OPEN

Plastic and Reconstructive Surgery Advance Online Article

DOI: 10.1097/PRS.0000000000010980

Myth-busting the zone of injury concept: a prospective study on the vascular response to high-energy lower extremity trauma

Adas Cepas, MD[1,2]; Juha Kiiski, MD, PhD[1]; Marja Majava MD, PhD[1]; Ivana Kholová MD, PhD[3]; Ilkka Kaartinen MD, PhD[1]

1. Department of Musculoskeletal Surgery and Diseases, Tampere University Hospital and University of Tampere, Faculty of Medicine and Health Technology, Tampere, Finland
2. Department of Plastic and Reconstructive Surgery, Hospital of Lithuanian University of Health Sciences, Kaunas Clinics, Kaunas, Lithuania
3. Department of Pathology, Fimlab Laboratories and University of Tampere, Faculty of Medicine and Health Technology, Tampere, Finland

Corresponding author: Adas Cepas, MD Niveltie 21 D 23 Tampere, Finland

adas.cepas@tuni.fi

Financial Disclosure Statement:

Adas Cepas – no financial conflicts of interests to disclose;

Juha Kiiski – no financial conflicts of interests to disclose;

Marja Majava – no financial conflicts of interests to disclose;

Ivana Kholova – no financial conflicts of interests to disclose;

Ilkka Kaartinen – received a grant (No. 9AB018) from Competitive State Research Financing of the Expert Responsibility Area of Tampere University Hospital which was used to cover expenses related to preparation and processing of histological slides as well as language editing service.

Short Running Head (no more than 40 characters in length): Zone of injury concept

Abstract

Background: Although the zone of injury concept is widely accepted, no histologic studies of vessel wall changes causing the phenomena are reported. This prospective study investigated the vascular response to high-energy lower extremity trauma to evaluate the validity of the zone of injury concept.

Methods: The histologic appearance of arterial and venous walls in the zone of injury was studied in 19 patients (median age 46 [interquartile range 29.5–62.5] years) who underwent osteosynthesis and free-flap reconstruction due to high-energy lower extremity open fracture. Vascular samples were harvested from the injured extremity and control samples were harvested from the free-flap donor site. Histologic and morphometric characteristics of the vessels were analyzed microscopically and using digital pathology QuPath software.

Results: Vascular samples were harvested on post-injury days 1–11. Intimal thickness was >3 times greater in arteries harvested from the zone of injury than in control samples ($P<0.01$) and the intima/media ratio was 2-fold that in control samples ($P=0.01$). Arterial intimal fibrosis was more evident in vessels harvested from the zone of injury ($P<0.01$), but medial fibrosis and medial thickness did not differ significantly between groups. Venous intimal thickening ($P<0.01$) and the intima/media ratio ($P=0.02$) were superior in samples from the zone of injury. Fibrosis-related changes did not differ between groups (*P=0.45).*

Conclusions: These findings support the validity of the zone of injury concept by providing a novel histologic basis for this phenomenon. Intimal thickening and arterial intimal fibrosis are prominent histologic features of vessels affected by major lower extremity trauma.

Introduction

In reconstructive plastic surgery of the lower extremities, the zone of injury refers to the area surrounding the injured tissue where reactive changes occur due to the inflammatory response.[1] This concept was established in the early 1980s to explain the 20%–30% flap failure rate encountered in lower extremity free-flap surgery.[2] Pioneers in this field considered that blood vessels in the zone of injury are more prone to vasospasm and lack the thromboresistant properties of healthy vessels,[2,3] and thus recommended that surgeons avoid performing the anastomosis in this area due to an increased risk of flap failure. This notion has guided plastic surgeons to perform the anastomosis proximal to the injury site, or outside the zone of injury. Although this is a well-known principle among plastic surgeons, its biologic basis has not been unequivocally described.[4] Moreover, the assumed safe distance from the injury site to the anastomoses has decreased from earlier long incisions and vein grafts to just a few centimeters above the bony fracture site.[1,5–10]

Despite wide acceptance of the zone of injury concept, histologic studies of the vessel wall changes causing the phenomena have not been reported. In addition to the absence of studies in humans, only a few animal model studies on arterial wall remodeling after endovascular injury have been published.[11–14] These studies described 3 different pathways of intimal thickening secondarily to increased myofibroblast and medial smooth muscle cell activity in the intimal layer. The mechanism and extent of vascular injury in these animal model studies, however, are not comparable to those of high-energy blunt trauma of the lower extremity.

In the present study, we investigated the relevant histologic changes of arterial and venous walls in the zone of injury after high-grade open fracture of the lower extremity.

Materials and Methods

This prospective study investigating human vessel walls was conducted at Tampere University Hospital and Tampere University, Finland. A total of 19 patients who sustained high-energy open fractures of the lower extremities requiring free-flap reconstruction were included in the study: 16 open tibial fractures (IIIB or worse), 1 open femur fracture (IIIB), and 2 open fractures of the foot. The patients were operated on at Tampere University Hospital between March 2020 and October 2022 according to the institutional algorithm on open lower extremity fracture management. Except for 5 multi-trauma patients with concomitant injuries that were not fit for reconstruction during the first week, patients with acute open fractures underwent initial debridement and bony stabilization within 24 h of hospital admission, followed by definitive fixation and soft tissue reconstruction within a week.

At the time of the soft tissue reconstruction, 1 arterial and 1 venous biopsy sample from the injury site of traumatized lower extremity and 1 venous and arterial control sample from the free-flap donor site were collected from each patient. In case the recipient artery was transected during the injury, the arterial stump at the level of injury was trimmed and harvested for the study, and end-to-end anastomosis was performed several centimeters proximal to the injury site. If the integrity of the recipient artery was preserved, a side branch of recipient vessel was harvested from the injury site and end-to-side anastomosis was performed proximally, based on clinical judgement of recipient vessel quality and patency of the flow (Fig. 1). Venous anastomoses were always performed end-to-end, allowing for sample harvest at the most affected zones. Control samples harvested from the free-flap donor sites were flap pedicle vessels or their side branches that were size-matched to the vessels harvested from the zone of injury.

Histology

Vessel wall samples were fixed in 10% formalin at room temperature for 24 h and further processed to paraffin blocks. Slides of 5-µm thick vessel walls were prepared and stained with hematoxylin and eosin, Elastic Stain Kit (Verhoeff Van Gieson, Abcam, Cambridge, MA, USA), and Masson's trichrome (Sigma, Merck Life Science, Espoo, Finland) for histologic analysis. The histologic slides were scanned (NanoZoomer S360, Hamamatsu Photonics, Hamamatsu City, Japan). Both microscopy and the open-source digital pathology software QuPath were used for pathology image analysis.[15] Intimal and medial thicknesses were measured on Elastic Van Gieson-stained slides at the thickest part of the vessel wall circumference using the "line" feature. Interstitial fibrosis of intimal and medial layers was quantified by the semi-automated artificial intelligence-guided pixel classifier function. The classifier was trained on multiple images before its application for the analysis. To detect fibrosis and smooth muscle cells, specific thresholds were set for blue (collagen) and red-stained (smooth muscle cells) components in Masson's trichrome-stained sections, as shown in Fig. 2. The fibrosis of the vessel walls was calculated as a percentage of the annotated intimal or medial area.

Statistical analysis

Quantified data are presented as median and interquartile range (IQR). Proportions are given as percentage or ratio. The Mann Whitney-U test was used to test the statistical significance for continuous variables. A *P*-value of 0.05 was considered statistically significant. All statistical analyses were performed using SPSS statistics 24.0 (IMB, Armonk, NY, USA).

Results

General characteristics of the study population

The median age of the patients included in the study was 46 (IQR 29.5–62.5) years with 84% being male and 16% female. Of the 19 patients, 21% had cardiovascular comorbidities and

26% were smokers. The median time frame from injury to definitive fixation and soft tissue reconstruction was 6 days (IQR 3.5–8.5), with 79% of the patients operated on within the first 7 days after trauma. The remaining 5 patients were multi-trauma patients with concomitant injuries who underwent reconstruction on post-injury days 8–11. The most common external cause of injury was road traffic accident (42%), followed by a fall from height (37%) and forestry or heavy industry-related trauma (21%) (see Table, Supplemental Digital Content 1, which provides case-by-case data regarding demographics, flap reconstructions, and vessels harvested for the analysis).

