

Imatinib attenuates neotissue formation during vascular remodeling in an arterial bioresorbable vascular graft

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ABSTRACT

Background: Bioresorbable vascular grafts (BVGs) can transform biologically into active blood vessels and represent an alternative to traditional synthetic conduits, which are prone to complications such as infection and thrombosis. Although platelet-derived growth factors and c-Kit positive cells play an important role in smooth muscle cell (SMC) migration and proliferation in vascular injury, atherosclerosis, or allograft, their roles in the vascular remodeling process of an arterial BVG remains unknown. Thus, we assessed the neotissue formation on arterial BVG remodeling by administering imatinib, which is both a platelet-derived growth factor receptor kinase inhibitor and c-Kit receptor kinase inhibitor, in a murine model.

Methods: BVGs were composed of an inner poly(L-lactic-co-ε-caprolactone) copolymer sponge layer and an outer electrospun poly(L-lactic acid) nanofiber layer, which were implanted into the infrarenal abdominal aortas of C57BL/6 mice. After graft implantation, saline or 100 mg/kg of imatinib was administered intraperitoneally daily for 2 weeks (n = 20 per group). Five mice in each group were scheduled to be humanely killed at 3 weeks and 15 at 8 weeks, and BVGs were explanted for histologic assessments.

Results: Graft patency during the 8-week observational period was not significantly different between groups (control, 86.7% vs imatinib, 80.0%; $P > .999$). Neotissue formation consisting of endothelialization, smooth muscle proliferation, and deposition of collagen and elastin was not observed in either group at 3 weeks. Similar endothelialization was achieved in both groups at 8 weeks, but thickness and percent area of neotissue formation were significantly higher in the control group than in the imatinib group, (thickness, $30.1 \pm 7.2 \mu\text{m}$ vs $19.6 \pm 4.5 \mu\text{m}$ [$P = .001$]; percent area, $9.8 \pm 2.7\%$ vs $6.8 \pm 1.8\%$ [$P = .005$]). Furthermore, SMC layer and deposition of collagen and elastin were better organized at 8 weeks in the control group compared with the imatinib group. The thickness of SMC layer and collagen fiber area were significantly greater at 8 weeks in the control group than in the imatinib group ($P < .001$ and $P = .026$, respectively). Because there was no difference in the inner diameter of explanted BVGs ($831.7 \pm 63.4 \mu\text{m}$ vs $841.8 \pm 41.9 \mu\text{m}$; $P = .689$), neotissue formation was thought to advance toward the outer portion of the BVG with degradation of the polymer scaffold.

Conclusions: Imatinib attenuates neotissue formation during vascular remodeling in arterial bioresorbable vascular grafts (BVGs) by inhibiting SMC layer formation and extracellular matrix deposition. (*JVS—Vascular Science* 2020;1:57-67.)

Clinical Relevance: This study demonstrated that imatinib attenuated neotissue formation during vascular remodeling in arterial Bioresorbable vascular graft (BVG) by inhibiting smooth muscle cell formation and extracellular matrix deposition. In addition, as imatinib did not modify the inner diameter of BVG, neotissue advanced circumferentially toward the outer portion of the neovessel. Currently, BVGs have not yet been clinically applied to the arterial circulation. The results of this study are helpful for the design of BVG that can achieve an optimal balance between polymer degradation and neotissue formation.

Keywords: Tyrosine kinase inhibitor; Smooth muscle cell; Neotissue formation; Vascular remodeling

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Vascular graft replacement and bypass surgery are common therapies for patients with aortic disease, coronary artery disease, and peripheral artery disease.¹ However, current synthetic grafts have limitations such as a lack of growth capacity and the risk of thromboembolism and infection. A bioresorbable vascular graft (BVG) composed of biodegradable polymer is reconstituted by host-derived cells and transforms into a native-like vessel with the degradation of polymer, and therefore does not carry the same risks as synthetic conduits.² A clinical trial has shown the safety and effectiveness of venous BVGs in pediatric patients.^{3,4} In contrast, BVGs have not yet been clinically applied to the arterial circulation. For arterial BVGs to succeed, they must achieve the proper balance between polymer degradation and vascular remodeling within the high-pressure system. As the polymeric scaffold degrades, vascular remodeling forms neotissue, which undergoes endothelialization, smooth muscle cell (SMC) proliferation and extracellular matrix (ECM) deposition. A well-organized neotissue is essential in the early phase of BVG remodeling as the graft's mechanical strength begins to decrease owing to polymer degradation.

Because it has been reported that platelet-derived growth factors (PDGFs) stimulated vascular SMC proliferation and migration *in vitro* in the 1980s,⁵ many researchers reported that PDGFs played a pivotal role in SMC migration and proliferation in injured vascular wall,⁶ atherosclerosis,^{7,8} and neointimal hyperplasia causing stent restenosis.⁹ Therefore, PDGFs were thought possibly to stimulate SMC proliferation and migration for neotissue formation during BVG remodeling. In our previous study, PDGF contributed to vascular remodeling of BVG in myeloid cell-specific PDGF-B knockout mice by regulating SMC proliferation and ECM deposition.¹⁰ In the study, release of PDGF was blocked only in cells differentiated from myeloid cells. Because PDGFs are also known to be released from other cells such as endothelial cells, SMCs, and epithelial cells, we sought to evaluate the role of PDGFs in the BVG remodeling process by widely blocking its function in the current study. We considered imatinib would be good candidate drug for the present study because it is a PDGF receptor kinase inhibitor with a known ability to inhibit SMC proliferation in a mouse model of restenosis after repeated vascular injury.¹¹ Additionally, imatinib also functions as a c-Kit receptor kinase inhibitor. Similar to the effects of PDGF on encouraging SMC proliferation, c-Kit-positive progenitor cells can differentiate into α -actin-positive vascular SMCs,¹² and recipient c-Kit lineage cells were recently shown to repopulate SMCs of transplant arteriosclerosis in mouse models.¹³ Therefore, by using imatinib as both a PDGF receptor kinase and c-Kit receptor kinase inhibitor, it should effectively inhibit SMC migration and proliferation during BVG remodeling in our study. Here, we assessed the effect of imatinib on BVG remodeling in a

ARTICLE HIGHLIGHTS

- **Type of Research:** Experimental study using arterial bioresorbable vascular graft (BVG) in a murine model
- **Key Findings:** Imatinib attenuated neotissue formation by inhibiting smooth muscle cell layer formation and extracellular matrix deposition into arterial BVGs.
- **Take Home Message:** Imatinib attenuates BVG remodeling by inhibiting smooth muscle cell layer formation and extracellular matrix deposition. As imatinib does not modify the inner diameter of BVG, neotissue advanced circumferentially toward the outer portion of the neovessel.

murine arterial BVG implantation model. The primary hypothesis is that imatinib attenuates neotissue formation by inhibiting SMC layer formation during BVG remodeling. In addition, the primary outcome was evaluated as the difference in the neotissue formation area with and without imatinib administration during BVG remodeling.

METHODS

BVG. All BVGs were composed of a poly(L-lactic-co- ϵ -caprolactone) sponge layer and an outer electrospun poly(L-lactic acid) nanofiber layer, and were provided by Gunze Ltd (Kyoto, Japan). Each graft was 3.0 mm in length with an inner diameter of 0.8 mm. The inner sponge layer was constructed by pouring a solution of 50:50 poly(L-lactic-co- ϵ -caprolactone copolymer sealant (Gunze Ltd) into a glass tube, then freeze drying it under a vacuum as previously described.¹⁴ Next, the outside of the inner sponge layer was reinforced by an electrospun poly(L-lactic acid) nanofiber.

