MEDICAL SCIENCE MONITOR

ANIMAL STUDY

e-ISSN 1643-3750
© Med Sci Monit, 2017; 23: 834-842
DOI: 10.12659/MSM.899107

Received: 2016.04.17
Accepted: 2016.06.15
Published: 2017.02.15

Flow Induced Microvascular Network Formation of Therapeutic Relevant Arteriovenous (AV) Loop-Based Constructs in Response to Ionizing Radiation

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ACDG 1 Volker J. Schmidt
BCD 2 Jennifer M. Covi
DEF 1 Christoph Koepple
B 3 Johannes G. Hilgert
AF 2 Elias Polykandriotis
A 1 Amir K. Bigdeli
AB 4 Luitpold V. Distel
A 2 Raymund E. Horch
ADE 1 Ulrich Kneser

1 Department for Hand-, Plastic- and Reconstructive Surgery, BG Unfallklinik Ludwigshafen, Universität Heidelberg, Heidelberg, Germany
2 Department of Plastic and Hand Surgery, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
3 Department of Nephrology and Hypertension, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
4 Department of Radiation Oncology, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

Corresponding Author: Volker J. Schmidt, e-mail: schmidtvolker@gmx.net
Source of support: Financial support for this study was granted by the Interdisciplinary Center for Clinical Research (IZKF, University Hospital of the University Erlangen-Nuremberg, TP-J15, to VJS)

Background: The arteriovenous (AV) loop model enables axial vascularization to gain a functional microcirculatory system in tissue engineering constructs *in vivo*. These constructs might replace surgical flaps for the treatment of complex wounds in the future. Today, free flaps are often exposed to high-dose radiation after defect coverage, according to guideline-oriented treatment plans. Vascular response of AV loop-based constructs has not been evaluated after radiation, although it is of particular importance. It is further unclear whether the interposed venous AV loop graft is crucial for the induction of angiogenesis.

Material/Methods: We exposed the grafted vein to a single radiation dose of 2 Gy prior to loop construction to alter intrinsic and angio-inductive properties specifically within the graft. Vessel loops were embedded in a fibrin-filled chamber for 15 days and radiation-induced effects on flow-mediated vascularization were assessed by micro-CT and two-dimensional histological analysis.

Results: Vessel amount was significantly impaired when an irradiated vein graft was used for AV loop construction. However, vessel growth and differentiation were still present. In contrast to vessel density, which was homogeneously diminished in constructs containing irradiated veins, vessel diameter was primarily decreased in the more peripheral regions.

Conclusions: Vascular luminal sprouts were significantly diminished in irradiated venous grafts, suggesting that the interposing vein constitutes a vital part of the AV loop model and is essential to initiate flow-mediate angiogenesis. These results add to the current understanding of AV loop-based neovascularization and suggest clinical implications for patients requiring combined AV loop-based tissue transfer and adjuvant radiotherapy.

MeSH Keywords: Arteriovenous Shunt, Surgical • Neovascularization, Physiologic • Radiation Effects • Tissue Engineering

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/899107

2961 — 6 28

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

The development of artificial bioengineered constructs largely depends on adequate vascularization to guarantee sufficient oxygen supply [1]. The arteriovenous (AV) loop model is an established method to obtain transplantable bioartificial tissue *in vivo*. Originally based on the findings of Erol and Spira [2] and subsequently modified by Murphy et al. [3] and Tanaka et al. [4] this model of benign angio-inductive behavior triggers the formation of novel vessel sprouts remarkably without additional extrinsic and angio-inductive factors. In brief, a femoral vein is dissected, harvested, and as an autologous graft, interposed between the contralateral femoral artery and vein. This results in an arteriovenous fistula, which is then embedded in a fibrin-filled Teflon chamber [5]. A mature microcirculatory system originates mainly from the venous graft, thereby facilitating cultivation, harvesting, and transfer of rudimentary organ-like structures to a secondary defect site, thus minimizing donor site morbidity [6–9].