Morphometric characteristics of the arterial walls

Histologic examination of the arterial walls revealed broad intimal hyperplasia in the zone of injury and control samples (18–206 μm vs 15–96 μm, respectively). The median intimal thickness of arteries harvested from the zone of injury, however, was >3 times greater than that in control samples (Table 1). Moreover, in the zone of injury, we observed a trend toward arterial intimal thickening related to the post-injury day of sampling (Fig. 3 a-h). Arterial intimal hyperplasia was mild in samples collected from the zone of injury on the first post-injury day, but already present in samples collected on post-injury day 3. The most prominent arterial intimal thickening was observed in samples harvested on days 6 and 7 following trauma, with a gradual decrease in intimal thickness in collected samples later. In contrast to these findings, median intimal thickness in the control samples remained unchanged regardless of the date of sampling or interindividual differences (Fig. 4). Although the arterial media thickness did not significantly differ between the zone of injury and control samples (P=0.11), the intima/media ratio was higher in the zone of injury samples (P=0.01) (Fig. 5). Despite the wide variation in arterial intimal fibrosis in the zone of injury (24%–83%) and control (5%–92%) samples, intimal fibrosis was more evident in arteries harvested from the zone of injury with a median fibrosis of

70% (IQR 60-80%) compared with 39% (IQR 21-55%) in control samples (*P*=0.01). Although median fibrosis of the arterial media was 51% (IQR 39-63%) in the zone of injury and 42% (IQR 31-54%) in control samples, the difference was not statistically significant (*P*=0.1).

There were no significant differences between the arterial walls of different donor sites. Moreover, recipient arteries in cases reconstructed with latissimus dorsi (LD) flaps or gracilis/anterolateral thigh (ALT) flaps showed no significant differences (see Table, Supplemental Digital Content 2. Morphometric characteristics of control sample arterial walls and recipient arteries in LD and gracilis & ALT flap reconstructions).

Morphometric characteristics of the venous walls

In contrast to the arteries, venous intimal hyperplasia was more evenly distributed between the zone of injury and control samples (13–70 μm vs 3–66μm). Despite the similar distribution range, the median venous intimal thickness was >2 times greater in the zone of injury than in the controls (*P*<0.01) (Table 2). In veins, unlike arteries, however, the median intimal thickness remained similar throughout the first 6 post-injury days with some outliers causing peaks on post-injury days 8 and 11 and valleys on post-injury days 7 and 10 (Fig. 6). Similar to arteries, venous medial thickness did not differ significantly between the zone of injury and control samples (*P*=0.23), but the intima/media ratio was higher in samples harvested from the zone of injury (*P*=0.01). Combined intimal and medial venous fibrosis ranged between 25%–74% in the zone of injury and 23%–85% in control samples, but no significant differences in the median intimal and medial fibrosis were detected between groups (*P*=0.45).

The medial thickness of LD flap pedicle vein was greater than that of the gracilis & ALT pedicle vein (p=0.03). Otherwise, no significant differences in vein wall morphometrics were observed (see Table, Supplemental Digital Content 3. Morphometric characteristics of control sample venous walls and recipient veins in LD and gracilis & ALT flap reconstructions).

Clinical outcomes

All vascular anastomoses were performed under microscope magnification. No free-flap losses or takebacks due to anastomotic problems were encountered in the study series. One patient required a kickstand frame to off-load and manage position-induced venous congestion, after which the flap healed uneventfully. The incidence of deep infection and non-union was 0%, but the follow-up period was short, ranging from 3 to 33 months. No major amputations were required.

Discussion

Existing literature on the zone of injury concept is based on observational studies and subjective interpretations addressing the quality of free-flap recipient vessels in traumatized lower extremities. Although the concept was first described in the 1980s, it still lacks biologic validity. This study was the first clinical study to evaluate histologic changes of the blood vessels affected by high-energy lower extremity trauma and provides the biologic basis of the zone of injury concept.

The most prominent finding in this study was vascular intimal hyperplasia, which was obvious in the samples harvested from the zone of injury. Our study data revealed that the vascular response to trauma had a sudden onset and resulted in intimal hyperplasia, which was particularly evident in arteries harvested on days 3–7 post-injury. These changes, however, were not as pronounced in veins (see Figure, Supplemental Digital Content 4, which shows evident arterial intimal thickening in the zone of injury sample (left) harvested on day 10 post-injury compared with a control sample (right) from a 31-year-old patient without concomitant comorbidities). In support of our study findings, intimal hyperplasia was reported in previous experimental animal model studies after simulated endovascular arterial injury.[11-14] Mechanical injury induced by the conventional balloon angioplasty procedure led to a shift of vascular

smooth muscle cells (VSMCs) from a contractile to a synthetic phenotype in the arterial media.[12] These synthetic VSMCs are present in the intima several days after vascular trauma and play a key role in intimal thickening by active migration, proliferation, and extracellular matrix secretion.[12,14] The arterial response to thermal injury seems to be different, although it also results in intimal thickening. In the Bayes-Genis experimental animal model study, monocytes/macrophages populated the neointima at early time-points after thermal endovascular injury, but at 4 weeks following injury, the thickened neointima was mostly populated with myofibroblasts and collagen fibers.[11] Bayes-Genis et al. hypothesized that blood-derived monocytes/macrophages are progenitors of collagen-secreting neointimal myofibroblasts rather than medial VSMCs or adventitial myofibroblasts as reported in previous studies.[11–13] Although intimal thickening mechanisms reported in animal model studies might also be valid in human vascular remodeling, our study results showed a more rapid vascular response to injury than described in the aforementioned animal model studies.

Another important finding regarding vessel wall quality was vessel wall fibrosis. Compared with control vessels, arteries harvested from the zone of injury were more fibrotic although the time from the injury to vessel harvest ranged from 1 to 11 days. High extracellular matrix deposits in the arterial intima suggest the presence and increased activity of pro-fibrotic cells such as myofibroblasts or fibroblasts. Although the incidence of arterial intimal fibrosis in samples harvested from the zone of injury was almost 2-fold greater than that in the control samples (P=0.01), those changes were not evident in the arterial media (P=0.1). In veins, surprisingly, combined medial and intimal fibrosis exhibited a lower trend in samples harvested from the zone of injury compared with controls (39% vs 48%, p=0.45), leading to a question of whether the onset of intimal fibrosis in the veins is slower, different, or nonexistent.

In our study the control samples were harvested from free flap pedicles of two different anatomical regions: trunk (LD flap) and thigh (Gracilis or ALT flap). One might suspect that vessel wall thickness is a consequence of the anatomical site. However, there were no significant differences noted between trunk and thigh flap arterial walls. This suggests that the vessel wall changes we described in this article are relevant to the zone of injury-concept and are in fact caused by the high energy trauma.

Although our study findings show gradual decrease in arterial intimal thickening past day 7 after the injury, our study did not investigate the endothelial function.

There is no clinical evidence suggesting that postponing reconstruction would result in better clinical/flap related outcomes.[16] On the other hand, delay in reconstruction is known to increase the risk of bony non-union, deep infection and major amputation rates and current guidelines advocate that the final soft-tissue reconstruction should be performed ideally within 72 hours after the injury.[17]

This study has several strengths. First, it is prospective in nature and is the first to describe the zone of injury concept from the vascular histology aspect. Second, we were able to compare the vascular morphology of injured vessels and healthy control vessels from the same patient, thereby avoiding interindividual differences. Third, variations in vessel harvest time-points allowed us to define the dynamics of the histologic changes in the vessel walls that seemed to be logical according to normal tissue remodeling after injury and findings from previous animal studies.

This study also has limitations. First, due to the rarity of this type of trauma, the sample size was relatively small. Second, the heterogeneity of the patients regarding age, sex, comorbidities, and other interindividual differences might affect the vascular wall histology. Third, because the extent of the zone of injury and the severity of the soft tissue injuries varied

among patients, it is possible that some samples were harvested from more severely injured sites compared with others and this could affect the extent of the vascular response, as observed by Shi et al.[13]

No flap losses were encountered after soft tissue reconstructions of severely injured lower extremities. Further studies at the cellular and molecular levels are needed to define the exact mechanisms of vascular intimal hyperplasia and especially how it might affect vascular patency in free-flap surgery in the setting of lower extremity trauma.