Animals and graft implantation. All animals received humane care in compliance with the National Institutes of Health Guideline for the Care and Use of Laboratory Animals. The Institutional Animal Care and Use Committee at Nationwide Children's Hospital approved the use of animals and all procedures described in this study. Eight-week-old female C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, Me). The BVGs were implanted into wild-type C57BL/6 mice as infrarenal abdominal aorta interposition conduits using standard microsurgical technique by two microsurgeons (T.Y. and Y.L.) as previously described (Fig 1, A and B).^{15,16} In brief, mice were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg), xylazine (10 mg/kg), and ketoprofen (5 mg/kg), a midline laparotomy incision was made, and the abdominal aorta was exposed. After transection of the abdominal aorta, a 3-mm graft was introduced with end-to-end anastomoses performed at both the proximal and distal ends using 10-0 monofilament nylon sutures in simple continuous stitches.

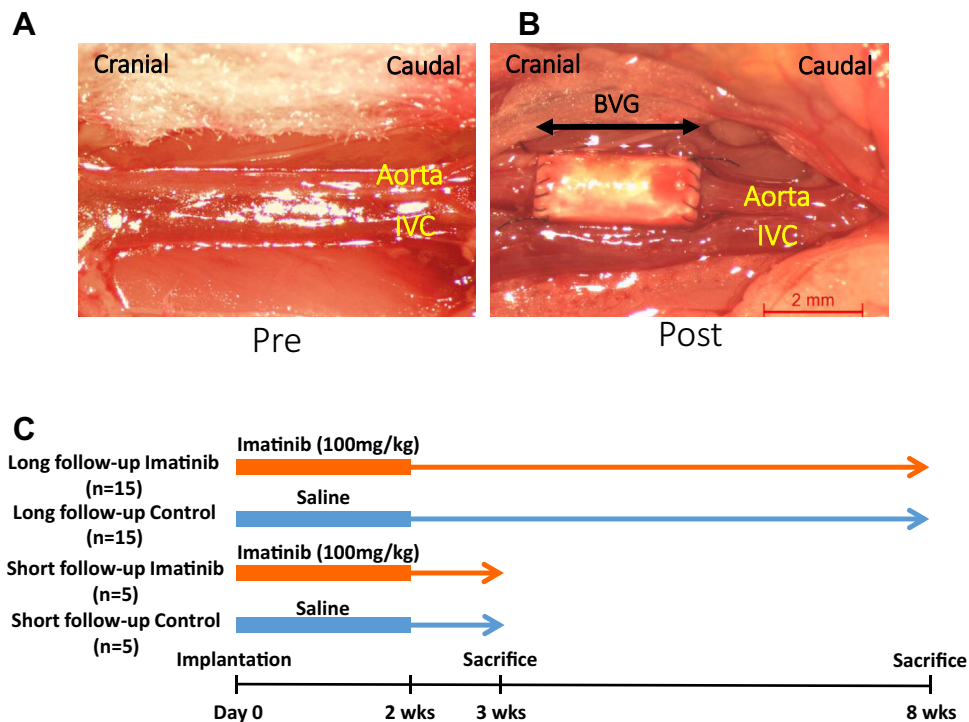


Fig 1. Macroscopic images of bioresorbable vascular graft (BVG) before implantation and after implantation (**A** and **B**) and study protocol (**C**). A total of 40 mice were administered 100 mg/kg of intraperitoneal imatinib or same volume of saline for 2 weeks after BVG implantation ($n = 20$, per group). Five mice were scheduled to be humanely killed at 3 weeks and 15 mice at 8 weeks in both groups. IVC, inferior vena cava.

During implantation, grafts were flushed with a heparin solution (1000 U/mL; Fresenius Kabi USA, Lake Zurich, Ill) to inhibit graft thrombosis. After anastomosis, the surgical clamps were removed, and the abdomen was closed with a running 5-0 Prolene suture. Postoperatively, mice did not receive anticoagulation or antiplatelet therapy. The mice were randomly administered 100 mg/kg of imatinib dissolved in water, or the same volume of saline, in the peritoneum once a day for 2 weeks after BVG implantation ($n = 20$ per group). Five mice were scheduled to be humanely killed at 3 weeks and 15 mice at 8 weeks in each group (Fig 1, C). To explant BVGs, mice were anesthetized with intraperitoneal injections of ketamine (100 mg/kg), xylazine (10 mg/kg), and ketoprofen (5 mg/kg). After the mice reached a surgical plane of anesthesia by lack of response to a stimulus, humane killing was performed by thoracotomy. Grafts were rinsed to remove blood by injecting 20 mL of saline intracardially after right atrial incision. After rinsing, the BVGs were harvested and then fixed in 4% paraformaldehyde.

Histology, immunohistochemistry, and immunofluorescence. Explanted grafts were embedded in paraffin, sliced in 5 μ m thick sections, and stained with hematoxylin and eosin, Masson's trichrome, Hart's, and

Picrosirius red. Collagen deposition was assessed by imaging Picrosirius red-stained slides using polarized light microscopy. The thickness and percent area of neovessel formation were assessed by quantifying two mid histologic sections per graft using ImageJ software (National Institutes of Health, Bethesda, Md). Based on a previous report,¹⁷ the percent area of collagen fibers in the BVG was also measured with ImageJ. Nuclei were counted to obtain the number of cells using ImageJ.

For immunofluorescent staining, endothelial cells (ECs) were stained using rabbit anti-CD31 primary antibody (1:50, ab28364; Abcam, Cambridge, UK). SMC staining was performed using mouse anti- α -SMA primary antibody (1:500; Dako, Glostrup, Denmark), followed by Alexa Fluor 488 anti-rabbit immunoglobulin G secondary antibody (1:300; Invitrogen, Carlsbad, Calif) and Alexa Fluor 647 anti-mouse immunoglobulin G secondary antibody (1:300; Invitrogen), respectively. To observe M1 macrophages, rat anti-CD1107b (Mac-3) primary antibody (1:75, 550292; BD Biosciences, San Jose, Calif) and rabbit anti-inducible nitric oxide synthase primary antibody (1:200, ab3523; Abcam) were double stained. Alexa Fluor 488 anti-rat IgG (1:300; Invitrogen) and Alexa Fluor 647 anti-rabbit IgG (1:300; Invitrogen) were used as the secondary antibodies. Images were obtained under a fluorescence