As vascularization is crucial to promote clinical implementation of bioengineered constructs, tremendous research efforts have been undertaken to further optimize vessel formation within the AV loop. The current understanding of angiogenesis is evolving, as the underlying nature and mediating signaling pathways fundamentally differ in various types of angiogenesis [10]. Watson et al. [11] demonstrated that vascular flow is essential for hypoxia-driven angiogenesis in embryonic development. Furthermore, we recently pointed out that distinct AV associated changes of the hemodynamic load – namely the shear rate – are responsible for triggering the angio-inductive behavior of endothelial cells within the venous graft [12]. Both of these observations fundamentally rely on the presence of angio-inductive endothelial cells and a viable venous graft. However, Polykandriotis et al. [13] and Westerband et al. [14] showed that venous grafts were exposed to considerable endothelial denudation. Moreover, Zdolsek et al. [15] demonstrated that utterly decellularized and avital vein allografts are continuously able to maintain angio-inductive properties within the AV loop.

Due to the constant progress in reconstructive surgery and due to improved and standardized microsurgical treatment algorithms, the range of medical indications for reconstructive vascularized tissue transfers has increased dramatically during the last decades. Thus, microvascular flaps are exposed to high-dose radiation after defect coverage with increasing frequency. For this reason, the vascular behavior of novel reconstructive approaches, such as intrinsically vascularized AV loop-based constructs, allows an excellent evaluation of the accompanying angiogenesis, and is of special interest in evaluating the response to radiation. Furthermore, the understanding of the AV loop associated angiogenesis is of special translational importance. There is an ongoing discussion as to whether the interposed venous graft is vitally important for the induction of angiogenesis under conditions of high flow.

In order to alter the cellular hemostasis specifically within the grafted vein we exposed the graft intraoperatively to 2 Gy of ionizing radiation (a level of radiation considered a relevant rat dose-rate [16]). We wanted to demonstrate that the graft possessed vital intrinsic factors that were at least partially responsible for triggering flow-mediated angiogenesis in the AV loop model, and we generally wanted to investigate the effect of radiation in the developing AV loop microvasculature. Beside three-dimensional micro-CT, a previously described observer-independent automatic software algorithm was used to assess vessel number, density, and area by analyzing two-dimensional histological cross sections in order to characterize the differences of angiogenesis in detail [17].

Material and Methods

Experimental design and surgical procedure

All operations were performed by the same investigator (JMC) using a surgical microscope (magnification 16×, OPMI IFC, Carl Zeiss, Oberkochen, Germany). 2 to 4 month old male Lewis rats (n=14) with an average body mass of 340 g were obtained from Charles River Laboratories (Sulzfeld, Germany). All experiments were approved by the Institutional Animal Care and Use Committee of the Regierungspräsidium Mittelfranken (AZ 54-2532.1-34/09) and in accordance with the German Animal Welfare Act. For induction of anesthesia, the rats received 5% isoflurane (Baxter, Vienna, Austria) inhalation. Before surgery, they received buprenorphin for analgesia, (0.3 mg/kg body weight, Temgesic, Essex Chemie AG, Luzern, Switzerland), as well as heparin anticoagulation (80 IU/kg Liquemin, Ratiopharm Ulm, Germany). The right femoral vascular bundle was exposed by a mid-ventral incision. The femoral vein was dissected and a 20 mm long venous graft was harvested. The grafts either underwent irradiation or were directly interposed as a loop (Figure 1) between the contralateral femoral artery and vein using 11/0 nylon sutures (Ethilon, Ethicon, Norderstedt, Germany) after similar incubation in sodium heparin solution (sham treatment, 10,000 IE/L). The AV loop was embedded in the isolation chamber, which was filled with 800 µL of fibrin sealant (Tissucol®, Baxter) composed of fibrinogen (10 mg/mL), thrombin (2 IU/mL) and aprotinin (1,500 KIE/mL) as described previously [5,18]. The chamber was sutured subcutaneously onto the underlying adductor fascia with 6-0 polypropylene (Prolene 6/0, Ethicon, Norderstedt, Germany). For wound closure, interrupted vertical mattress sutures with Vicryl 4-0 (Ethicon, Norderstedt, Germany) were used. All rats received buprenorphin and heparin (80 IU/kg Liquemin, Ratiopharm