Conclusions

Our study findings support the validity of the zone of injury concept. The most prominent histologic features of the vessels affected by major lower extremity trauma are intimal thickening and arterial intimal fibrosis. Despite these histologic findings in affected vessels, excellent free-flap outcomes were achieved by placing the anastomoses based on clinical judgment of the vessel quality.

References

1. Isenberg JS, Sherman R. Zone of injury: a valid concept in microvascular reconstruction of the traumatized lower limb? *Ann Plast Surg*. 1996;36(3):270-272. doi:10.1097/00000637-199603000-00007
2. Godina M, Lister G. Early microsurgical reconstruction of complex trauma of the extremities. *Plast Reconstr Surg*. 1986;78(3):285-292. doi:10.1097/00006534-198609000-00001
3. Acland RD. Refinements in Lower Extremity Free Flap Surgery. *Clin Plast Surg*. 1990;17(4):733-744. doi:https://doi.org/10.1016/S0094-1298(20)30655-6
4. Loos MS, Freeman BG, Lorenzetti A. Zone of Injury: A Critical Review of the Literature. *Ann Plast Surg*. 2010;65(6). https://journals.lww.com/annalsplasticsurgery/Fulltext/2010/12000/Zone_of_Injury__A_Critical_Review_of_the.12.aspx.
5. Godina M, Arnez ZM, Lister GD. Preferential use of the posterior approach to blood vessels of the lower leg in microvascular surgery. *Plast Reconstr Surg*. 1991;88(2):287-291. doi:10.1097/00006534-199108000-00019
6. Bayramiçli M, Tetik C, Sönmez A, Gürünlüoğlu R, Baltaci F. Reliability of primary vein grafts in lower extremity free tissue transfers. *Ann Plast Surg*. 2002;48(1):21-29. doi:10.1097/00000637-200201000-00003
7. Kruse ALD, Luebbers HT, Grätz KW, Obwegeser J a. Factors influencing survival of free-flap in reconstruction for cancer of the head and neck: a literature review. *Microsurgery*. 2010;30(3):242-248. doi:10.1002/micr
8. Heller L, Levin LS. Lower Extremity Microsurgical Reconstruction. *Plast Reconstr Surg*. 2001;108(4).

https://journals.lww.com/plasreconsurg/Fulltext/2001/09150/Lower_Extremity_Microsurgical_Reconstruction.36.aspx.

9. Yazar S, Lin CH. Selection of recipient vessel in traumatic lower extremity. *J Reconstr Microsurg*. 2012;28(3):199-204. doi:10.1055/s-0032-1306366

10. Stranix JT, Borab ZM, Rifkin WJ, et al. Proximal versus Distal Recipient Vessels in Lower Extremity Reconstruction: A Retrospective Series and Systematic Review. *J Reconstr Microsurg*. 2018;34(5):334-340. doi:10.1055/s-0037-1621746

11. Bayes-Genis A, Campbell JH, Carlson PJ, Holmes DR, Schwartz RS. Macrophages, myofibroblasts and neointimal hyperplasia after coronary artery injury and repair. *Atherosclerosis*. 2002;163(1):89-98. doi:10.1016/S0021-9150(01)00771-7

12. Thyberg J. Phenotypic modulation of smooth muscle cells during formation of neointimal thickenings following vascular injury. *Histol Histopathol*. 1998;13(3):871-891.

13. Shi Y, O'Brien JE, Fard A, Mannion JD, Wang D, Zalewski A. Adventitial myofibroblasts contribute to neointimal formation in injured porcine coronary arteries. *Circulation*. 1996;94(7):1655-1664. doi:10.1161/01.CIR.94.7.1655

14. Wu W, Zhang W, Choi M, et al. Vascular smooth muscle-MAPK14 is required for neointimal hyperplasia by suppressing VSMC differentiation and inducing proliferation and inflammation. *Redox Biol*. 2019;22(January):101137. doi:10.1016/j.redox.2019.101137

15. Bankhead P, Loughrey MB, Fernández JA, et al. QuPath: Open source software for digital pathology image analysis. *Sci Rep*. 2017;7(1):1-7. doi:10.1038/s41598-017-17204-5

16. D'alleyrand JCG, Manson TT, Dancy L, et al. Is time to flap coverage of open tibial fractures an independent predictor of flap-related complications? *J Orthop Trauma*. 2014;28(5):288-293. doi:10.1097/BOT.0000000000000001

17. Eccles S, Handley B, Khan U, McFadyen I, Nanchachal S. Standards for the Management of Open Fractures - New BAPRAS guidelines. 2020.

Figure legends

Table 1. Morphometric characteristics of arterial walls in the zone of injury and control samples.

Table 2. Morphometric characteristics of venous walls in the zone of injury and control samples.

Figure 1. Harvest of arterial samples before anastomosis of the free-flap. (Left) Sample harvest procedure and anastomosis in arteries that were transected during the injury. (Right) Anastomosis and sample harvest procedure if the arterial integrity was preserved.

Figure 2. Measuring vessel wall fibrosis in Masson's trichrome-stained sections. (Left) arterial section with annotated intimal and medial layers. (Right) Same image after application of pixel classifier function (blue color represents collagen fibers).

Figure 3. Post-injury dynamics of arterial intimal thickening. Photos (a-h) represent paired arterial wall sections harvested from the zone of injury and controls at different time-points post-injury. Photos (a) and (b) show the zone of injury sample and control sample harvested on post-injury day 1 (c) and (d) post-injury day 3, (e) and (f) on post-injury day 6, (g) and (h) on post-injury day 10.

Figure 4. Dynamics of arterial intimal thickening.

Figure 5. Arterial intima/media ratio and its relation to the day of sampling.

Figure 6. Venous intimal thickening and its relation to the post-injury day of sampling.

Table, Supplemental Digital Content 1. Detailed characteristics of the study population.

Table, Supplemental Digital Content 2. Morphometric characteristics of control sample arterial walls and recipient arteries in LD and gracilis & ALT flap reconstructions.

Table, Supplemental Digital Content 3. Morphometric characteristics of control sample venous walls and recipient veins in LD and gracilis & ALT flap reconstructions.

Figure, Supplemental Digital Content 4. Obvious arterial intimal thickening in the zone of injury in 31-year-old patient w/o concomitant comorbidities on day 10 post-injury. (Left) – zone of injury sample. (Right) – control sample harvested from gracilis flap pedicle.

Table 1. Morphometric characteristics of arterial walls in the zone of injury and control samples.

Variable	Zone of injury	Control	*P* value
Artery intimal thickness M (IQR)	92µm (47-137µm)	30µm (17-43µm)	<0.01
Artery medial thickness M (IQR)	320µm (162-478µm)	235µm (169-301µm)	0.11
Arterial intima/media ratio (IQR)	0.26 (0.09-0.43)	0.12 (0.08-0.17)	0.01

Table 2. Morphometric characteristics of venous walls in the zone of injury and control samples.