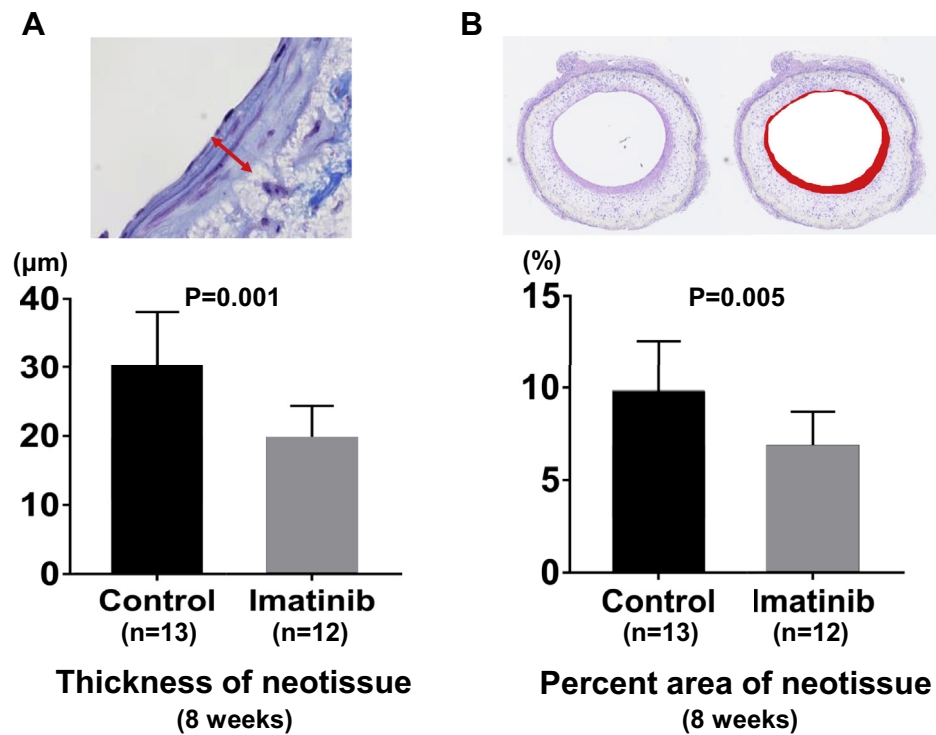


Fig 2. Thickness (**A**) and percent area (**B**) of neotissue formation. The thickness and percent area of neotissue formation at 8 weeks were measured manually by a microscopic image ($n = 13$; imatinib group, $n = 12$; control group). The thickness and percent area in the imatinib group were significantly smaller than in the control group ($P = .001$ and $P = .005$, respectively).

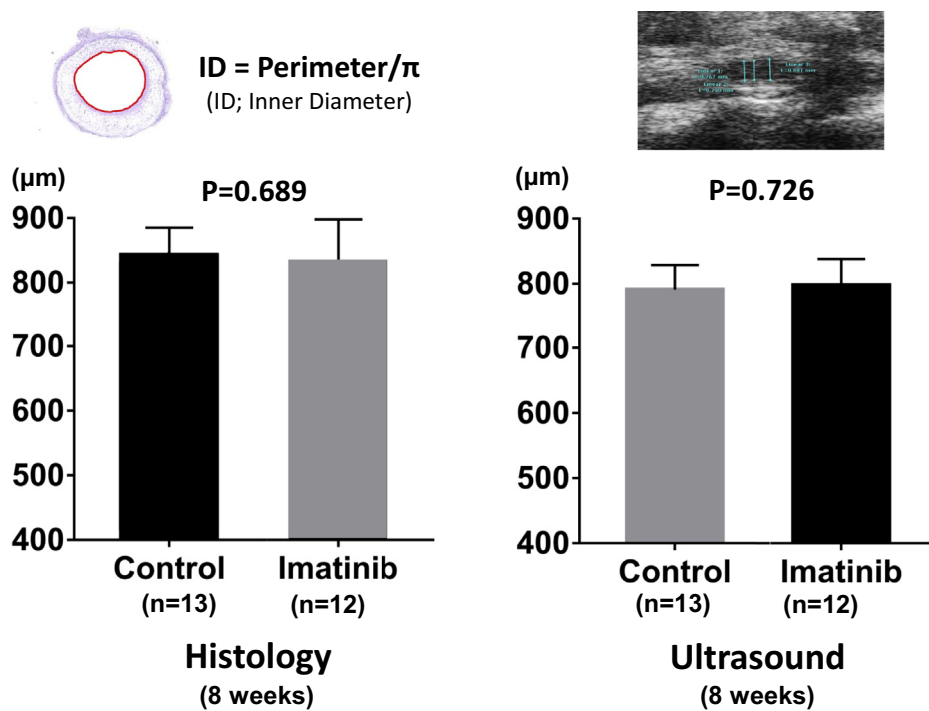


Fig 3. Inner diameter of explanted bioresorbable vascular graft (BVG). The inner diameter was measured manually by microscopic and ultrasound findings. The inner diameter was statistically similar in both groups.

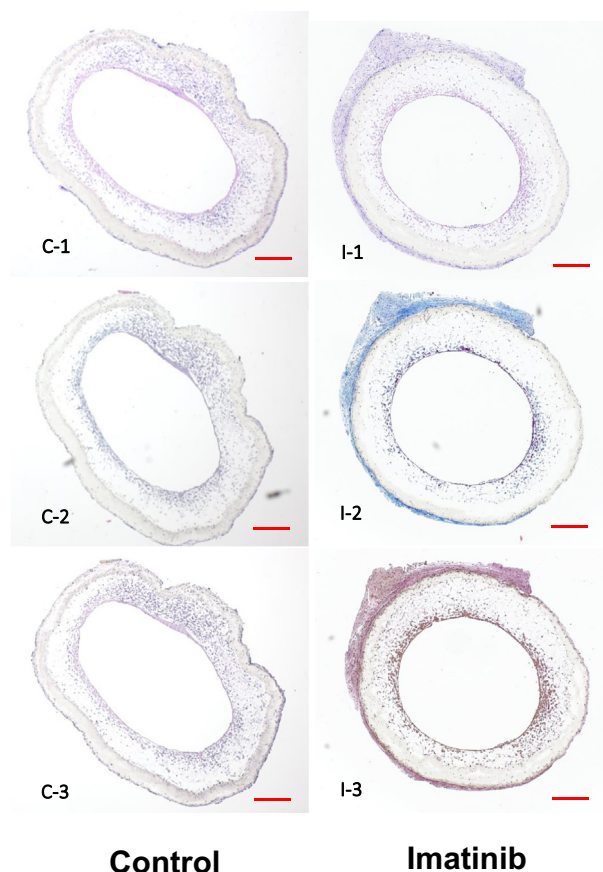


Fig 4. Histomorphologic images of bioresorbable vascular graft (BVG) explanted at 3 weeks in the control and imatinib group (**C-1, I-1** stain: hematoxylin and eosin; **C-2, I-2** stain: Masson's trichrome; **C-3, I-3** stain: Hart's). Bar scale = 200 μ m.

microscope (Zeiss AXIO Observer Z1, Oberkochen, Germany).

Assessment of BVGs after implantation. At 8 weeks after BVG implantation, the inner diameter of BVGs were measured by ultrasound examination (Vevo Visualsonics 770; Visualsonics, Toronto, Ontario, Canada). Before ultrasound examination, mice were anesthetized with 1.5% inhaled isoflurane. In a nonblind process, the thickness and percent area of neotissue formation and inner diameter of BVGs were assessed from explanted BVGs at 8 weeks. To obtain the thickness of neotissue formation, assuming that the neovessel was a circle, the outer and inner perimeters of the neovessel were manually measured from hematoxylin and eosin staining using ImageJ. Next, the outer and inner diameters were acquired from the outer and inner perimeters using the ratio of the circumference of a circle to its diameter. Then the thickness were measured as the half of the difference between outer and inner diameter. In addition, neotissue formation area and BVG area were also manually measured to obtain the percent area of neotissue

formation. The measurements were performed from two histologic section per graft in the center part of the explanted BVG. In this study, neotissue formation was defined as the cell layer on the luminal side of BVG. In addition, thickness of SMC layer were manually measured in each part of BVG divided in quarters.