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

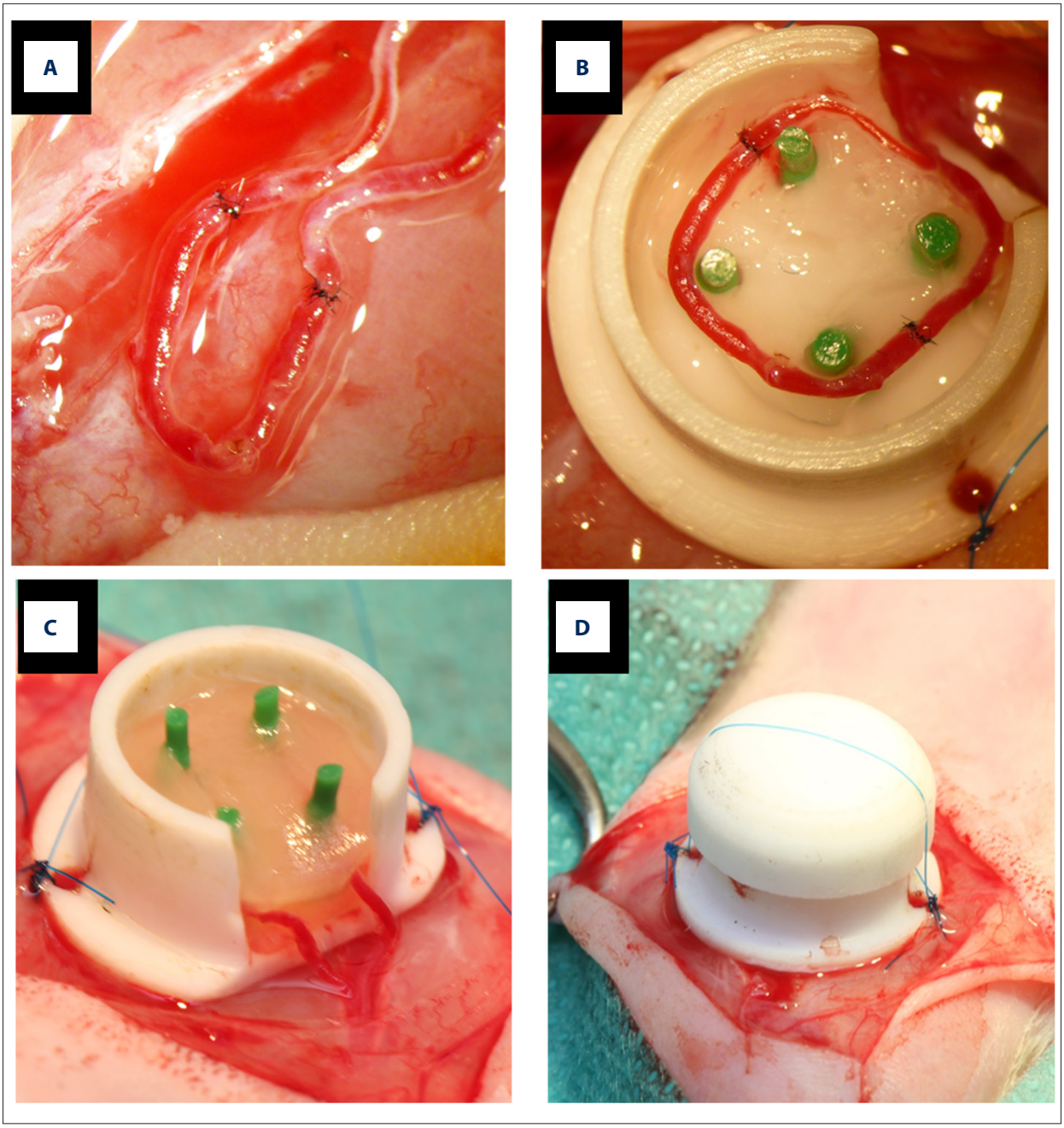

Figure 1. Surgical procedure and implementation of the AV loop constructs. The right femoral vein was dissected and used as an interposing graft between the left femoral artery and vein (**A**). The resulting AV shunt was then embedded in an isolating Teflon chamber (**B**), which was filled with a fibrin clot (**C**). After a lid was placed on top, the chamber was subsequently attached to the underlying muscles of the thigh (**D**).

Ulm, Germany) postoperatively. The rats were kept at a 12-hour dark/light cycle in the animal facility of the University of Erlangen Medical Centre with free access to standard chow (Sniff) and water. At the end of the experiment, the rats were sacrificed under deep anesthesia (5% isoflurane) by exsanguination, and subsequent reperfusion with India Ink or Microfil® (Flowtech, MA, USA).

Irradiation of the interpositional venous graft

The autologous venous graft was harvested and rinsed in sodium heparin solution (10,000 IE/l). A dose of 2 Gy ionizing radiation was applied to the vein graft within 30 seconds using an Isovolt Titan x-ray machine with a 120 kV power supply (GE Sensing & Inspection Technologies, Ahrensburg, Germany).