Variable	Zone of injury	Control	*P* value
Vascular histology			
Vein intimal thickness M (IQR)	24µm (6.5-41.5µm)	9µm (5-13µm)	<0.01
Vein medial thickness M (IQR)	152µm (69-235µm)	139µm (84-194µm)	0.23
Vein intima/media ratio (IQR)	0.21 (0.14-0.28)	0.07 (0.01-0.13)	0.02
Fibrosis			
Vein intimal and medial fibrosis (combined) % (IQR)	39% (26-52%)	48% (41-53%)	0.45

Figure 1

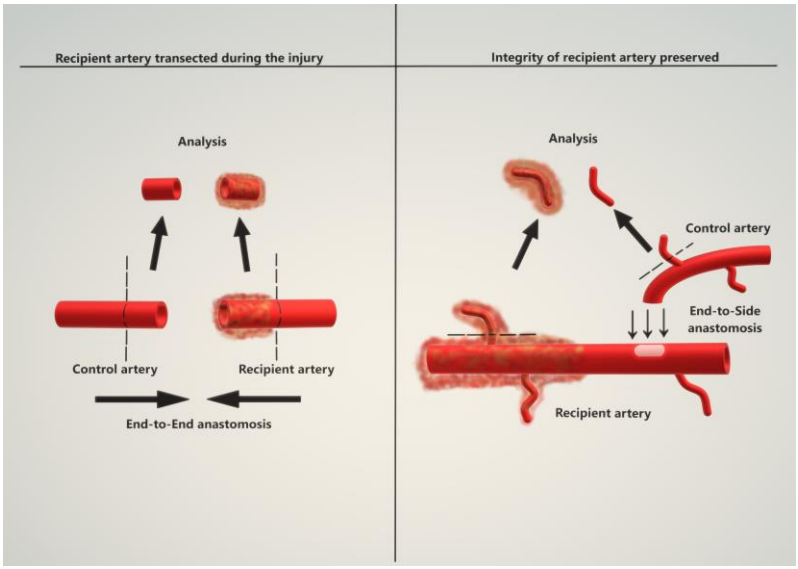

Recipient artery transected during the injury
Integrity of recipient artery preserved
Analysis
Control artery
Recipient artery
End-to-End anastomosis
Analysis
Control artery
End-to-Side
anastomosis
Recipient artery