Statistical analysis. The sample size estimation was based on the following assumptions: a two-side alpha level of 0.05 and power of 80% and percent area of neotissue formation of 10% in the control group and 7% in the imatinib group, and a standard deviation of 2.5%. Accordingly, a sample size of 10 mice in each group was calculated. We prepared a total of 30 mice for 8-week follow-up to accommodate for missing data. Numeric values are listed as mean \pm standard deviation. The number of experiments is shown in each case. When applicable, the nonparametric Mann-Whitney *U* test was used to measure the statistical significance between two groups. Fisher's exact probability test was used for dichotomous variables between two groups (graft patency). A *P* value of less than .05 indicated statistical significance. All statistical analyses were performed by GraphPad Prism version 7.03 (GraphPad Software, Inc, La Jolla, Calif).

RESULTS

Graft patency. The mortality rate for mice followed for 3 weeks was 0%, and none of the grafts were occluded on histology. In contrast, of the mice followed for 8 weeks, three in the imatinib group and two in the control group were humanely killed within several days of implantation, because of paraplegia owing to acute BVG thrombosis. Therefore, the patency rate of the mice followed for 8 weeks was 80.0% (12/15) in the imatinib group, compared with 86.7% (13/15) in the control (*P* > .999). There were no occurrences of severe stenosis (defined as >50% stenosis), aneurysmal formation, or graft rupture in the mice that survived for 3 and 8 weeks.

Neotissue formation and luminal diameter of explanted BVGs. The neotissue in the control group was significantly thicker than in the imatinib group ($30.1 \pm 7.2 \mu$ m vs $19.6 \pm 4.5 \mu$ m [*P* = .001] *n* = 13 control group; *n* = 12 imatinib group; Fig 2, A). The percent area of neotissue formation in the control group was also significantly larger than in the imatinib group ($9.8 \pm 2.7\%$ vs $6.8 \pm 1.8\%$ [*P* = .005]; *n* = 13 control group; *n* = 12 imatinib group; Fig 2, B). In contrast, the inner diameter of explanted BVGs was statistically similar in both groups (Fig 3). Based on these findings, neotissue formation is believed to advance toward the outer surface as the scaffold polymer degrades. In this study, we confirmed the repeatability of the results by measuring and comparing the data three times.

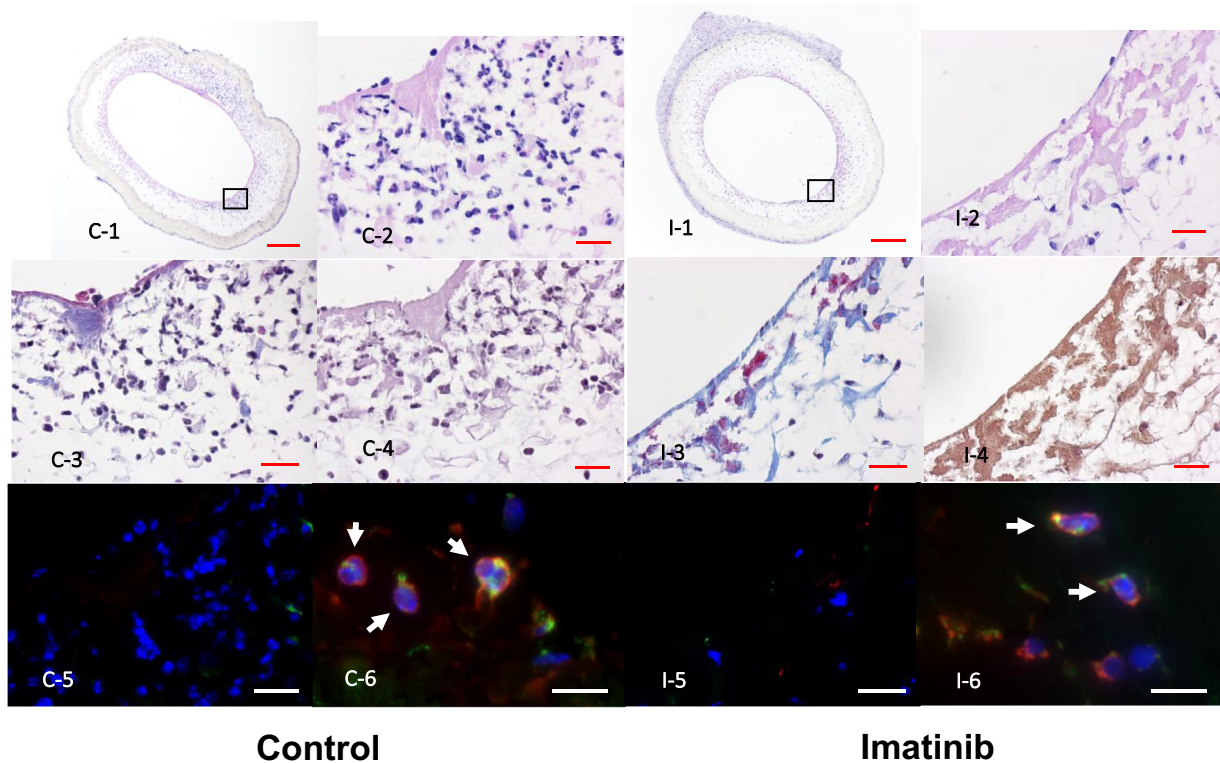


Fig 5. Histomorphologic images of bioresorbable vascular graft (BVG) explanted at 3 weeks in the control and imatinib groups (C-1, C-2, I-1, I-2 stain: hematoxylin and eosin; C-3, I-3 stain: Masson's trichrome; C-4, I-4 stain: Hart's; C-5, I-5: immunofluorescent staining; green = CD31, red = α -SMA; C-6, I-6: high magnitude immunofluorescent staining; green = Mac-3, red = inducible nitric oxide synthase). Bar scale = 200 μ m (C-1, I-1), 20 μ m (C-2-6, I-2-6).

BVG remodeling. Immunohistologic staining with low- and high-power magnifications showed some cells with nuclei had migrated into the BVG of both groups at 3 weeks (Figs 4 and 5). At the very early phase, a majority of the cells that have migrated into BVG are inflammatory cells, which also include monocytes and macrophages.¹⁸ In this study, Mac-3 and inducible nitric oxide synthase double-positive cells defined as M1 macrophage were also observed at 3 weeks in both groups (Fig 5, C-6, I-6). At 3 weeks, typical vascular structures such as intima, media, and adventitia did not form in either group. Cell number was evaluated by counting nuclei, and there was no significant difference in cell number between two groups at 3 weeks (2123 ± 528 cells/BVG vs 2270 ± 416 cells/BVG in the imatinib group [$n = 12$] vs the control group [$n = 13$]; $P = .960$). In contrast, histomorphologic images with low- and high-power magnification showed neotissue formation on the luminal side of BVG in both groups at 8 weeks (Figs 6 and 7); however, the thickness was quite different between both groups, as described elsewhere in this article. However, cell numbers were not significantly different between two groups at 8 weeks (2859 ± 933 cells/BVG vs 3094 ± 576 cells/BVG in the imatinib group [$n = 5$] vs the control group [$n = 5$]; $P = .152$).

