each animal was randomly assigned to 1 of the 4 groups mentioned above. An interposition with an 8-mm-diameter, 3-cm-long prosthetic vascular graft (Gelweave, Vascutek Ltd, Scotland, United Kingdom) was carried out with a 5–0 polypropylene over-and-over running suture after transection of the aorta. The animals were allowed to recover from the anesthesia and the procedures for the subsequent 6 months.
enhance neovascularization in the media based on the deduction from our previous observations.16,17

Commercial aqueous recombinant human bFGF solution equivalent to 50 μg was added to rectangular PGA felt strips and allowed to stand for 1 hour at 25°C to incorporate the bFGF into the gelatin hydrogel completely.

Biomechanical Evaluation: Measurement of Compliance With Tensile Tester

Differences in aortic biomechanical properties between the groups were examined. Vascular compliance could be assessed in 3 directions, which contain longitudinal, transmural, and circumferential directions. Of these, the circumferential direction was the most significant and reliable for evaluation of the aortic compliance that accommodates the dynamic component during the cardiac systolic and diastolic phases. The stress-strain relationship was therefore examined in the circumferential direction.

A rectangular aortic strip 10×20 mm in size was resected from the reinforced site and suspended on the above-mentioned tensile tester (Figure 2B). Each specimen was tested at a constant elongation rate of 1 mm/min up to a physiological upper limit of load (50 g; equivalent to 120 mm Hg blood pressure). The stress-strain curve was recorded. The modulus of elasticity and subsequently compliance were calculated within the load range corresponding to a blood pressure of 80 to 120 mm Hg. The modulus of elasticity was calculated as the slope of the stress-strain curve, and compliance was calculated by taking the inverse of the modulus.

Histological Evaluation: Retrieval of Factors Associated With Compliance

Transmural sections were stained with elastica-Masson (EM) and hematoxylin and eosin for microscopic examination to determine the thickness of the elastic fiber layer in the media (Te-m), and collagen fiber layer in the false lumen (Tc-f) and adventitia (Tc-ad) in each segment (Figure 3A). The vessel density in the false lumen (D-f) and adventitia (D-ad) was also quantified. The numbers of vessels from 5 fields were averaged and used to assess the vessel density (Figure 3B). The mean thickness of each histological component and the average number of vessels per slide were used to assess Te-m, Tc-f, Tc-ad, D-f, and D-ad. These histological measurements were performed using Image-Pro Plus (v 4.0; Media Cybernetics, Silver Spring, MD) for Windows (Microsoft, Redmond, WA).

Statistical Analysis

Statistical analyses were performed using Excel for Windows (Microsoft, Redmond, WA) with the add-in software StatMate III (ATMS Co, Ltd, Tokyo, Japan) and JMP Pro (SAS Institute Inc, Cary, NC). All numeric data were analyzed by Tukey-Kramer test to examine a difference between the groups. Homoscedasticity was confirmed by F test for deviations in the data on each parameter from the experimental groups. The results are expressed as mean±SD. A difference with a probability value of <0.05 was considered significant.
Results

Failure Force Measured by Ex Vivo Tensile Test of Suture Pull-Out Strength on the Repaired Aortic Dissection

The failure forces in the AFm, AFm+bF, and AFm+bF/E groups were essentially the same, but all were significantly higher than in the control group (1.86±0.67 N in control, 7.95±1.82 N in AFm, 8.74±1.46 N in AFm+bF, 8.15±1.85 N in AFm+bF/E, respectively; exact probability values against control were 1.7×10^{-5}, 3.3×10^{-6}, 5.9×10^{-6}, respectively). Single asterisk shows significant difference against control. Values are given as mean±SD.

Overall Survival Outcome in the Chronic Model

Two animals in the AFm/bF/E group died of intraoperative arrhythmia and postoperative strangulation ileus. The number of animals that survived to the time of assessment in each group was 10 in control, 7 in AFm and AFm/bF, and 5 in AFm/bF/E.