Figure 2 Left

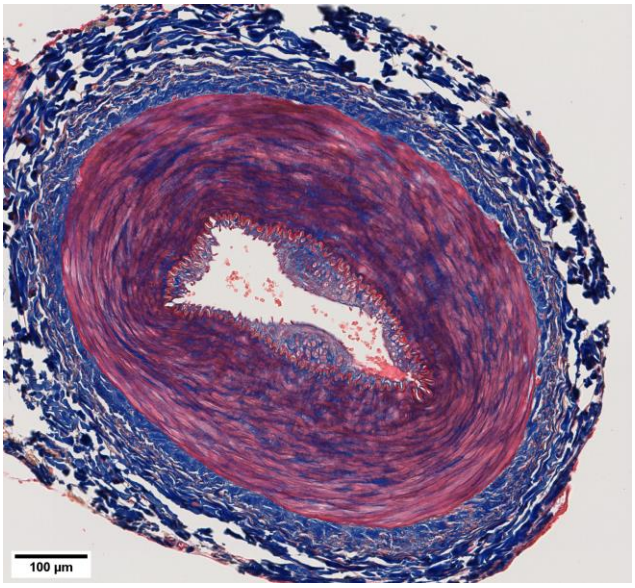


Figure 2 right

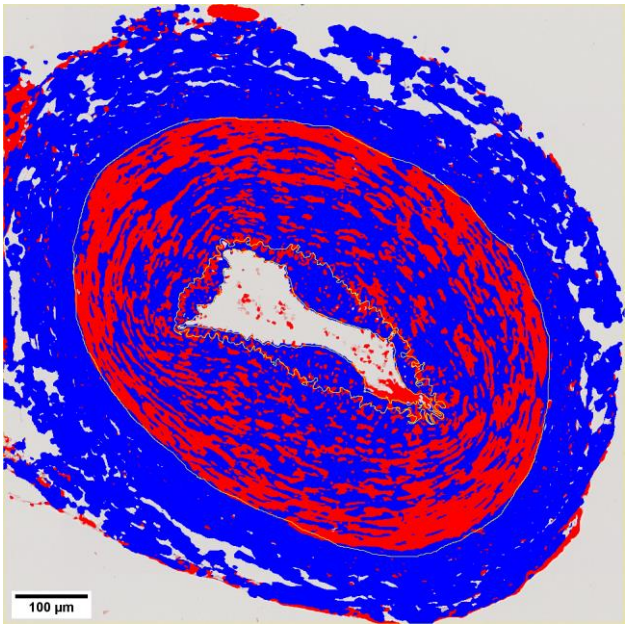


Figure 3a

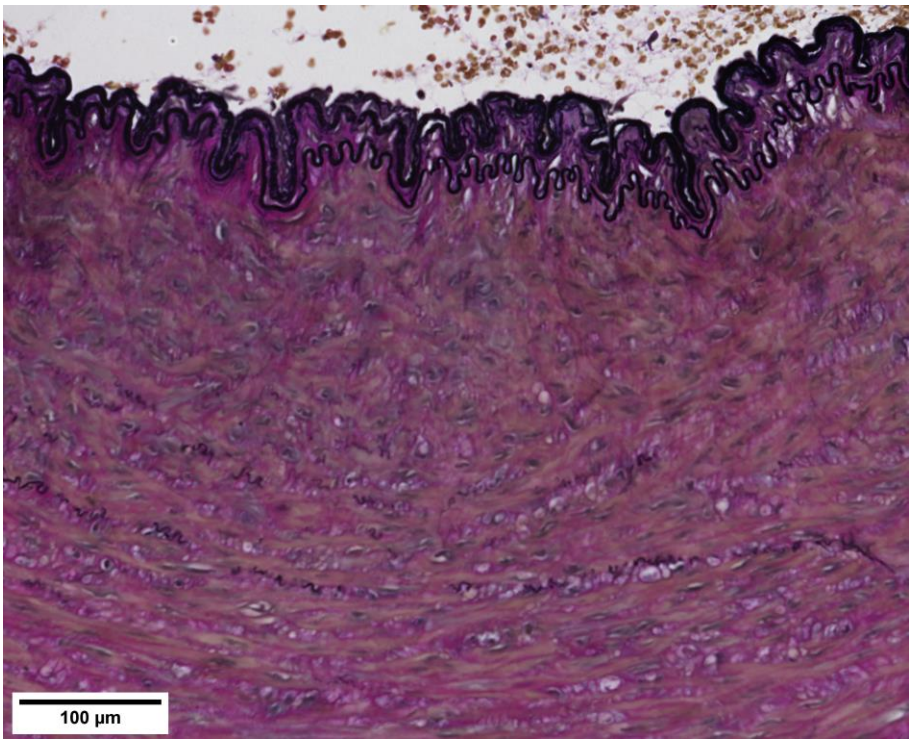


Figure 3b

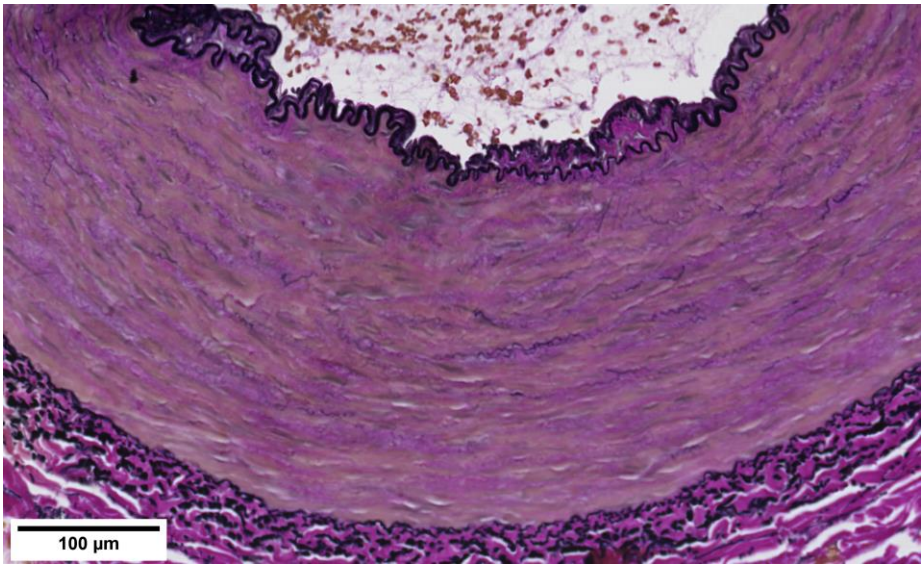


Figure 3c

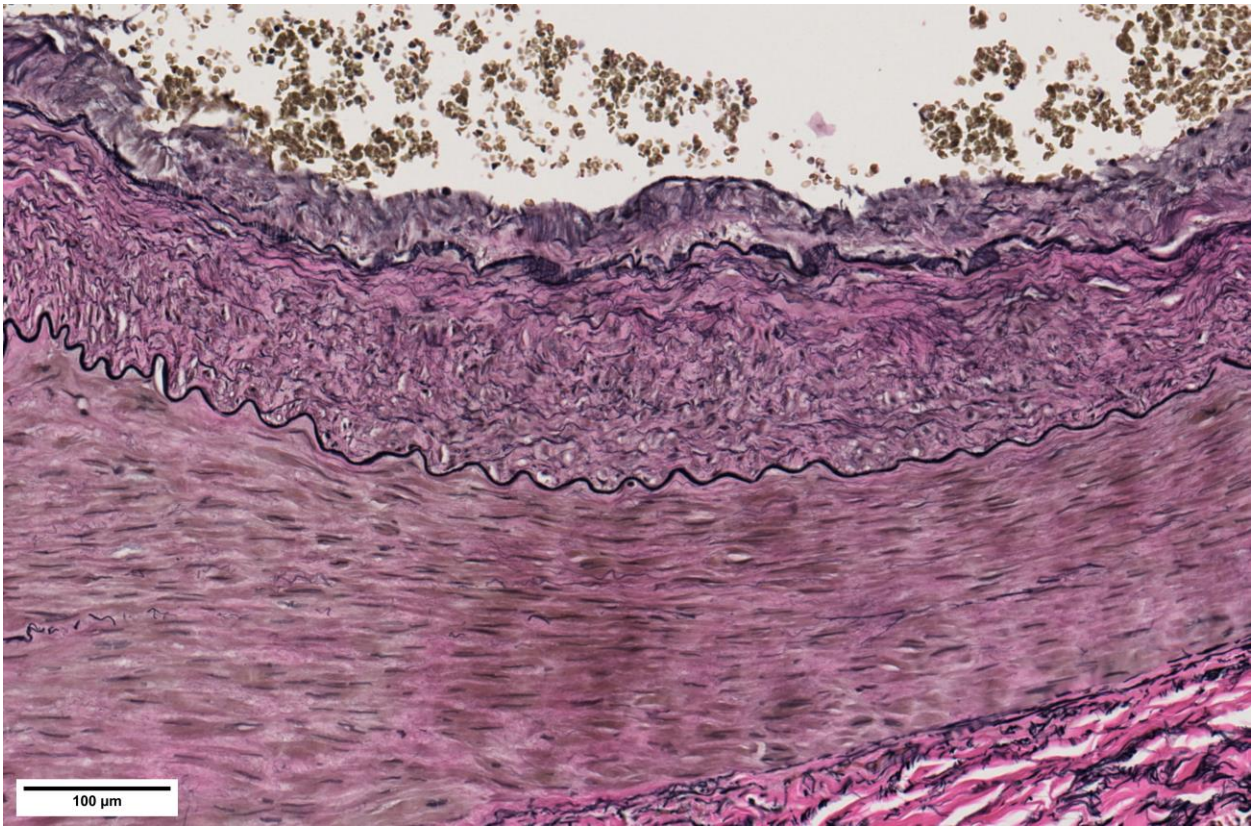