BVG remodeling at 8 weeks showed endothelialization and development of a SMC layer. Endothelialization can prevent BVG thrombosis and the SMC layers can control vascular tonus and maintain vascular durability. ECs and SMCs were evaluated by immunofluorescent staining with CD 31 and α -SMA. On immunofluorescent staining, ECs and SMCs were not observed at 3 weeks in either group (Fig 5, C-5, I-5). However, ECs were partially covering the luminal side of BVG at 8 weeks in both groups (Fig 7, C-5, I-5). SMCs were subjectively thinner in the imatinib group than in the control group at 8 weeks (Fig 7, C-5, I-5). Furthermore, SMC layers were significantly thicker in the control group than in the imatinib group (Fig 8, A).

ECM deposition is also essential for BVG remodeling because it maintains vascular durability in collaboration with SMCs. ECMs are composed of proteoglycans, collagen, elastin, and other entities. In particular, collagen and elastin fibers provide the structural and biochemical support for vessel cells and tissues. In this study, collagen and elastin fibers were visualized by Masson's trichrome and Hart's stains, respectively. At 3 weeks, collagen fibers are rare in both groups (Fig 5, C-3, I-3), and elastin fibers are not observed in either group (Fig 5, C-4, I-4). At 8 weeks, however, collagen fibers were located

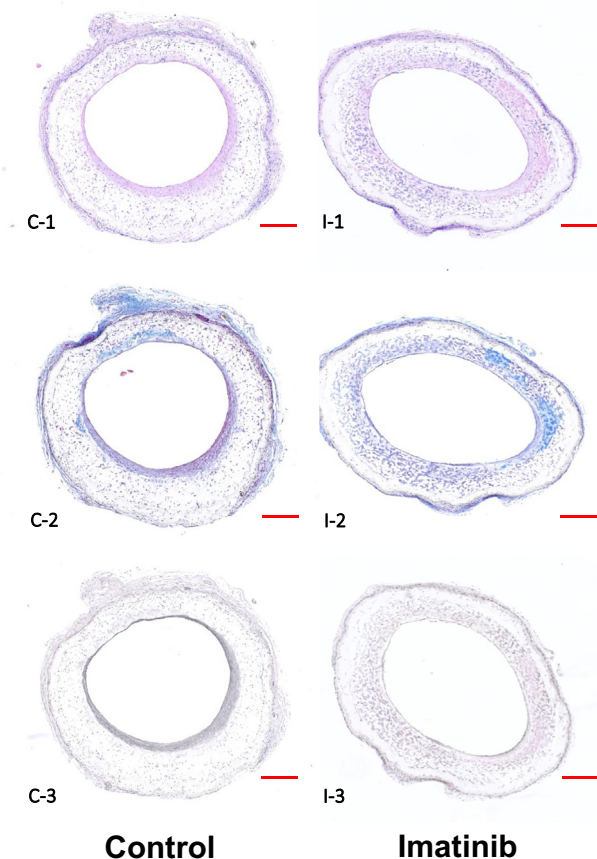


Fig 6. Histomorphologic images of bioresorbable vascular graft (BVG) explanted at 8 weeks in the control and imatinib groups (stain: stain hematoxylin and eosin; **C-2, I-2** stain: Masson's trichrome; **C-3, I-3** stain: Hart's). Bar scale = 200 μ m.

surrounding SMCs on the luminal side of BVG in both groups (Fig 7, C-3, I-3). In addition, collagen fibers in the control group are subjectively thicker than in the imatinib group. Also, elastin fibers were observed between SMC layers, and the elastin fibers in the control group were thicker with more layers than in the imatinib group (Fig 7, C-4, I-4). The percent area of collagen fiber in BVG area were significantly larger in the control group than in the imatinib group (Fig 8, B). However, the elastin fiber was not evaluated quantitatively in the Hart's staining, because the fiber volume was extremely small.

DISCUSSION

We demonstrated that the tyrosine kinase inhibitor, imatinib, attenuated neotissue formation during the vascular remodeling of arterial BVGs. Although the molecular mechanism of BVG remodeling remains unclear, these results suggest that imatinib attenuates the SMC layer formation and ECM deposition, which are the main components of neotissue formation in arterial BVG remodeling. Furthermore, we demonstrated that, as polymer degrades from the luminal side of BVGs,

neotissue advanced circumferentially toward the outer portion of the neovessel (Fig 9). These findings are important for the design of BVG that can achieve an optimal balance between polymer degradation and neotissue formation.

In the present study, all BVG except for those with acute thrombosis were patent without excessive neotissue formation at 8 weeks. However, the effect of PDGFs on arterial BVG in the late phase remains unknown. The objective of arterial BVG is to achieve well-organized vascular remodeling with an optimal trade-off between polymer degradation and neotissue formation to withstand arterial pressure. However, excessive vascular remodeling could lead to late-term BVG stenosis. SMCs are heterogeneous populations, including the typical contractile differentiated SMCs, which comprise the normal vascular wall, and synthetic dedifferentiated SMCs, which exist during development. The synthetic SMCs lead to SMC proliferation and ECM accumulation during the repair of vascular wall injury¹⁹ and PDGFs are among the factors implicated in SMC phenotype, switching from the contractile to the synthetic phenotype. In the early phase of BVG remodeling, synthetic SMCs are thought to be necessary for neotissue formation, and synthetic SMCs change to the contractile phenotype as the BVG remodels and matures. However, persistent PDGFs stimulation to SMCs may keep SMCs in the synthetic phenotype through the entire remodeling period. As the limitation in this study, synthetic SMCs were not stained. Thus, the switching time course from synthetic to contractile during the BVG remodeling was not elucidated. The effects of PDGFs on BVG remodeling may vary from positive neotissue formation to negative BVG stenosis, depending on the phase of vascular remodeling.

PDGF signaling is known to stimulate vascular SMCs proliferation and migration in atherosclerosis, and PDGF receptor kinase inhibitor reduced the intimal hyperplasia, characterized as SMC proliferation, in various restenosis models.^{9,11,20-22} Thus, we believe that imatinib, a PDGF receptor inhibitor, inhibits the SMC proliferation during BVG remodeling, as shown in various restenosis models. However, the present study did not elucidate whether SMC layer formation inhibition by imatinib was due to the death of SMCs, SMC proliferation inhibition, or less SMC migration into this BVG model. In contrast with various restenosis animal models, a double-blind randomized controlled clinical trial revealed that systemic imatinib therapy did not reduce the risk of in-stent restenosis. The authors postulated that the dose of imatinib (600 mg oral administration) might be insufficient to antagonize the local effects at the site of vascular injury.²³ In contrast, another study reported that an imatinib-incorporated nanoparticle-eluting stent attenuated in-stent neointimal formation in porcine coronary arteries.⁹

