Diverse Contribution of Bone Marrow Cells to Neointimal Hyperplasia After Mechanical Vascular Injuries

Kumie Tanaka, Masataka Sata, Yasunobu Hirata, Ryozo Nagai

Abstract—We and others have suggested that bone marrow–derived progenitor cells may contribute to the pathogenesis of vascular diseases. On the other hand, it was reported that bone marrow cells do not participate substantially in vascular remodeling in other experimental systems. In this study, three distinct types of mechanical vascular injuries were induced in the same mouse whose bone marrow had been reconstituted with that of GFP or LacZ mice. All injuries are known to cause smooth muscle cell (SMC) hyperplasia. At 4 weeks after wire-mediated endovascular injury, a significant number of the neointimal and medial cells derived from bone marrow. In contrast, marker-positive cells were seldom detected in the lesion induced by perivascular cuff replacement. There were only a few bone marrow–derived cells in the neointima after ligation of the common carotid artery. These results indicate that the origin of intimal cells is diverse and that contribution of bone marrow–derived cells to neointimal hyperplasia depends on the type of model.

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Key Words: bone marrow □ smooth muscle cells □ atherosclerosis □ restenosis □ inflammation

There is accumulating evidence that adult tissues may contain multipotent stem cells that can differentiate into various lineages, beyond their differentiative and regenerative potential for the tissues in which they reside. Many studies documented that somatic stem cells contributed to regeneration and remodeling of remote organs. On the other hand, recent reports have cast doubt on the pluripotential of adult tissues in vivo under physiological conditions. Similarly, it was reported that bone marrow does not contribute substantially to vascular remodeling in some experimental systems, whereas we and others have suggested that bone marrow cells can participate in the pathogenesis of vascular diseases in models of graft vasculopathy, postangioplasty restenosis, and hyperlipidemia-induced atherosclerosis.

We are concerned that such opposite conclusions have been drawn using different experimental systems to test hypotheses.

The present study was undertaken to determine whether bone marrow contribution to neointimal hyperplasia depends on the type of model. After three distinct types of mechanical injuries were induced in a single mouse, the proportion of bone marrow–derived cells in the vascular lesions was compared. Results demonstrate that the origin of neointimal cells is diverse and that the mode of injury is important for the recruitment of bone marrow cells to vascular remodeling.

Materials and Methods

Animals

ROSA26 mice, which are knock-in mice expressing the LacZ gene in essentially all tissues (C57BL/6×129S background), were originally purchased from Jackson Laboratory (B6; 129S-Gtrosa26, Stock Number 002073, Bar Harbor, Maine). ROSA26 mice were maintained in our animal facility and intercrossed with C57BL/6J mice. The resulting littermates were used for this study. All wild-type mice were purchased from SLC (Shizuoka, Japan). Transgenic mice (C57BL/6 background) that ubiquitously express enhanced green fluorescent protein (GFP mouse) were a generous gift from Dr Masaru Okabe (Osaka University, Osaka, Japan). ApoE-deficient mice (ApoE−/− mice) were purchased from Jackson Laboratory. All procedures involving experimental animals were performed in accordance with protocols approved by the institutional committee for animal research of The University of Tokyo and complied with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1985).

Bone Marrow Reconstitution

Bone marrow transplantation (BMT) was performed as described previously. Bone marrow cells were harvested from femora and tibias of donor mice as already described. Eight- to 10-week-old male wild-type mice or ApoE-deficient mice were lethally X-irradiated with a total dose of 9.5 or 8.8 Gy (MBR-1520RB, Hitachi), respectively. One day later, the recipient mice received unfractionated bone marrow cells (3×10⁶) suspended in 0.3 mL PBS by tail vein injection. Twenty-seven to 30 weeks after BMT, three distinct types of mechanical injuries were induced in the recipient...
mice. Peripheral leukocytes (80% to 90%) had been reconstituted as determined by flow cytometry (BMT<sup>GFP</sup>→apoE mice or BMT<sup>GFP</sup>→Wild mice) or FISH (fluorescence in situ hybridization) for Y-chromosome (BMT<sup>GFP</sup>→Fmales mice).