Macroscopic Findings

There were no aneurysmal changes in any animals. PGA felts inserted into the false lumen were completely absorbed and replaced with autologous tissue. One animal in the control group developed a redissection. An intimal tear was present at the suturing site on the distal anastomosis. The dissection developed distally and extended to a far-off point beyond the reinforced site (Figure 5).

Compliance

Compliance in the AFm+bF/E group was significantly lower compared with the control (Figure 6). Compliance in the medial reinforcement groups (AFm and AFm+bF) remained equivalent to that in controls.

Thickness of the Elastic Fiber Layer in the Media

There were no significant differences in Te-m among the 4 groups. However, the Te-m in the AFm+bF/E group tended to be less compared with that in control (Figure 7A through 7E).

Figure 4. Failure force measured by a tension of suture pull-out at first break test in the aortic specimen. Failure was defined as the appearance of an intimal tear more than 2 mm long. The failure force measured at the time of failure was significantly higher in all 3 reinforced groups than in the control group (1.86±0.67 N in control, 7.95±1.82 N in AFm, 8.74±1.46 N in AFm+bF, 8.15±1.85 N in AFm+bF/E, respectively; exact probability values against control were 1.7×10^{-5}, 3.3×10^{-6}, 5.9×10^{-6}, respectively). Single asterisk shows significant difference against control. Values are given as mean±SD.

Figure 5. Cross-sectional view of redissection. A redissection developed in 1 animal in the control group. The dissected aorta was transected at the distal aorta beyond the anastomotic site. An intimal tear was located at the suturing site.

Thickness of the Collagen Fiber Layer in the Healed False Lumen

There were no statistically significant differences between the groups, but the mean Tc-f in the AFm+bF/E group also tended to be less than those in other groups (Figure 7A through 7D and 7F).

Thickness of the Collagen Fiber Layer in the Adventitia

The Tc-ad in the AFm+bF/E group also exhibited a tendency to be less than control. The medial interposition groups without external reinforcement preserved the same amount of adventitial collagen fiber as control (Figure 7A through 7D and 7G).

Vessel Density in the Healed False Lumen

A significantly lower D-f was obtained in the AFm+bF/E group relative to those in the control and AFm groups. The AFm+bF group also had a diminished vessel density in this model. The difference in D-f between AFm and control was not statistically significant (Figure 8A through 8E).

Figure 6. Compliance of the repaired aortic wall. Compliance in the AFm+bF/E group (2.98±0.69 mm²/N) was significantly lower compared with the control (6.75±1.78 mm²/N, P=0.0075). Compliance in the medial reinforcement groups AFm (5.56±2.37 mm²/N) and AFm+bF (4.88±1.75 mm²/N) remained equivalent to control. Single asterisk shows significant difference compared to control. Values are given as mean±SD.
Vessel Density in the Adventitia

The D-ad in the AFm + bF/E group tended to be lower than that of control. In the medial reinforcement groups without external reinforcement, vessel density in the adventitia was relatively preserved (Figure 8A through 8D and 8F).

Discussion

We first proved that medial reinforcement provides sufficient augmented strength for aortic surgical repair using an ex vivo tensile test of suture pull-out strength on the repaired aortic dissection. We have also demonstrated that medial regeneration using a biodegradable felt as a scaffold preserves histological integrity and compliance of the canine dissected aorta. Our investigation may stimulate further research on regeneration of the media after aortic dissection.