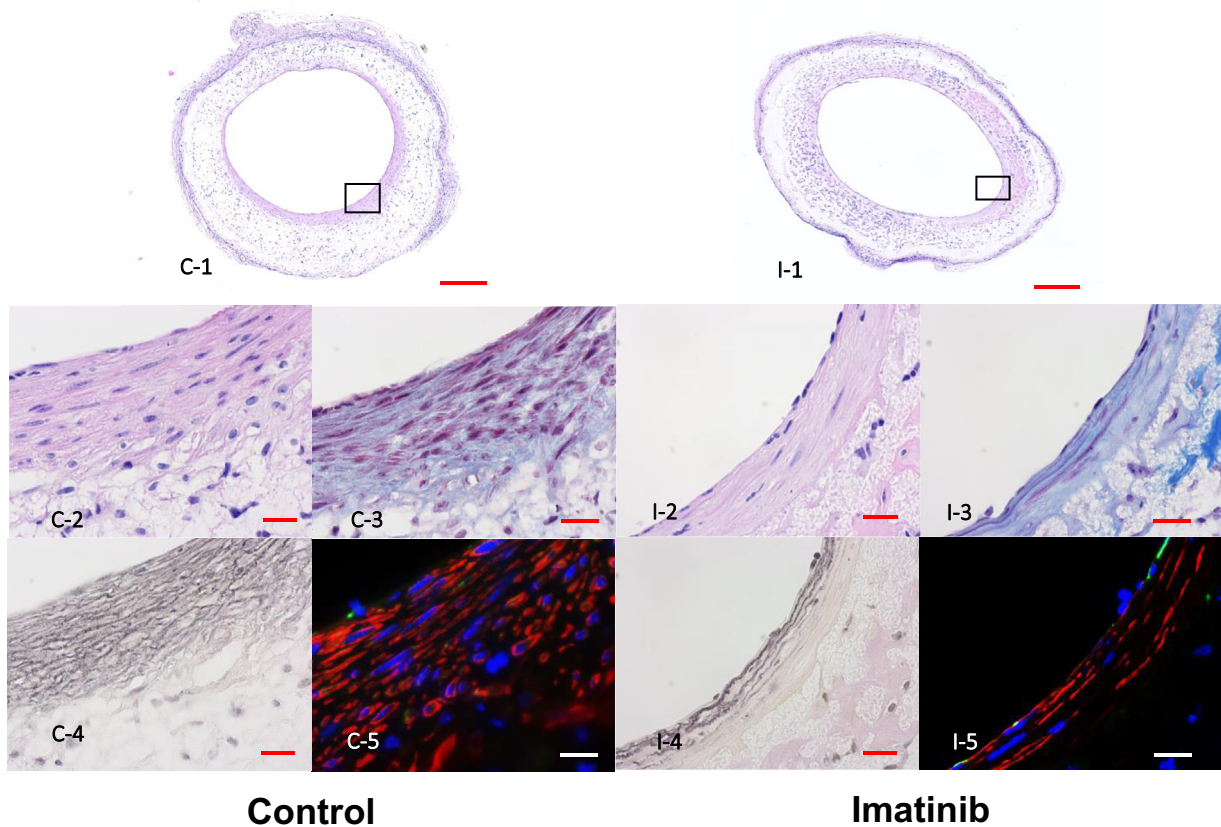


Fig 7. Histomorphologic images of bioresorbable vascular graft (BVG) explanted at 8 weeks in the control and imatinib groups (C-1, C-2, I-1, I-2 stain: hematoxylin and eosin; C-3, I-3 stain: Masson's trichrome; C-4, I-4 stain: Hart's; C-5, I-5: immunofluorescent staining, green = CD31, red = α -SMA) Bar scale = 200 μ m (C-1, I-1), 20 μ m (C-2-5, I-2-5).

In this study, SMCs formed on the luminal surface of BVG at 8 weeks. Several theories concerning the origin of SMC migration for neotissue formation in BVG remodeling have been suggested, such as transanastomotic infiltration of SMCs,²⁴ macrophages from the circulating blood,²⁵ bone marrow-derived vascular progenitor cells,¹² endothelial-mesenchymal transition as the transdifferentiation of ECs,^{15,18} and myofibroblasts derived from adventitia.²⁶ Although these possible mechanisms can explain the formation of the SMC layer in BVG remodeling, the significance and temporal change of these processes according to an advance of vascular remodeling has not been established. Augmentation of the SMC layer in the early phase of BVG remodeling through these processes is essential to achieving a physical balance between an advance of vascular remodeling and polymer degradation in a high-pressure environment. In addition, a recent study revealed that c-Kit-positive stem/progenitor cells repopulated neointimal SMCs and contributed to neointimal formation in an aortic allograft model.¹³ Thus, the negative effect of imatinib on SMC proliferation may be due to not only a PDGF receptor inhibitor, but also to a c-Kit receptor inhibitor.

We acknowledge some limitations in the present study. First, the present study is limited to short-term BVG remodeling data within 8 weeks after BVG implantation. The long-term natural history of arterial BVG remodeling remains unknown in this model and may yield different results. Second, we previously showed aneurysmal rupture was observed in 46% of implanted monolayered arterial BVGs.²⁷ Thereafter, we developed a bilayered BVG that contained an inner sponge layer and outer small pore electrospun layer to decrease the risk of aneurysmal rupture, and showed favorable results in murine model.²⁸ The present study showed that a large number of cells migrated into the luminal side of BVG and there were few cells on the adventitial side of BVG (Figs 4 and 6). On the basis of these histomorphologic findings in the present study and the previous venous BVG model study,²⁹ we conclude that the electrospun outer layer of the bilayered arterial BVG may inhibit cell migration into BVG owing to the small porosity of the outer layer. Thus, vascular remodeling of our arterial BVG may be delayed compared with a BVG without an outer electrospun layer. The vasa vasorum is thought to be another cell source for BVG remodeling. However, the vasa vasorum, defined as

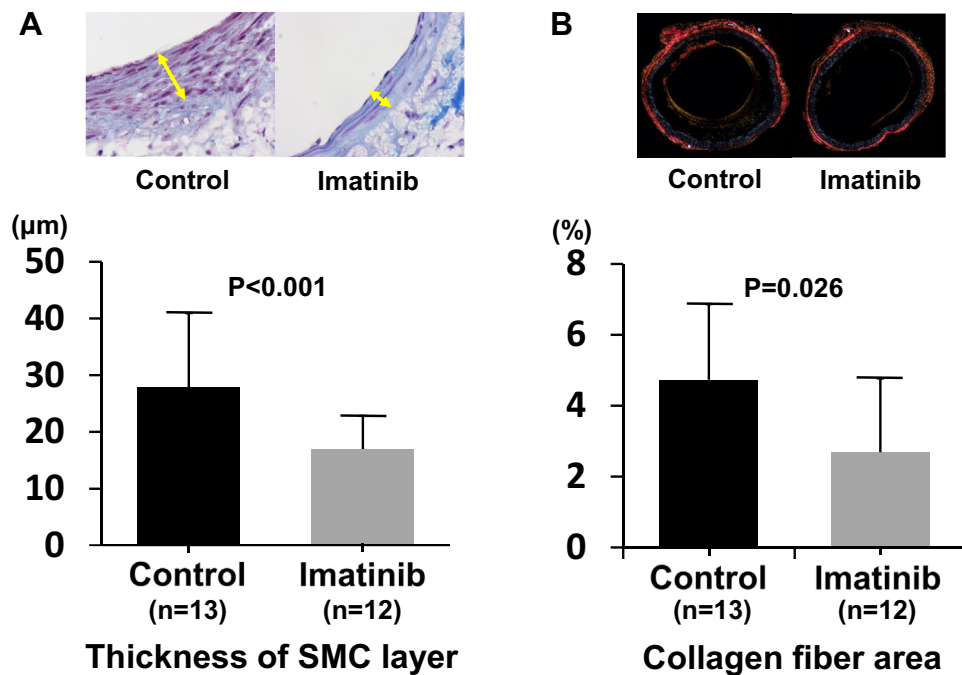


Fig 8. Thickness of the smooth muscle cell (SMC) layer (**A**) and collagen fiber area (**B**). The thickness of the SMC layer and collagen fiber area were measured using ImageJ from a microscopic image. The thickness of SMC layer and collagen fiber area in the imatinib group were significantly smaller than in the control group ($P < .001$ and, $P = .026$, respectively).