**Wire-Mediated Endovascular Injury**
Transluminal arterial injury was induced by inserting a straight spring wire (0.38 mm in diameter, No. C-SF-15-15, COOK) into the left femoral artery as already described. After the wire was left in place for 1 minute to denude and dilate the artery. A copy of the tutorial video of the surgical procedure can be seen on request; alternatively, the video can be viewed at http://plaza.umin.ac.jp/~msata/.

**Cuff-Mediated Perivascular Injury**
A cuff-mediated vascular injury was induced by placing a polyethylene tube around the right femoral artery. The distal left common carotid artery was completely ligated just proximal to the bifurcation with a spring wire (0.38 mm in diameter, No. C-SF-15-15, COOK) into the right femoral artery from the surrounding tissues, a tube (2-mm PE-50; Becton-Dickinson) was opened longitudinally, loosely placed around the artery and then closed with sutures.

**Flow-Restriction Vascular Injury**
A flow-restriction vascular injury was caused by ligating the bifurcation of the left common carotid artery. The distal left common carotid artery and its bifurcation into the external and internal carotid arteries were exposed using minimal dissection. The common carotid artery was completely ligated just proximal to the bifurcation with a 6-0 silk suture. All animals recovered and showed no symptom of a stroke.

**Plastic Embedding to Detect GFP Signal**
Four weeks after surgery, mice were euthanized with overdose of pentobarbital and perfused at a constant pressure via the left ventricle with 0.9% sodium chloride solution, followed by perfusion fixation with 4% paraformaldehyde in PBS. The injured arteries were further fixed in 4% paraformaldehyde overnight at 4°C. The arteries were illuminated with a GFP-lighting system (Illumino atom Tunnelable Lighting System, LT-9800, Lightools Research) and observed using a cooled CCD camera (VB-6010, Keyence). To preserve GFP signal for histological analyses, the arteries were embedded in plastic resin (Technovit 8100, Heraeus Kulzer) according to the manufacturer’s instructions. Briefly, the arteries were washed overnight in PBS containing 6.8% sucrose at 4°C, dehydrated in 100% acetone, and embedded in a 2% Energy Beam Sciences, Inc) as a mold. The polymerized block was fixed onto a block (Histobloc, Heraeus Kulzer) with an adhesive agent (EP001, Semedain) and cut using a rotary microtome (HM335E, Microm International GmbH) with a disposable knife (Histoknife, Heraeus Kulzer). Thin sections (3 to 4 μm) were stretched in a water bath, mounted on silanized slides (Matsumani), and dried for 2 hours at 37°C. The sections were washed in PBS and used for immuno-fluorescence studies.

**Detection of LacZ**
LacZ was detected as described. After perfused with 0.9% sodium chloride solution, the arteries were excised and stained with X-gal solution containing 1 mg/mL X-gal, 2 mmol/L MgCl, 5 mmol/L K<sub>2</sub>Fe(CN)<sub>6</sub>, 5 mmol/L K<sub>3</sub>Fe(CN)<sub>6</sub>, 0.01% sodium deoxycholate, and 0.02% NP40 in PBS at 37°C overnight, further fixed in 4% paraformaldehyde overnight at 4°C, and embedded in paraffin. Thin sections (5 μm) were deparaffinized and blocked with 0.5% horse serum. Endogenous biotin and binding proteins were blocked with a blocking kit (Vector Laboratories). Sections were counterstained with hematoxylin.

**Immunohistochemistry**
Paraffin-embedded sections (5 μm thick) were deparaffinized and blocked with 0.5% horse serum. Endogenous biotin and binding proteins were blocked with a blocking kit (Vector Laboratories). The sections were incubated with anti-VWF (clone F8/86, BD Biosciences; anti-CD45, clone 30-F11, BD Biosciences; anti–von Willebrand cell antigen, clone MECA-32, BD Biosciences; anti-CD34, clone 30-F11, BD Biosciences; or anti-smooth muscle myosin heavy chain, clone HSM-5, Sigma; anti–smooth and skeletal myosin polyclonal antibody, Sigma) followed by incubation with FITC, Rhodamine, or Cy3-conjugated secondary antibodies (Jackson ImmunoResearch). When mouse monoclonal antibody was used, the M.O.M immunodetection kit (Vector) was used. Endothelial cells were identified using the biotinylated Bandeiraea simplicifolia Lectin 1 (BS-Lectin 1, Vector Laboratories) followed by incubation with Rhodamine-conjugated streptavidin (Immunotech, Cedex 9). Nuclei were counterstained with Hoechst 33258 (Sigma). The sections were mounted with the ProLong Antifade Kit (Molecular Probes) and observed under a confocal microscope (FLUOVIEW FX300, Olympus).