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

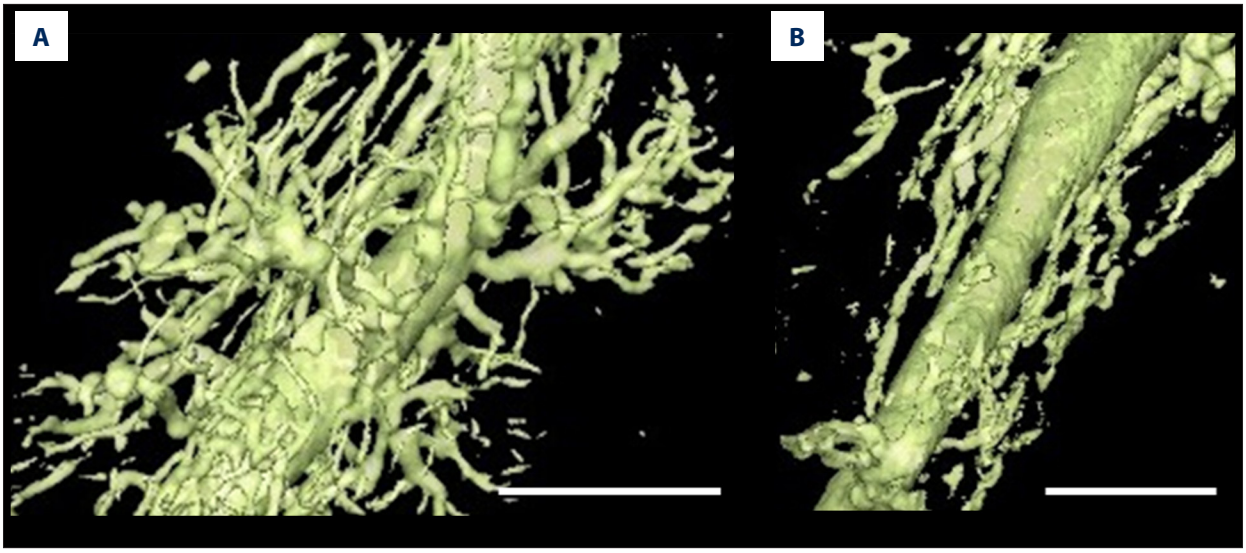

Figure 2. Negative effects of graft irradiation on neovessel formation analyzed by Micro-CT. At day 15 animals were sacrificed and micro-CT images were acquired after reperfusion with an appropriate contrasting dye (Microfil®). Abundant vessel sprouting was only present in control constructs containing non-irradiated grafts (**A**). However, neovessels evidently originate from the interposition graft. In contrast, irradiated grafts showed a marked reduction in vascular sprouts (**B**). Scale bars: 1000 µm.

Histological and micro-CT analysis

After 15 days, an abdominal midline incision was performed and newly formed vessels along the main vessel axis were detected by cannulating the distal descending aorta using a 24-gauge catheter. The descending vascular system was cleansed with 100 mL of prewarmed (37°C) isotonic salt solution containing heparin (100 IE/mL) and subsequently perfused with 30 mL of prewarmed (37°C) India ink solution [50% v/v India ink (Rohrer & Klinger)] in 5% gelatin and 4% mannitol in order to detect additional vessels around the constructed AV loop. Micro-CT analysis required the reperfusion of the circulatory system with 20 mL of warmed yellow Microfil (MV-122) containing 5% of MV Curing Agent (Flowtech Inc., Carver, USA) [19]. Incubation at 4°C for 24 hours allowed the gelatin and mannitol to solidify before continuing with downstream applications.

For histological analysis, the chambers with the AV loop were explanted. Formalin-fixed, paraffin-embedded tissue sections were obtained using standard protocols. Then 5 µm histological sections in standardized planes perpendicular to the longitudinal AV loop axis were produced using a microtome (Leica Microsystems, Wetzlar, Germany). Hematoxylin and eosin (H&E) staining was performed using an autostainer (ST5010, Leica Microsystems) according to standard protocol. Isolectin B4 (Sigma L 2140, Sigma-Aldrich, Hamburg, Germany) staining was used to detect luminal endothelial cells by means of immunohistochemistry as described elsewhere [17]. Histological cross sections were visualized using an inversed microscopy (Olympus IX81, 10× magnification, Olympus Corporation, Hamburg, Germany) and analyzed using an automatic two-dimensional quantification algorithm established previously by our group for superior morphometric quantification [17].