Evaluation in Ex Vivo Tensile Test of Suture Pull-Out Strength on the Repaired Aortic Dissection

During surgical repair of aortic dissection, reinforcement of the anastomotic site is crucial in reconstructing an integrated aorta from dissected friable aortic wall. Residual dissection or redissection secondary to an intimal tear at a suturing point can lead to a lethal condition both during and after surgery. Thus, evaluation of suture pull-out strength at the time of anastomosis is important when various techniques for aortic repair are assessed. To our knowledge, there have been no reports concerning the suture-holding capacity of the repaired aorta immediately after the onset of dissection. From the data on failure force, primary suture closure of the dissecting lumen performed in controls yielded a lower suture-holding capacity than any other reparative techniques. The PGA felt
applied in the media provided 4-fold greater suture pull-out strength than controls. The use of a reinforcing material in the media of the dissected aortic wall is therefore a reasonable procedure of choice. Additional reinforcement using nonbiodegradable felt from the adventitial side is considered to augment suture-holding capacity at the anastomotic site. Our results showed no increase in breaking strength in the AFm/H11001bF/E group; however, this negative result is not surprising, considering the thinness of the ePTFE sheet we used. Clinically, a thicker PTFE Teflon felt is frequently used for external reinforcement, but it was necessary to use thinner ePTFE in our experiments because the Beagle’s comparatively small aorta did not accommodate a thick PTFE felt. It would be reasonable to expect that applying Teflon felt would augment suture-holding capacity considerably.

Compliance

To assess the mechanical properties of vessel walls, compliance and strength are essential indicators and clearly represent distinct characteristics. When we investigate pathological conditions of the aortic wall biomechanically, both indicators must be taken into account. We consider that aortic mechanical properties must be analyzed under physiological conditions. In general, the load on the aortic wall under normal blood pressure is born by elastic fiber, which is the main component\(^{19}\) of the media. The volume and quality of elastic fiber dictate compliance of a vessel.\(^{20–22}\) Therefore, we elected to measure compliance as the most crucial parameter to characterize aortic mechanical properties. In our investigation, we found that the nonbiodegradable material ePTFE, used as a reinforcement material, lowered local aortic compliance. We speculate that diminished aortic compliance is associated with the reduction of elastic fiber volume as a result of compression by nonbiodegradable material. Medial reinforcement with biodegradable felt preserved vascular compliance to the same degree as in controls in the long term.

Histological Changes

In our present study, the elastic and collagen fiber layers were measured both in the media and adventitia: elastic fiber because it is the largest component in the media and also determines vascular compliance,\(^{20}\) and collagen fiber because, despite its relatively small volume, it has a high elastic modulus of approximately 1000 MPa, almost 1000 times the value\(^{20}\) for elastic fiber.

Kinefuchi et al and Fujiwara et al\(^{8,16,17}\) have reported damage to aortic tissue from using nonbiodegradable materials, and, in the present study, we have also confirmed similar effects including thinning of the medial and adventitial layers in the group using external reinforcement by ePTFE.
When biodegradable PGA alone was used, the volume of elastic fiber was well preserved. We therefore deduce that PGA itself did not have any negative effects on vascular structures even before the material was completely absorbed, nor was any infection associated with the use of this material.

Basic FGF administered in the false lumen was initially expected to increase vessel density, in line with our previous data. Surprisingly, the opposite result was observed in the AFm + bFGF group. The underlying mechanism of this reduced vascularity is unclear. The pharmacokinetics of bFGF under the unique conditions of our experimental model must be elucidated further. One can speculate that vessel density might have increased transiently at an early stage of the healing process. Evaluation at an early postoperative period would reveal this phenomenon; however, the reason for diminishing vascularity in the longer term remains unknown.