**Transmission Electron Microscopy**
The femoral arteries were excised, stained with X-gal solution overnight, and fixed in 2% glutaraldehyde and 2.5% paraformaldehyde. Samples were postfixed in 1% osmium tetroxide, dehydrated, and embedded in epoxy resin (Epon 812). Thin sections were stained with 3% uranyl acetate and examined with an electron microscope (H-7000, Hitachi, Tokyo). No lead citrate was used for counterstaining.

**Statistics**
All data are expressed as mean±SEM.

**Results**
Diverse Contribution of Bone Marrow Cells to Neointimal Hyperplasia
BMT was performed from GFP mice to wild-type (BMT<sup>GFP</sup>→Wild mice) or ApoE-deficient mice (BMT<sup>GFP</sup>→ApoE mice). After 12 to 32 weeks, three models of vascular injuries were induced in a single BMT mouse. At 4 weeks after surgery, GFP-positive (GFP<sup>+</sup>) cells accumulated in the injured arteries of BMT<sup>GFP</sup>→Wild mice under a fluorescence illuminator (Figure 1A). No GFP<sup>+</sup> cell was observed in uninjured artery of BMT<sup>GFP</sup>→ApoE mice (Figure 1B). In the left
femoral artery where the wire had been inserted, GFP+ cells were detected in the neointima (38.9 ± 5.8%) and media (61.4 ± 5.8%) (n = 4, Table 1). A significant percentage of α-smooth muscle actin-positive (α-SMA+) cells expressed GFP in neointima (26.6 ± 6.4%) and media (35.4 ± 9.6%) (Figure 1C, Table 1). Cuff-replacement around the right femoral artery induced neointimal hyperplasia that was exclusively composed of SMCs. Notably, most of the medial cells were negative for α-SMA. A smaller number of GFP+ was found in the neointima (7.0 ± 2.1%) and media (15.1 ± 2.2%). Few GFP+ cells expressed α-SMA. In the common carotid artery, neointimal hyperplasia was caused by proliferation of α-SMA-positive cells. GFP+ cells were found in the neointima (24.1 ± 5.3%) and media (33.1 ± 8.2%). A few GFP+ cells expressed α-SMA. Consistent with the results obtained in BMTGFP→ApoE mice, there were no LacZ+ cells in uninjured artery of BMTLacZ→Wild mice. A significant percentage of LacZ+ cells were found in the neointima (56.3 ± 7.8%) and media (54.3 ± 8.0%) after the wire injury (Figure 1C) (n = 5). We could readily detect LacZ+ cells that expressed α-SMA. In the right femoral artery, around which a polyethylene cuff had been placed, bone marrow–derived cells were seldom detected in the neointima, whereas many inflammatory cells in the adventitia expressed LacZ. There were only a few LacZ+ cells in the neointima of the common carotid artery after ligation.

Differentiation of Bone Marrow–Derived Cells to Vascular Cells After Wire Injury

Next, we characterized the bone marrow–derived cells observed in the femoral arteries after wire injury. In BMTGFP→ApoE mice, GFP+ cells on the luminal side were positive for endothelial markers (BS-lectin, CD31, or VWF) (Figure 2A). 42.9 ± 8.5% of VWF+ cells were GFP+ (Table 2). In neointima, abundant GFP+ cells expressed α-SMA (Figure 2B). In BMTLacZ→ApoE mice, LacZ+ cells were readily detected to express endothelial markers on the luminal side (Figure 2C) or α-SMA in the neointima. The ultrastructure of bone marrow–derived neointimal cells was examined in BMTLacZ→Wild mice. Without lead staining after X-gal staining, electron-dense crystallloid precipitates could be observed in cytoplasm of the cells from LacZ mice (Figure 2E), but not from wild-type mice (Figure 2D). In the injured artery of BMTLacZ→Wild mice, LacZ+ cells were readily identified (Figure 2G), whose morphology was quite distinct from that of peripheral leukocytes (Figure 2F). These results suggest that bone marrow–derived cells differentiate into certain cell-types that contribute to vascular remodeling after wire injury.
Characterization of Bone Marrow–Derived α-SMA–Positive Cells