Figure 3d

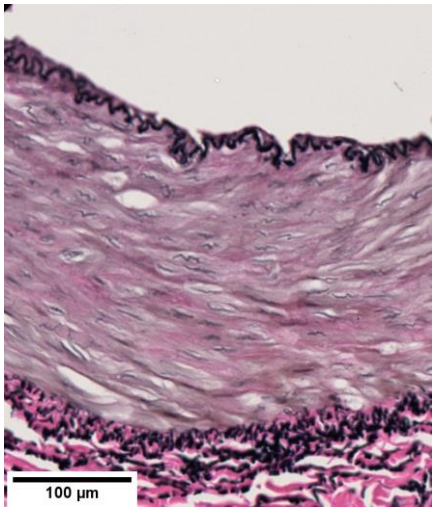


Figure 3e

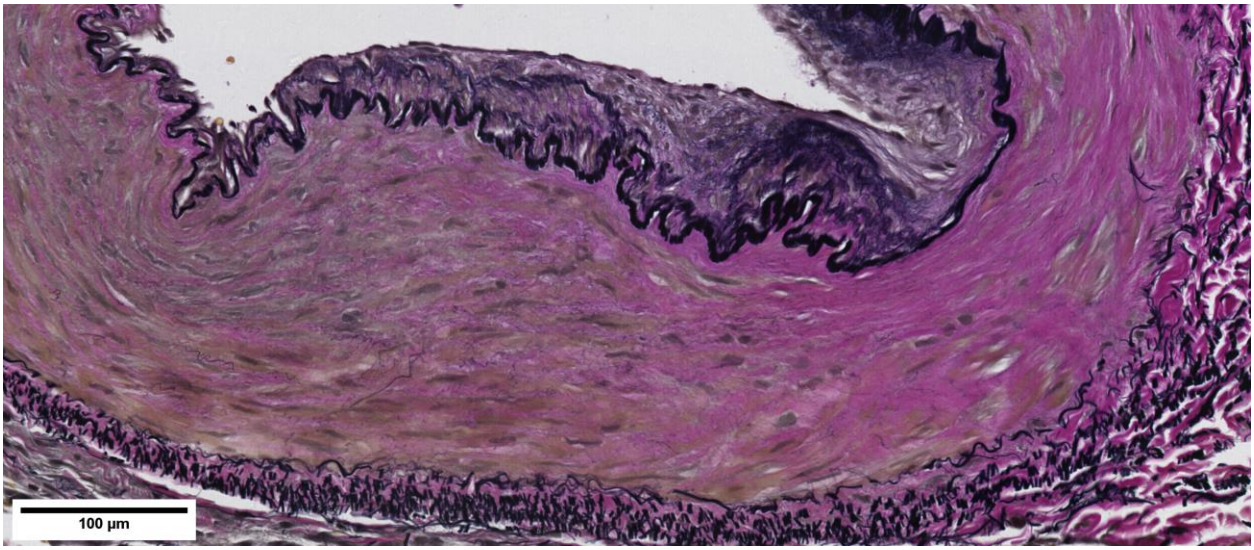


Figure 3f

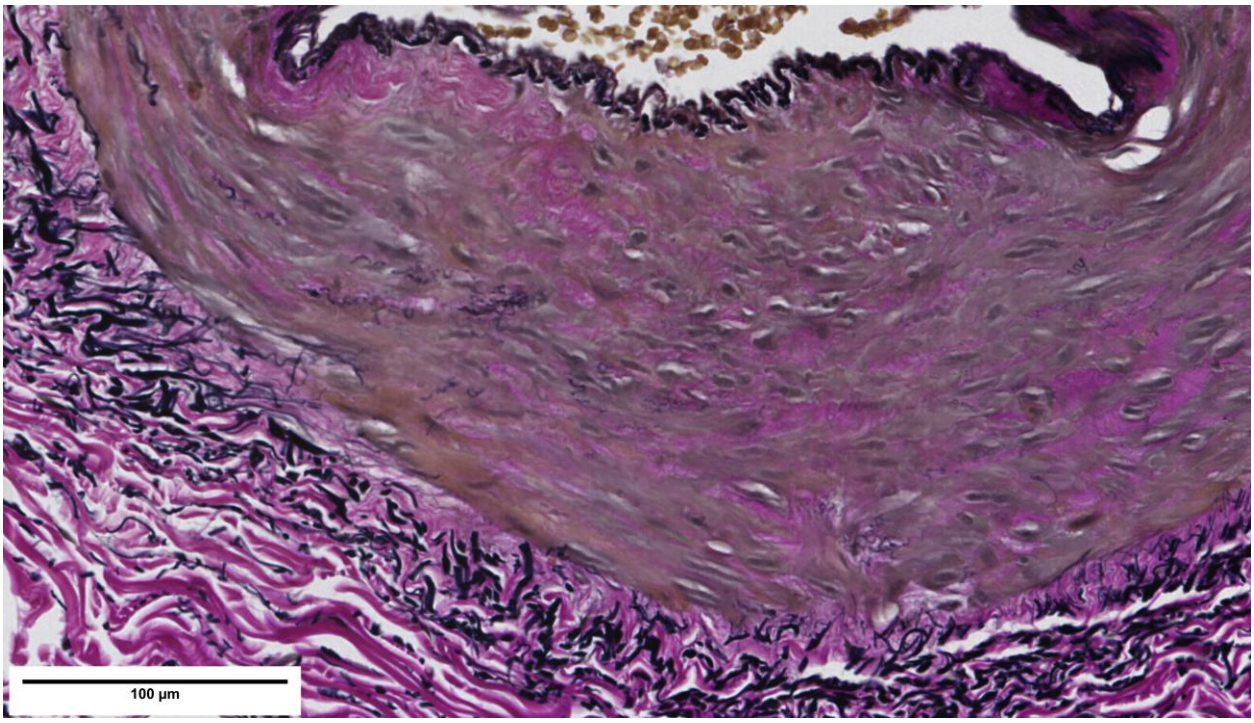


Figure 3g

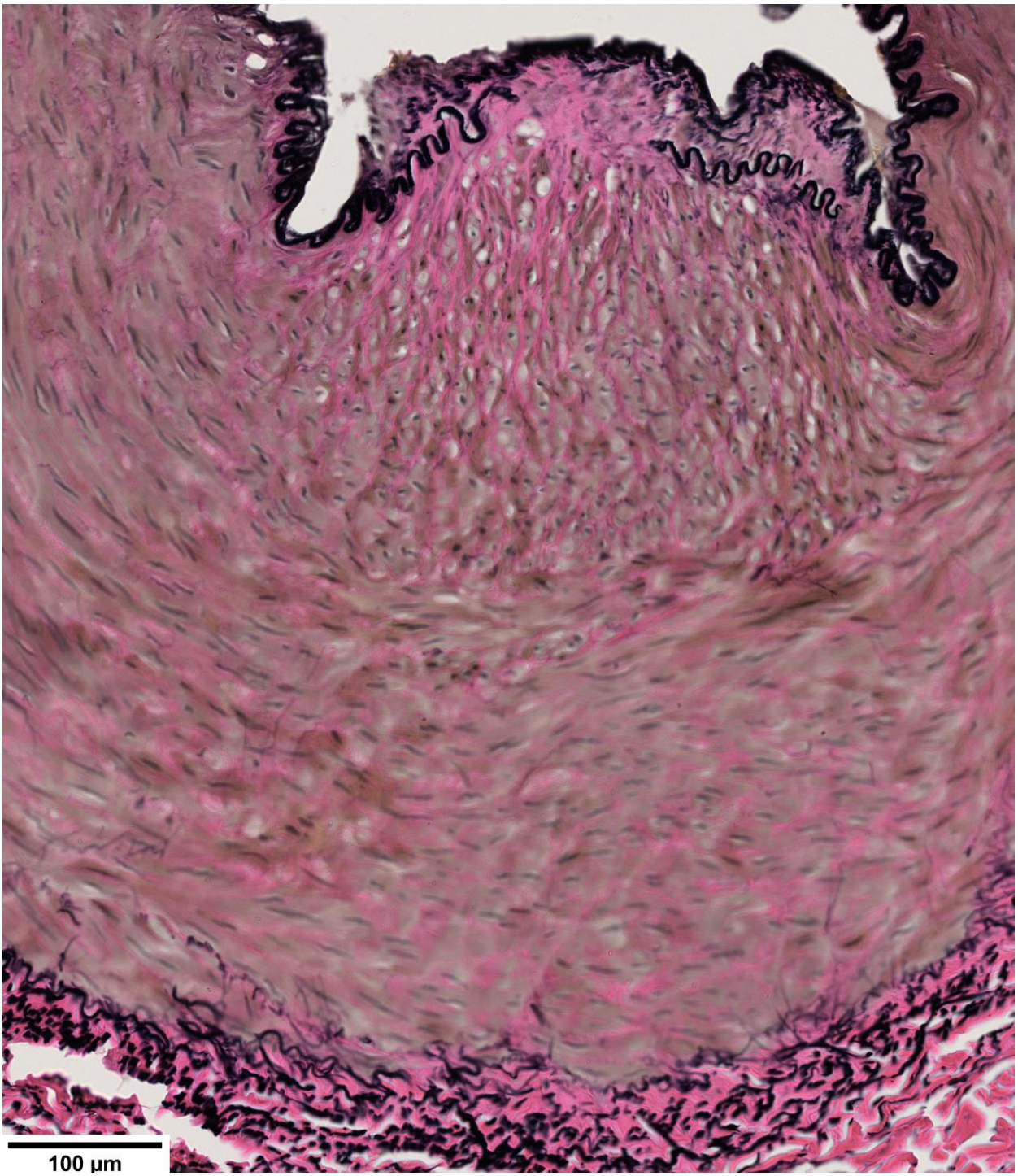


Figure 3h

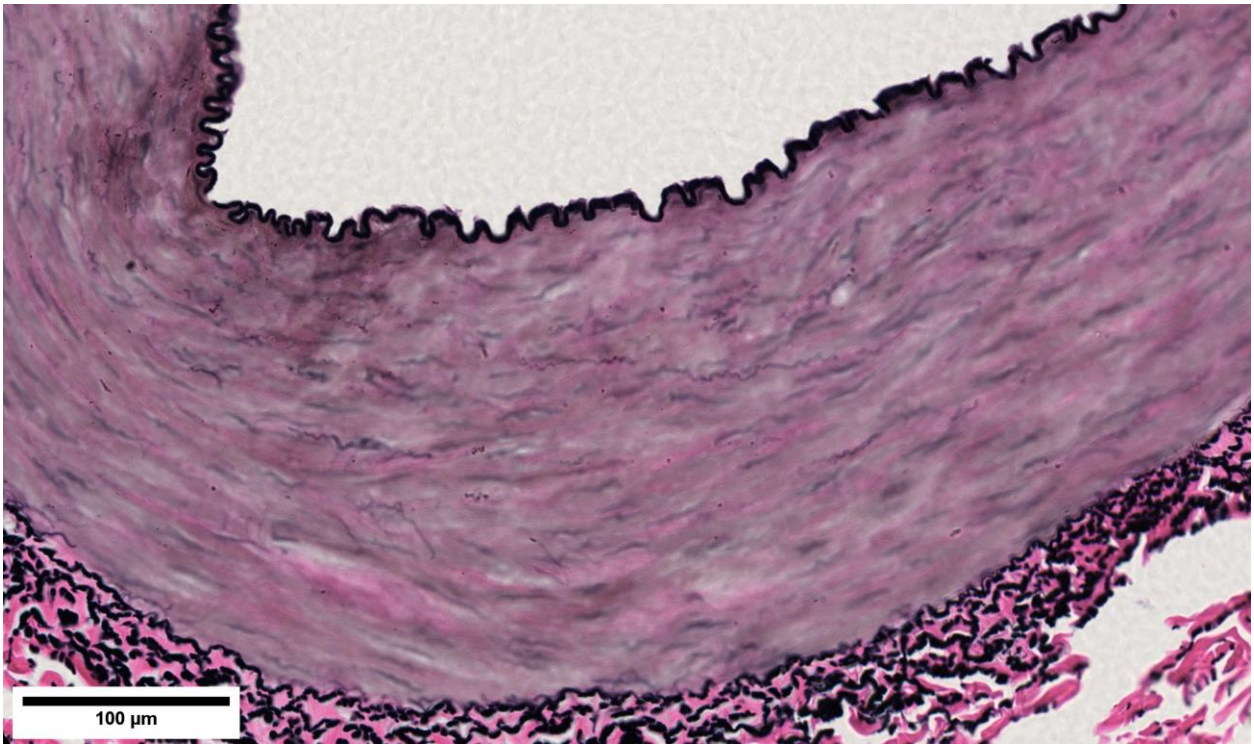


Figure 4