High-resolution micro-CT scans were acquired on a cone-beam micro-CT scanner at the Institute of Medical Physics, University Erlangen, Germany (ForBild scanner). Further technical specifications and specialized algorithms for optimization and enhancement of images obtained were described in detail elsewhere [19].

Statistical analysis

For statistical analysis and figures, OriginPro 2015 (b9.2.214, OriginLab Corp., Northampton, MA, USA) was used. Morphometric data were calculated using MATLAB (MathWorks). All data are presented as mean ±SEM and compared using unpaired *t*-test. For analysis of more than two groups, analysis of variance (one-way ANOVA) was used. All data were distributed normally. Differences were considered significant if a corrected error probability was $p<0.05$.

Results

Surgery, patency, and macroscopic appearance upon explantation

All rats survived the experiments and tolerated the anesthesia and surgical procedures well (n=14). There were no surgical complications like wound dehiscence, infection, or hematomas. One rat in the control group had a significant swelling

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

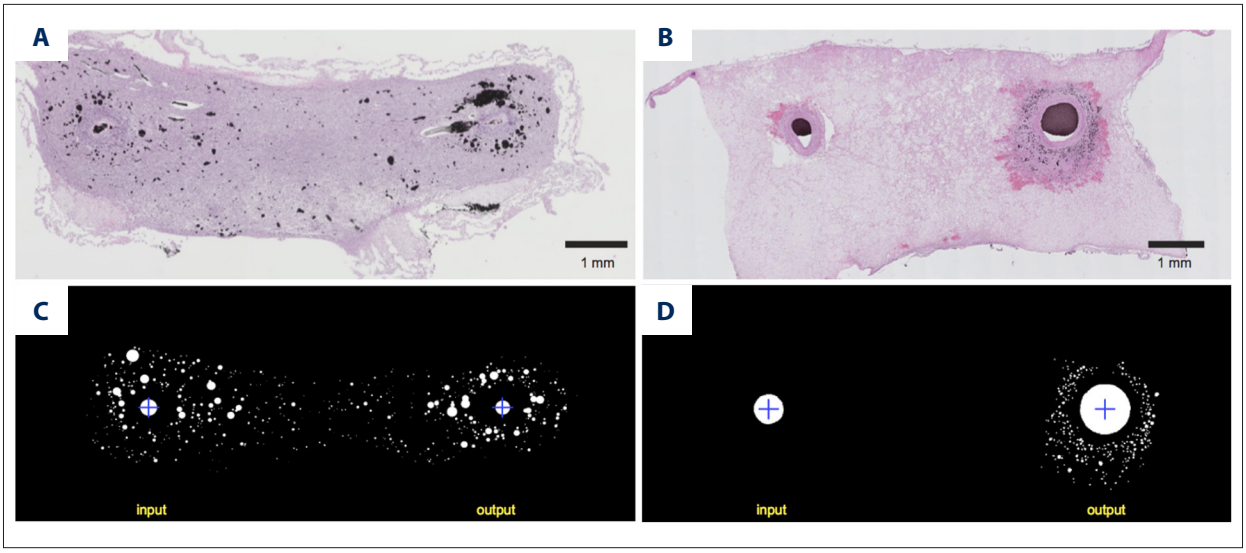

Figure 3. Graft irradiation inhibits AV loop-based neovascularization as assessed by histological cross sections. Newly formed vessels of control constructs (**A**) were more evenly distributed than those of constructs with irradiated vein grafts (**B**) as visualized by India Ink stained histological cross sections. Ideal maps (**C, D**) of image samples shown in **A** and **B** respectively were required for the observer-independent morphometric quantification algorithm.

in the groin in the first week after surgery most likely due to seroma formation. After serous fluid puncture, this rat showed no further clinical signs or symptoms. A fibrous capsule surrounding the isolation chamber could be observed in all rats.

After explantation of the chamber, the peripheral tissue was examined by macroscopic assessment. The matrix embedding the AV loop within the chambers had a milky translucent appearance at implantation. The fibrin clot appeared red at the time of explantation. Homogenous consistency, however, was unaltered. No indications of clot degradation or fibrinolysis were apparent in the explanted matrices. Stained main vessel axis was visible in all rats indicating a successful reperfusion and patency of the AV loop at the time of reperfusion.