CD31-positive cells from capillaries formed into the neotissue or BVG layer, was not observed in the present study. We considered that that tightly knit electrospun fibers with small pores in our model might restrict the infiltration of vasa vasorum from outer side, in addition to inhibition of cellular migration. Third, imatinib is known to inhibit not only the PDGF receptor kinase and the c-kit receptor kinase, but also the bcr-abl tyrosine kinase.^{12,13} A study has reported that c-Abl tyrosine kinase is involved in angiotensin II-induced vascular SMCs hypertrophy.³⁰ Thus, other factors may also have an effect on BVG remodeling, including SMC proliferation.

Fourth, a direct effect of imatinib on the deposition of collagen and elastin in BVGs was shown in this study. The deposition of collagen and elastin were not observed at 3 weeks after BVG implantation, regardless of 2-week imatinib administration. However, imatinib administration resulted in the difference of thickness of collagen fibers between the groups at 8 weeks. Therefore, further studies are required to elucidate the indirect effect of imatinib on the deposition of collagen and elastin. Similarly, SMCs were also not observed at 3 weeks. A previous study revealed that α -SMA-positive cells were distributed loosely in the BVG within 14 days in a rat model.²⁶ The previous study's result is in contrast with our present study. The difference seems to be due to the structure of the two BVGs. As described elsewhere in this article, our bilayered arterial BVG may inhibit cell migration into the BVG owing to the small porosity of its outer layer.

Therefore, a study with delayed administration of imatinib should be performed to further provide information to the process of SMC layer formation. Fifth, this study demonstrated that neotissue advances circumferentially toward the outer portion of the neovessel; however, polymer degradation was not evaluated in this study. Furthermore, quantification and measurement were made on two histologic sections per graft in the center portion of the explanted BVGs. However, this may be insufficient, because the ends of the BVG will be possibly affected by cell migration from the adjacent native aorta. Thus, the process of BVG remodeling may be different among the middle and end portions of the BVGs. Sixth, all BVG implantations were performed in female mice. Female mice were preferred anatomically, because they do not have two testicular arteries that go down along the abdominal inferior vena cava. However, potential sex differences may exist. Finally, the optimal administration period of PDGFs to encourage BVG remodeling remains unknown. Thus, imatinib administration for 2 weeks may be inappropriate. As well as the period, the optimal dose of imatinib remains unknown. In previous studies, imatinib dose-dependently inhibited the proliferation of vascular SMC in rat injured arteries (6.0, 12.5, 25.0, and 50.0 mg/kg).^{11,31,32} However, intraperitoneal administration of 100 mg/kg imatinib in this study is much more than a clinically relevant dosage (10 mg/kg).³³ Thus, a 100 mg/kg dosage may result in unknown side effects.

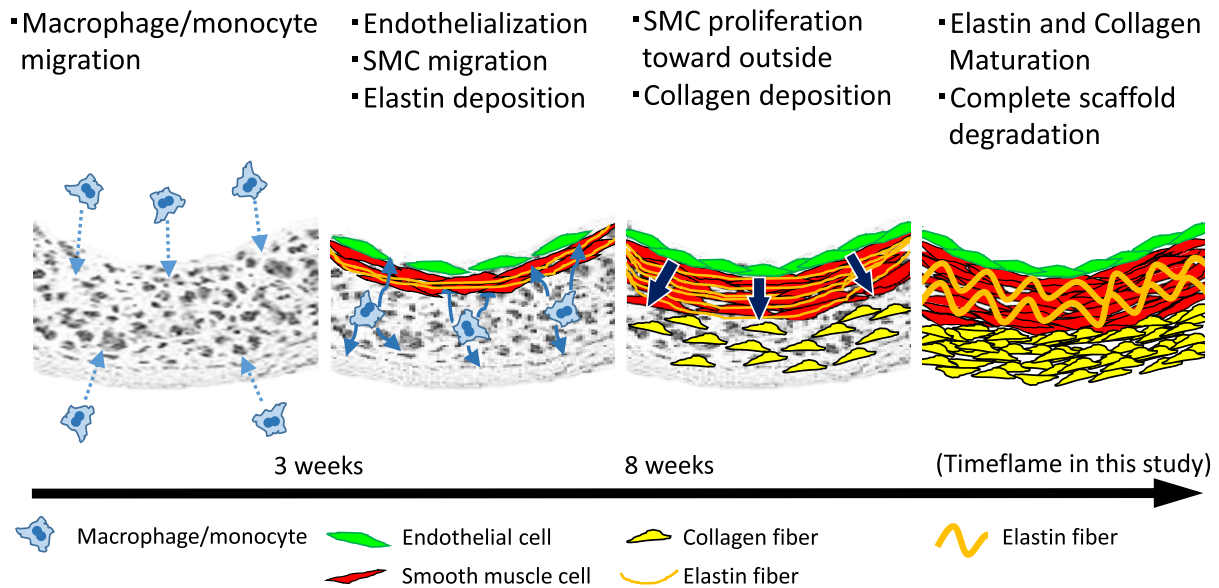


Fig 9. Proposed process of bioresorbable vascular graft (BVG) remodeling. (1) In the first step of the inflammatory process, macrophages/monocytes infiltrate into BVG. (2) Infiltrating macrophages/monocytes release multiple angiogenic cytokines and growth factors, which recruit SMCs and endothelial cells (ECs) to the luminal side of the scaffold. (3) ECs and SMCs organize into a mature blood vessel structure. In particular, SMCs proliferate and migrate circumferentially toward the outside of the BVG. (4) As the scaffold degrades completely, elastin and collagen mature to create an autologous neovessel.

CONCLUSIONS

Imatinib, a tyrosine kinase inhibitor, attenuated neotissue formation by inhibiting SMC layer formation and ECM deposition into arterial BVGs. In addition, as polymer degrades from the luminal side of BVGs, neotissue advanced circumferentially toward the outer portion of the neovessel.

The Morphology Core at Nationwide Children's Hospital performed sample embedding, sectioning, and immunohistochemical staining.

AUTHOR CONTRIBUTIONS

Conception and design: HM, ST, SO, SM, TSu, CB, TShi
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REFERENCES