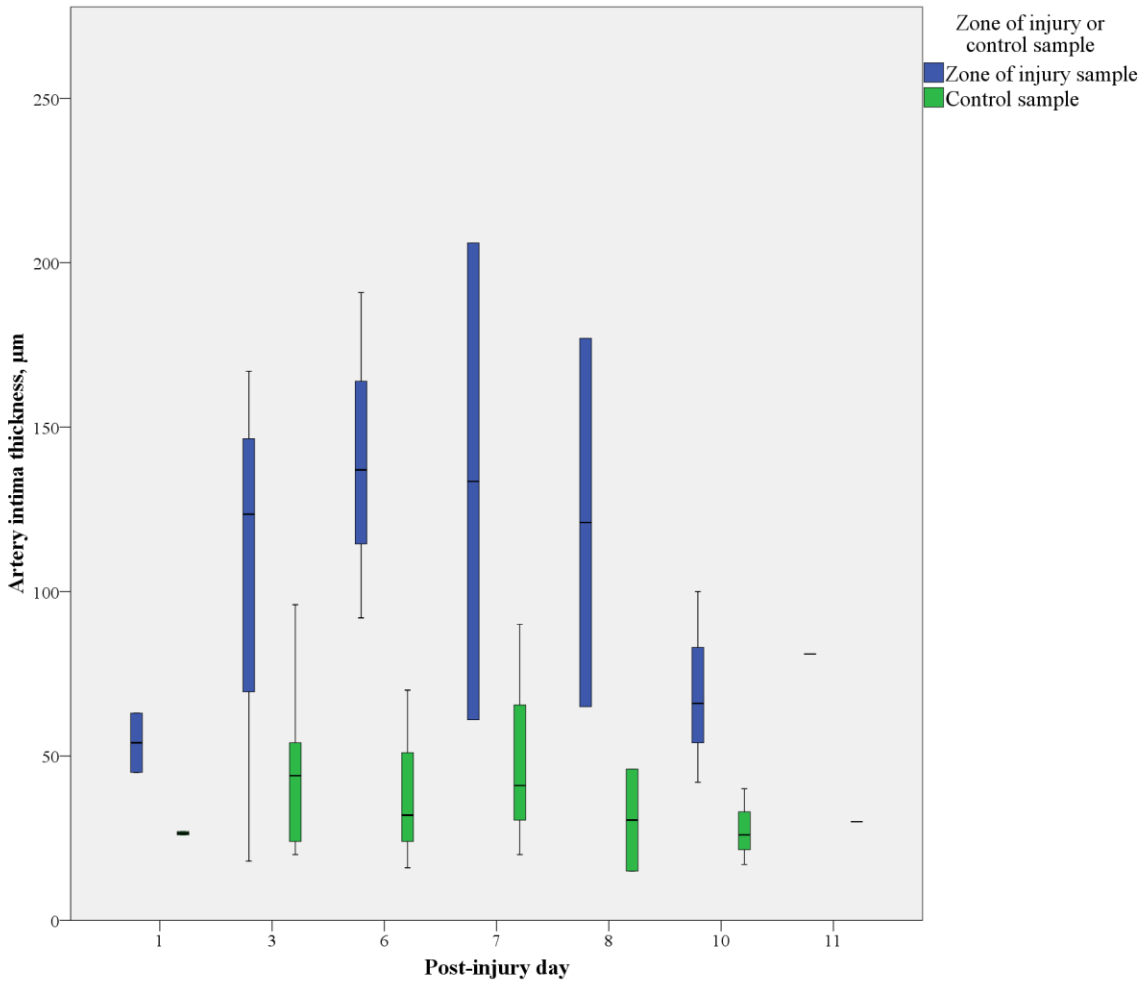

Zone of injury or control sample
Zone of injury sample
Control sample
Artery intima thickness, µm
0
50
100
150
200
250
1
3
6
7
8
10
11
Post-injury day

Figure 5

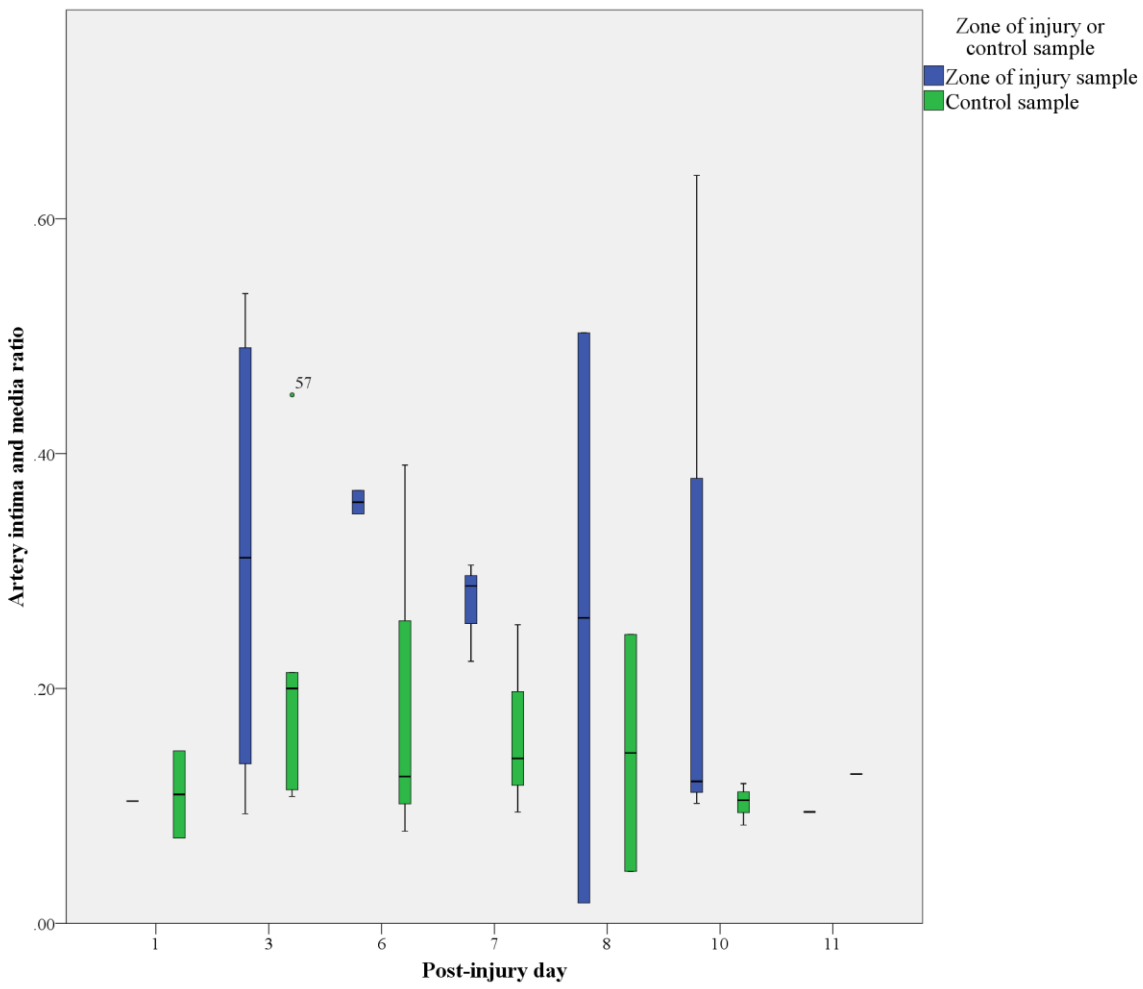
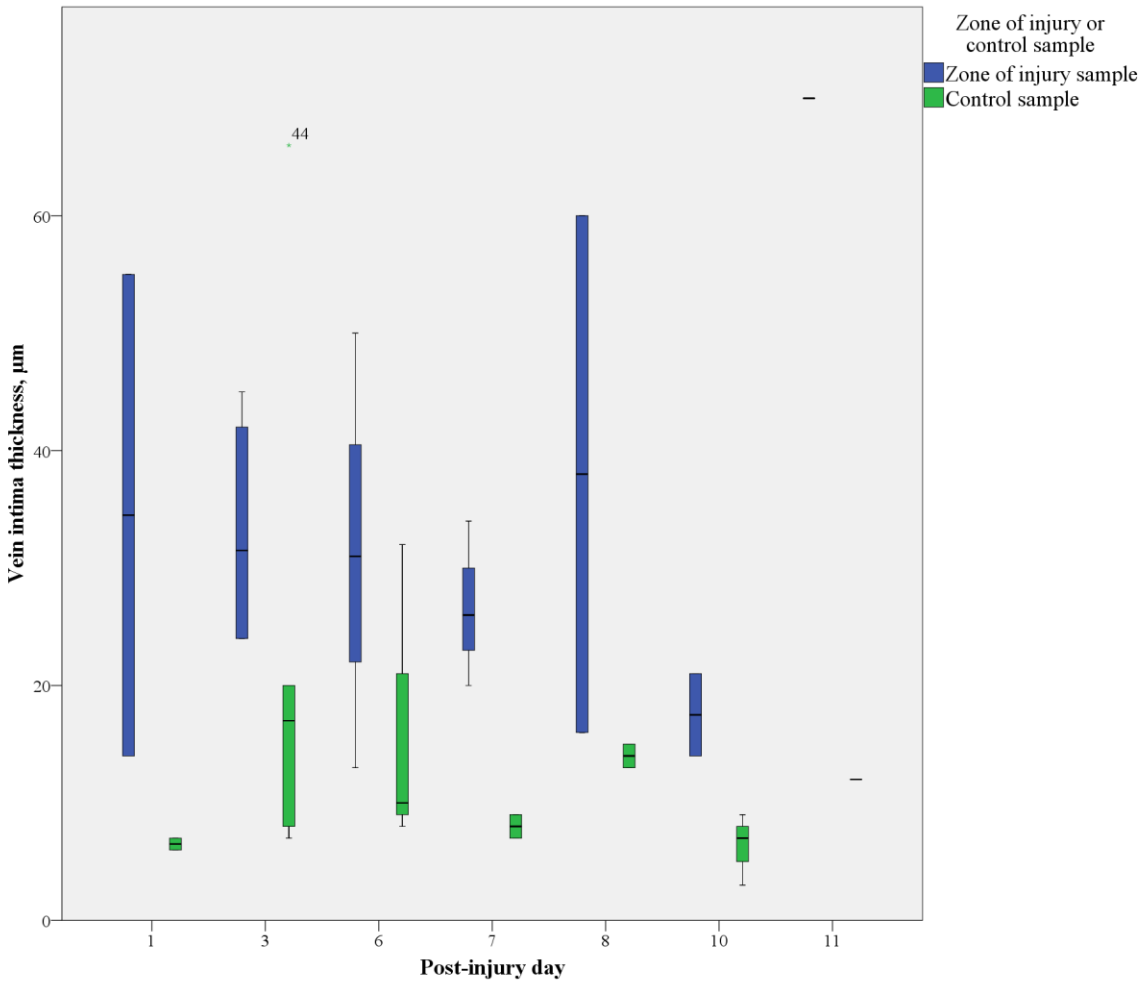