Bone marrow–derived α-SMA–positive cells were further characterized. Cross sections of BMT\(^{\alpha\text{-SMA}^{+}}\)Wild mice were stained for LacZ and various markers of SMCs. An anti–α-SMA antibody (clone 1A4) recognized more than half of the LacZ cells in the neointima (Figure 3A). On the other hand, a few LacZ-positive cells were stained for myosin (clone HSM-V, or polyclonal antibody) (Figure 3A).\(^1\) Double-immunofluorescence study revealed that most of the medial cells expressed both α-SMA and myosin in the uninjured artery, whereas a few α-SMA\(^{+}\) cells were positive for smooth muscle myosin in injured arteries (Figure 3B). Furthermore, immunoreactive α-SMA was expressed by some CD45-positive cells in the neointima (Figure 3C).

Vascular Changes Induced by the Vascular Injury

To understand the mechanism by which contribution of bone marrow cells to vascular remodeling depends on type of injury, the vascular changes induced by the injuries were investigated in C57BL/6 mice (n = 3). At 6 hours, wire injury caused complete endothelial denudation, marked enlargement of the lumen, and thinning of the media (Figure 4). TUNEL staining revealed massive apoptosis of the medial cells. Most of the medial SMCs were eliminated by the injury as determined by immunostaining for α-SMA.\(^6\) The cellularity of the media remained low until 1 or 2 weeks after injury. In the opposite femoral artery, we found some apoptotic cells in the media (39.1±9.9%) at 6 hours after cuff-replacement. The medial smooth muscle layer remained thick. The luminal side was coated with a monolayer of endothelial cells. Six hours after flow-restriction vascular injury, the endothelium and media remained almost intact with few TUNEL-positive cells (0.3±0.3%).

One week after wire injury, MCP-1, SDF-1, and VEGF were highly expressed in the left femoral artery (Figure 5). After cuff-replacement, chemokines were abundantly expressed in adventitia, but not in the vessel wall. After ligation of carotid artery, those factors were slightly expressed on the luminal side.

Inflammatory Response Induced by Vascular Injuries

At 4 weeks, inflammatory cell accumulation were identified by immunostaining for CD3ε (T cells) or F4/80 (macrophages) antigen in BMT\(^{\alpha\text{-SMA}^{+}}\)Wild mice (Table 3) (n=4). Inflammatory cells were predominantly macrophages in all models.\(^{16,18}\) Robust infiltration of macrophages was observed

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**Table 1. Frequency of GFP-Positive Cells in Neointima and Media per Cross Section 4 Weeks After Vascular Injuries in BMT\(^{\alpha\text{-SMA}^{+}}\)**