Our aim was to regenerate autologous aortic wall without thinning of the elastic fiber in the media. In general, the formation of elastic fiber requires various elements, including fibrillin, which provides a scaffold, lysyl oxidase, which mediates the cross-linking reaction of several components including elastin, and DANCE protein as an organizer for elastogenesis. Because of the complexity of the components necessary for the formation of elastic fiber, there is still controversy about which additive agents or methods work best for its regeneration. On the other hand, Takahashi et al have reported that the regeneration of normal vascular wall can be achieved in the pulmonary artery using a structure of reinforcing materials without either cell seeding or inducive differentiating factors. This successful report encouraged us to apply absorbable PGA as a scaffold in the media with the expectation of modest regeneration of elastic fiber. However, medial regeneration was mainly composed of collagen fibers, and the addition of bFGF did not influence the volume of elastic fiber. In fact, the environment of the prosthetic scaffold was fundamentally different between Takahashi’s experiments and ours: the pulmonary scaffold was directly exposed to blood circulation, whereas our medial scaffold was placed in an isolated dissected lumen. Taking this critical difference into consideration, we speculate that circulating progenitor cells (CPCs) may have a role in regenerating the entire vascular structure. CPCs have been reported to be multipotential, that is, able to differentiate into various tissues. Contact of a prosthetic scaffold with CPCs may play a role in local tissue regeneration corresponding to the specific surroundings.

Potential Benefits of Using Biodegradable PGA Felt
We anticipated that the use of PGA for reinforcement would reduce the risk of infection or foreign body reaction at an anastomotic site. As expected, complete absorption of the PGA felt was achieved in the chronic phase, and no infection was encountered. Because infection remains an unsolved clinical issue with prosthetic material, the use of biodegradable PGA may provide a solution.

In contrast, the biodegradable nature of PGA felt may give rise to concern over diminishing biomechanical strength. The present study, however, showed that there was no aneurysmal change or redissection even after complete absorption of PGA. We therefore concluded that excessive reinforcement is not always necessary once tissue at the reinforced site has healed and that medial reinforcement with PGA may be sufficient to reinforce a dissected aortic wall.

Study Limitations and Future Directions
The present study had some limitations. In the chronic model, we defined the euthanasia time point as 6 months after surgical repair, based on the fact that more than 3 months were needed for complete absorption of the biodegradable felt inserted in the false lumen, according to the preliminary results of our pilot study. We focused on examination only at the time of euthanasia and did not carry out serial observations at multiple time points. A time-dependent multiple assessment study may be necessary to elucidate the detailed healing process in the reinforced false lumen of the dissected aorta.

We used a standard size of PGA and administered a single dose of bFGF equivalent to that used in previous experiments. The ideal thickness and structure of PGA and optimal dose of bFGF may need to be determined to maximize the beneficial effects of these agents when used specifically in the dissected lumen.

PTFE Teflon felt has commonly been applied as an external reinforcing material. However, the Beagle’s relatively small aorta did not accommodate a clinically available 1.6-mm-thick PTFE Teflon felt. We had to use a less rigid ePTFE sheet as a nonbiodegradable reinforcing material in our animal model. The use of larger animals may allow us to apply a PTFE Teflon felt to the aorta, which may alter the results of the compliance test.

Additionally, we expect that similar studies with larger sample sizes will ensure our results in the future.

Conclusions
Medial reinforcement provides augmented strength sufficient for surgical repair in a canine model with a dissected descending thoracic aorta. Medial regeneration using biodegradable felt as a scaffold preserves histological integrity and compliance of the dissected aorta. Basic FGF, when applied with PGA in the dissected lumen, failed to yield additional modifications in this animal model. External reinforcement with nonbiodegradable material induced medial thinning and diminished vascularity in the media and the adventitia and resulted in poor compliance at the anastomotic site of the dissected aorta. Hence, medial reinforcement with biodegradable material may prove to be valuable as a novel reparative procedure for acute aortic dissection.

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Disclosures

None.

References


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Results of the additional experiments on failure force to prove the robustness of the repaired aortic dissection using an intramedial PGA felt compared with those of aortic samples repaired with clinically relevant techniques. Exact failure forces of these groups were 8.56±0.76 N in external PTFE group, 7.54±1.25 N in GRF glue group, 8.51±1.01 N in Bio glue group, 7.55±0.93 N in fibrin glue group, and 8.26±0.90 N in

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intact aorta, respectively. No statistically significant differences were seen between the groups.