Zone of injury or control sample
Zone of injury sample
Control sample
Artery intima and media ratio
.00
.20
.40
.60
.57
1
3
6
7
8
10
11
Post-injury day

Figure 6


Zone of injury or control sample
Zone of injury sample
Control sample
Vein intima thickness, µm
0
20
40
60
44
1
3
6
7
8
10
11
Post-injury day

Supplemental Digital Content 1. Detailed characteristics of the study population.

Case	Age	Comorbidities, smoking status	Reconstruction and vessel harvest day (post-injury)	Injury	Free flap	Zone of injury sample origin	Control sample origin
1	62	Smoker	6	Open tibial fracture IIIB	Latissimus dorsi	Posterior tibial	Thoracodorsal pedicle
2	56	-	7	Open tibial fracture IIIB	Latissimus dorsi	Anterior tibial	Thoracodorsal pedicle
3	29	-	11	Open tibial fracture IIIB	Gracilis	Posterior tibial	Gracilis pedicle
4	48	Smoker	3	Open tibial fracture IIIB	Gracilis	Posterior tibial	Gracilis pedicle
5	62	-	6	Open tibial fracture IIIB	Gracilis	Posterior tibial	Gracilis pedicle
6	44	Asthma	3	Open tibial fracture IIIB	Latissimus dorsi	Posterior tibial	Thoracodorsal pedicle
7	44	Arterial hypertension, diabetes type I	6	Open tibial fracture IIIB	Gracilis	Posterior tibial	Gracilis pedicle
8	33	-	1	Open calcaneal fracture	ALT	Posterior tibial	Descending branch of lateral circumflex femoral artery
9	66	-	8	Open tibial fracture IIIB	Latissimus dorsi	Posterior tibial	Thoracodorsal pedicle
10	75	Coronary artery disease	3	Open tibial fracture IIIB	Gracilis	Posterior tibial	Gracilis pedicle
11	78	Persistent atrial fibrillation	7	Open tibial fracture IIIB	Latissimus dorsi	Posterior tibial	Thoracodorsal pedicle
12	29	Smoker	10	Open tibial fracture IIIC	Latissimus dorsi	Medial superior genicular	Thoracodorsal pedicle
13	23	-	10	Open Lisfranc fracture	Latissimus dorsi	Dorsalis pedis	Thoracodorsal pedicle
14	48	-	8	Open tibial fracture IIIB	Gracilis	Posterior tibial	Gracilis pedicle
15	31	Smoker	10	Open tibial fracture IIIB	Gracilis	Posterior tibial	Gracilis pedicle
16	28	-	3	Open tibial fracture IIIB	Latissimus dorsi	Medial sural	Thoracodorsal pedicle
17	19	Smoker	1	Open tibial fracture IIIB	Latissimus dorsi	Posterior tibial	Thoracodorsal pedicle
18	56	Arterial hypertension	3	Open tibial fracture IIIB	Gracilis	Posterior tibial	Gracilis pedicle
19	49	-	7	Open femur fracture IIIB	Latissimus dorsi	Branch of profunda femoris	Thoracodorsal

Table, Supplemental Digital Content 2. Morphometric characteristics of control sample arterial walls and recipient arteries in LD and gracilis & ALT flap reconstructions.

Control sample arteries			
Variable	LD flap pedicle vessels n=10	Gracilis & ALT flap pedicle vessels n=9	p
Artery intimal thickness M (IQR)	36µm (14-50 µm)	27µm (12-42µm)	0.32
Artery medial thickness M (IQR)	240µm (199-281µm)	231µm (124-338µm)	0.36
Arterial intimal fibrosis % (IQR)	35% (13-57%)	42% (20-64%)	0.71
Arterial medial fibrosis % (IQR)	40% (17-63%)	42% (34-50%)	0.69
Recipient arteries			
Variable	LD flap pedicle vessels n=10	Gracilis & ALT flap pedicle vessels n=9	p
Artery intimal thickness M (IQR)	81µm (11-151µm)	96 µm (62-130µm)	0.82
Artery medial thickness M (IQR)	320µm (198-442µm)	332µm (147-517µm)	0.76
Artery intimal fibrosis % (IQR)	62% (38-86%)	70% (61-79%)	0.28
Artery medial fibrosis % (IQR)	42% (30-54%)	51% (36-66%)	0.39

Table, Supplemental Digital Content 3. Morphometric characteristics of control sample venous walls and recipient veins in LD and gracilis & ALT flap reconstructions.

Control sample veins			
Variable	LD flap pedicle vessels n=10	Gracilis & ALT flap pedicle vessels n=9	p
Venous intimal thickness M (IQR)	9 µm (6-12µm)	15 µm (3-27µm)	0.73
Venous medial thickness M (IQR)	172 µm (133-211µm)	97 µm (39-155µm)	0.03
Venous intimal and medial fibrosis % (IQR)	49% (31-54%)	45% (38-53%)	0.61
Recipient veins			
Variable	LD flap pedicle vessels n=10	Gracilis & ALT flap pedicle vessels n=9	p
Venous intimal thickness M (IQR)	29µm (12-46µm)	24µm (8-40µm)	0.61
Venous medial thickness M (IQR)	147µm (69-221µm)	152µm (72-232µm)	0.37
Venous intimal and medial fibrosis % (IQR)	39% (26-52%)	41% (28-54%)	0.73

SDC 4