<table>
<thead>
<tr>
<th></th>
<th>Neointima</th>
<th>Media</th>
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<tbody>
<tr>
<td></td>
<td>No. of GFP(^{+}) Cells/No. of Cells Examined per Section</td>
<td>α-SMA+</td>
</tr>
<tr>
<td>Wire injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMT(^{\alpha\text{-SMA}^{+}}) mouse 1</td>
<td>137/308</td>
<td>65/181</td>
</tr>
<tr>
<td>BMT(^{\alpha\text{-SMA}^{+}}) mouse 2</td>
<td>38/85</td>
<td>29/74</td>
</tr>
<tr>
<td>BMT(^{\alpha\text{-SMA}^{+}}) mouse 3</td>
<td>79/366</td>
<td>32/236</td>
</tr>
<tr>
<td>BMT(^{\alpha\text{-SMA}^{+}}) mouse 4</td>
<td>140/314</td>
<td>49/278</td>
</tr>
<tr>
<td>Cuff</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMT(^{\alpha\text{-SMA}^{+}}) mouse 1</td>
<td>4/72</td>
<td>5/26</td>
</tr>
<tr>
<td>BMT(^{\alpha\text{-SMA}^{+}}) mouse 2</td>
<td>5/39</td>
<td>9/95</td>
</tr>
<tr>
<td>BMT(^{\alpha\text{-SMA}^{+}}) mouse 3</td>
<td>10/360</td>
<td>23/164</td>
</tr>
<tr>
<td>BMT(^{\alpha\text{-SMA}^{+}}) mouse 4</td>
<td>7/106</td>
<td>32/181</td>
</tr>
<tr>
<td>Ligation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMT(^{\alpha\text{-SMA}^{+}}) mouse 1</td>
<td>53/307</td>
<td>50/143</td>
</tr>
<tr>
<td>BMT(^{\alpha\text{-SMA}^{+}}) mouse 2</td>
<td>195/495</td>
<td>61/130</td>
</tr>
<tr>
<td>BMT(^{\alpha\text{-SMA}^{+}}) mouse 3</td>
<td>20/87</td>
<td>97/237</td>
</tr>
<tr>
<td>BMT(^{\alpha\text{-SMA}^{+}}) mouse 4</td>
<td>7/42</td>
<td>1/103</td>
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Three distinct types of mechanical injuries were induced in BMT\(^{\alpha\text{-SMA}^{+}}\) mice. Injured arteries were harvested at 4 weeks and embedded in plastic resin. Sections were stained with Cy3-conjugated anti–α-SMA antibody, followed by counterstaining with Hoechst 33258. Sections were observed under a fluorescence microscope. Cell number was counted in the neointima and media of a cross section of each artery. Frequency of GFP\(^{+}\) cells among α-SMA–positive cells in the wire-induced lesions is reported in right columns. Wire indicates endovascular injury model prepared by inserting a large wire; cuff, perivascular injury model prepared by placing a polyethylene tube; ligation, flow-restriction vascular injury model prepared by ligating the bifurcation of the left common carotid artery.
in neointima and adventitia after cuff-replacement. On the other hand, a few macrophages were detected in the vascular lesions induced by wire or ligation.

**Discussion**

In this study, three distinct types of mechanical injuries were induced in the same mouse whose bone marrow had been reconstituted with that of GFP or LacZ mice. After wire-mediated endovascular injury, a significant number of the neointimal and medial cells derived from bone marrow. In contrast, marker-positive cells were seldom detected in the lesion induced by perivascular cuff replacement. There were only a few bone marrow–derived cells in the neointima after ligation of the common carotid artery. The results of the present study suggest that the mode of injury is crucial for the recruitment of bone marrow–derived cells to tissue remodeling and that the potential participation of bone marrow cells in organ regeneration may be underestimated in certain types of experiments.

It remains unknown why contribution of bone marrow–derived cells to neointimal hyperplasia depends on the type of model. There was no correlation between the number of inflammatory cells in vessel wall and the degree of bone marrow cell contribution. Vascular inflammation should be essential, but may not be sufficient to recruit bone marrow–derived cells to lesions. After perivascular cuff-replacement or flow-restriction by ligation, endothelial cells and medial cells remained relatively intact with mild expression of MCP-1, SDF-1, and VEGF. Those minimal changes in vessel wall were associated with little contribution of bone marrow cells to neointimal hyperplasia. In contrast, wire injury induced complete endothelial denudation and medial cell loss due to apoptosis. The injury induces expression of MCP-1, SDF-1, and VEGF that may be important for homing of bone marrow–derived cells. It was observed that neointimal hyperplasia developed when the media remained acellular. It is most likely that bone marrow–derived cells must be recruited to repair the

**We induced wire-mediated endovascular injury in BMTGFp–Apoe mice.** Injured arteries were harvested at 4 weeks after the injuries and embedded in plastic resin. After endogenous mouse Ig was blocked, the sections were stained with anti–von Willebrand factor (VWF) antibody and Cy3-conjugated anti-mouse Ig antibody. Sections were counterstained with Hoechst 33342 and observed under a fluorescence microscope.
injured artery, when there are not enough local mesenchymal cells for the process.

Recent advances in gene-manipulating techniques have produced various genetically modified mice to determine the role of specific molecules in vascular remodeling, such as post-PCI restenosis. However, mouse arteries, unlike those of larger animals, are too small for transluminal injury with a balloon. Alternatively, several models of vascular injury have been shown to produce neointima-like hyperplasia and are used to evaluate the susceptibility of transgenic/knock-out mice to vascular lesion formation. Our findings suggest that we should be cautious about the difference in the mechanisms of neointimal hyperplasia when we compare findings obtained in different experimental systems.