- Suma H. Arterial grafts in coronary bypass surgery. *Ann Thorac Cardiovasc Surg* 1999;5:141-5.
- Shin'oka T, Imai Y, Ikada Y. Transplantation of a tissue-engineered pulmonary artery. *N Engl J Med* 2001;344:532-3.
- Hibino N, McGillicuddy E, Matsumura G, Ichihara Y, Naito Y, Breuer C, et al. Late-term results of tissue-engineered vascular grafts in humans. *J Thorac Cardiovasc Surg* 2010;139:431-6. 436.e1-436.e2.
- Drews JD, Miyachi H, Shinoka T. Tissue-engineered vascular grafts for congenital cardiac disease: clinical experience and current status. *Trends Cardiovasc Med* 2017;27:521-31.
- Ross R, Raines EW, Bowen-Pope DF. The biology of platelet-derived growth factor. *Cell* 1986;46:155-69.
- Deguchi J, Namba T, Hamada H, Nakaoka T, Abe J, Sato O, et al. Targeting endogenous platelet-derived growth factor B-chain by adenovirus-mediated gene transfer potently inhibits in vivo smooth muscle proliferation after arterial injury. *Gene Ther* 1999;6:956-65.
- Andrae J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. *Genes Dev* 2008;22:1276-312.
- He C, Medley SC, Hu T, Hinsdale ME, Lupu F, Virmani R, et al. PDGFRbeta signalling regulates local inflammation and synergizes with hypercholesterolaemia to promote atherosclerosis. *Nature Commun* 2015;6:7770.
- Masuda S, Nakano K, Funakoshi K, Zhao G, Meng W, Kimura S, et al. Imatinib mesylate-incorporated nanoparticle-eluting stent attenuates in-stent neointimal formation in porcine coronary arteries. *J Atheroscler Thrombos* 2011;18:1043-53.
- Onwuka E, Best C, Sawyer A, Yi T, Heuer E, Sams M, et al. The role of myeloid cell-derived PDGF-B in neotissue formation in a tissue-engineered vascular graft. *Regen Med* 2017;12:249-61.
- Myllarniemi M, Frosen J, Calderon Ramirez LG, Buchdunger E, Lemstrom K, Hayry P. Selective tyrosine kinase inhibitor for the platelet-derived growth factor receptor in vitro inhibits smooth muscle cell proliferation after reinjury of arterial intima in vivo. *Cardiovasc Drugs Ther* 1999;13:159-68.
- Sata M, Saiura A, Kunisato A, Tojo A, Okada S, Tokuhisa T, et al. Hematopoietic stem cells differentiate into vascular

- cells that participate in the pathogenesis of atherosclerosis. *Nat Med* 2002;8:403-9.
13. Ni Z, Deng J, Potter CMF, Nowak WN, Gu W, Zhang Z, et al. Recipient c-Kit lineage cells repopulate smooth muscle cells of transplant arteriosclerosis in mouse models. *Circ Res* 2019;125:223-41.
 14. Roh JD, Nelson GN, Brennan MP, Mirensky TL, Yi T, Hazlett TF, et al. Small-diameter biodegradable scaffolds for functional vascular tissue engineering in the mouse model. *Biomaterials* 2008;29:1454-63.
 15. Duncan DR, Chen PY, Patterson JT, Lee YU, Hibino N, Cleary M, et al. TGFbetaR1 inhibition blocks the formation of stenosis in tissue-engineered vascular grafts. *J Am Coll Cardiol* 2015;65:512-4.
 16. Mirensky TL, Nelson GN, Brennan MP, Roh JD, Hibino N, Yi T, et al. Tissue-engineered arterial grafts: long-term results after implantation in a small animal model. *J Pediatr Surg* 2009;44:1127-32; discussion: 1132-3.
 17. Junqueira LC, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem J* 1979;11:447-55.
 18. Lee YU, de Dios Ruiz-Rosado J, Mahler N, Best CA, Tara S, Yi T, et al. TGF-beta receptor 1 inhibition prevents stenosis of tissue-engineered vascular grafts by reducing host mononuclear phagocyte activation. *FASEB J* 2016;30:2627-36.
 19. Muto A, Fitzgerald TN, Pimiento JM, Maloney SP, Teso D, Paszkowiak JJ, et al. Smooth muscle cell signal transduction: implications of vascular biology for vascular surgeons. *J Vasc Surg* 2007;45(Suppl A):A15-24.
 20. Kimura S, Egashira K, Nakano K, Iwata E, Miyagawa M, Tsujimoto H, et al. Local delivery of imatinib mesylate (STI571)-incorporated nanoparticle ex vivo suppresses vein graft neointima formation. *Circulation* 2008;118(14 Suppl): S65-70.
 21. Ferns GA, Raines EW, Sprugel KH, Motani AS, Reidy MA, Ross R. Inhibition of neointimal smooth muscle accumulation after angioplasty by an antibody to PDGF. *Science* 1991;253:1129-32.
 22. Banai S, Wolf Y, Golomb G, Pearle A, Waltenberger J, Fishbein I, et al. PDGF-receptor tyrosine kinase blocker AG1295 selectively attenuates smooth muscle cell growth in vitro and reduces neointimal formation after balloon angioplasty in swine. *Circulation* 1998;97:1960-9.
 23. Zohlnhofer D, Hausleiter J, Kastrati A, Mehilli J, Goos C, Schühlen H, et al. A randomized, double-blind, placebo-controlled trial on restenosis prevention by the receptor tyrosine kinase inhibitor imatinib. *J Am Coll Cardiol* 2005;46: 1999-2003.
 24. Hibino N, Villalona G, Pietris N, Duncan DR, Schoffner A, Roh JD, et al. Tissue-engineered vascular grafts form neovessels that arise from regeneration of the adjacent blood vessel. *FASEB J* 2011;25:2731-9.
 25. Talacua H, Smits AI, Muylaert DE, van Rijswijk JW, Vink A, Verhaar MC, et al. In Situ tissue engineering of functional small-diameter blood vessels by host circulating cells only. *Tissue Eng Part A* 2015;21:2583-94.
 26. Wang Z, Cui Y, Wang J, Yang X, Wu Y, Wang K, et al. The effect of thick fibers and large pores of electrospun poly(epsilon-caprolactone) vascular grafts on macrophage polarization and arterial regeneration. *Biomaterials* 2014;35: 5700-10.
 27. Tara S, Kurobe H, Maxfield MW, Rocco KA, Yi T, Naito Y, et al. Evaluation of remodeling process in small-diameter cell-free tissue-engineered arterial graft. *J Vasc Surg* 2015;62:734-43.
 28. Sugiura T, Tara S, Nakayama H, Yi T, Lee YU, Shoji T, et al. Fast-degrading bioresorbable arterial vascular graft with high cellular infiltration inhibits calcification of the graft. *J Vasc Surg* 2017;66:243-50.
 29. Miyachi H, Reinhardt JW, Otsuru S, Tara S, Nakayama H, Yi T, et al. Bone marrow-derived mononuclear cell seeded bioresorbable vascular graft improves acute graft patency by inhibiting thrombus formation via platelet adhesion. *Int J Cardiol* 2018;266:61-6.
 30. Ushio-Fukai M, Zuo L, Ikeda S, Tojo T, Patrushev NA, Alexander RW. cAbl tyrosine kinase mediates reactive oxygen species- and caveolin-dependent ATP receptor signaling in vascular smooth muscle: role in vascular hypertrophy. *Circ Res* 2005;97:829-36.
 31. Leppanen O, Rutanen J, Hiltunen MO, Rissanen TT, Turunen MP, Sjoblom T, et al. Oral imatinib mesylate (STI571/gleevec) improves the efficacy of local intravascular vascular endothelial growth factor-C gene transfer in reducing neointimal growth in hypercholesterolemic rabbits. *Circulation* 2004;109:1140-6.
 32. Makiyama Y, Toba K, Kato K, Hirono S, Ozawa T, Saigawa T, et al. Imatinib mesilate inhibits neointimal hyperplasia via growth inhibition of vascular smooth muscle cells in a rat model of balloon injury. *Tohoku J Exp Med* 2008;215: 299-306.
 33. Peng B, Hayes M, Resta D, Racine-Poon A, Druker BJ, Talpaz M, et al. Pharmacokinetics and pharmacodynamics of imatinib in a phase I trial with chronic myeloid leukemia patients. *J Clin Oncol* 2004;22:935-42.

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