Given the complexity of human atherosclerotic lesions, none of the vascular injury models would represent the exact pathogenesis of human vascular diseases. Previous reports suggested that SMCs in human vascular lesions are composed of cells of diverse origin and that the cellular constituents should differ depending on the type of vascular injury. Our findings suggest that bone marrow cells would substantially contribute to lesion formation when arteries are subjected to severe injuries. Advanced atherosclerotic lesions exhibit a higher incidence of internal elastic rupture and intimomedial interface damage, which are associated with focal intraplaque microhemorrhage. PCI denudes endothelium completely and mechanically dilates atherosclerotic lesions with a tear in the luminal surface. Circulating progenitors would be recruited to those severely injured human vessels. Consistent with this notion, an analysis of sex-mismatched bone marrow transplant subjects revealed that SMCs throughout the atherosclerotic vessel wall can derive from donor

**Figure 3.** Differentiation of bone marrow–derived cells in vascular lesions. A, Left femoral arteries of BMTLacZ mice were snap-frozen at 8 weeks after the wire injury and stained for LacZ (green) and markers of smooth muscle cells (red). Bar=10 μm. Anti-LacZ polyclonal antibody was used with monoclonal anti-myosin antibodies (clone 1A4, α-SMA; clone HSM-V, myosin heavy chain). An anti-LacZ monoclonal antibody (clone GAL-13) was used with anti-myosin polyclonal antibody. Arrowheads indicate double-positive cells. B, Uninjured and wire-injured femoral arteries of BMTLacZ mice were snap-frozen and stained for α-SMA (red) and myosin (green). Arrowheads indicate double-positive cells. Bar=20 μm. C, Wire-injured femoral arteries were stained for α-actin (red) and CD45 (D; green). Bar=5 μm. Arrow indicates a double-positive cell.

**Figure 4.** Tissue damage induced by the vascular injury. Cross sections of the arteries of C57BL/6J mice 6 hours after the surgery were analyzed. Total nuclei and apoptotic nuclei were stained with propidium iodide (PI, red) and TUNEL (green), respectively. Smooth muscle cells and endothelial cells were identified by immunostaining for α-SMA and CD31, respectively.16 Arrows and arrowheads indicate the internal and external elastic laminas, respectively. Scale bar=20 μm. Endothelial layer was preserved 6 hours after cuff injury and ligation (insets).
bone marrow\textsuperscript{2} and that these cells are extensively recruited in diseased compared with undiseased segments.

Most of the bone marrow–derived cells expressed α-SMA, but not markers for highly differentiated SMCs. Some CD45-positive cells also expressed α-SMA. These results indicate that bone marrow–derived cells present in the neointima easily express α-SMA even when they remain positive for hematopoietic markers. In contrast, it seems a rare event, if not at all, for bone marrow–derived cells to express markers of highly differentiated SMCs, at least within a few months after a wire injury. Consistently, it was reported that most of the bone marrow–derived cells detected in human atherosclerotic plaques expressed α-SMA-positive cells, but not calponin, a marker for differentiated SMCs.\textsuperscript{2}

In summary, our data clearly demonstrate that the contribution of bone marrow cells to vascular remodeling highly depends on the type of arterial injury. When we extrapolate data obtained in animal experiments to the pathogenesis of human diseases, we should be aware that the origin of vascular lesions is diverse and that distinct mechanisms may regulate neointimal formation in different models of vascular injury.

Acknowledgments

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TABLE 3. Number of Macrophages per Cross Section of Injured Arteries 4 Weeks After Vascular Injuries in BMT\textsuperscript{LacZ} Mice

<table>
<thead>
<tr>
<th></th>
<th>Neointima</th>
<th>Media</th>
<th>Adventitia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wire</td>
<td>3.3±0.3</td>
<td>4.0±0.6</td>
<td>23.3±4.9</td>
</tr>
<tr>
<td>Cuff</td>
<td>25.7±13.1</td>
<td>7.3±2.8</td>
<td>73.7±20.7</td>
</tr>
<tr>
<td>Ligation</td>
<td>16.7±4.1</td>
<td>2.0±1.2</td>
<td>8.3±1.7</td>
</tr>
</tbody>
</table>

In BMT\textsuperscript{LacZ} mice, the injured arteries were harvested at 4 weeks and embedded in paraffin. Macrophages were identified by immunohistochemistry using an anti-F4/80 antibody. Data are presented as the mean±SEM.

References


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