Non-quantitative assessment of the effect of irradiation on loop angiogenesis by means of micro-CT and histological cross sections

Fifteen days after the loop implantation, angiogenesis was visualized by three-dimensional micro-CT analysis in two rats from each group. In line with previous results, control loops showed homogenous vessel sprouting that originated predominantly from the lumen of the graft (Figure 2A). Local graft irradiation considerably impaired neovessel formation along the graft (Figure 2B). Similar observations were made by macroscopic evaluation of H&E cross section slides. Lumina of the microcirculatory system along the graft appeared darkly stained in H&E and lectin stained cross sections of India Ink-or Microfil®-perfused AV loop constructs. The microcirculatory system along irradiated grafts was predominantly aligned in close proximity to the main vessel axis. When a non-irradiated control graft was used, however, newly formed vessels extended further peripherally from the main vessel axis (Figure 3).

Automatic morphometric quantification of the AV loop vascularization in histological cross sections

Morphometric analysis was performed on lectin-stained histological cross sections. The degree of vascular sprouts was assessed by an automatic observer-independent quantification algorithm, as described earlier, and yielded a total amount of 739±111 (n=7) vessels per cross section. No significant morphometrical differences were found between the inflow (431±83, n=7) and the outflow tract (308±43, n=7) of non-irradiated control grafts (Figure 4A). Corresponding results were obtained in irradiated venous grafts. The amount of vessels of the graft inflow (174±58, n=7) was not statistically different from the graft outflow (137±38 n=7). Mean vessel area (Figure 4B) assessed by cross section analysis within constructs where control grafts were used amounted to 238,924±63,126 μm^2 and, similar to the vessel number, there were no statistical significant differences among control grafts with an area of 151,730±51,975 μm^2 (n=7) at inflow segments and 84,337±22,964 μm^2 (n=7) at outflow segments. Irradiated grafts behaved correspondingly with an area of 21,998±6,850 μm^2, n=7 (graft inflow) and 21,140±5,897 μm^2, n=7 (graft outflow) respectively.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

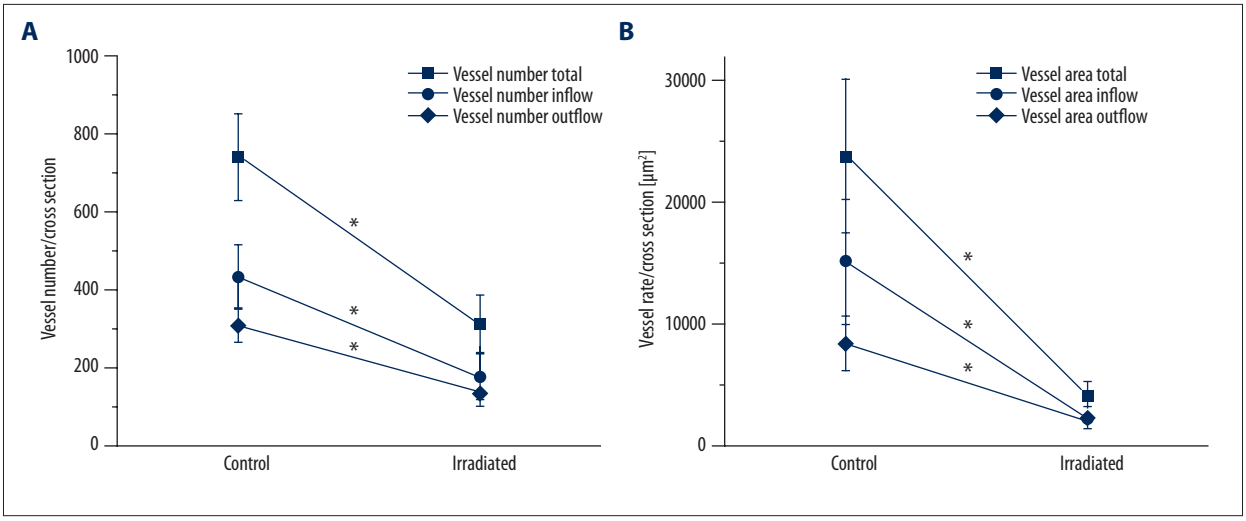

Figure 4. Local graft irradiation inhibits vessel sprouting in the AV loop construct. Irradiation impaired neovessel formation as the total number of luminal sprouts (**A**) and total vessel area (**B**) were significantly reduced after graft-specific irradiation. Corresponding morphometrical data of the graft inflow and outflow respectively did not show statistically significant differences. Asterisk indicates a statistically significant difference at $p<0.05$, n.s. not significant.

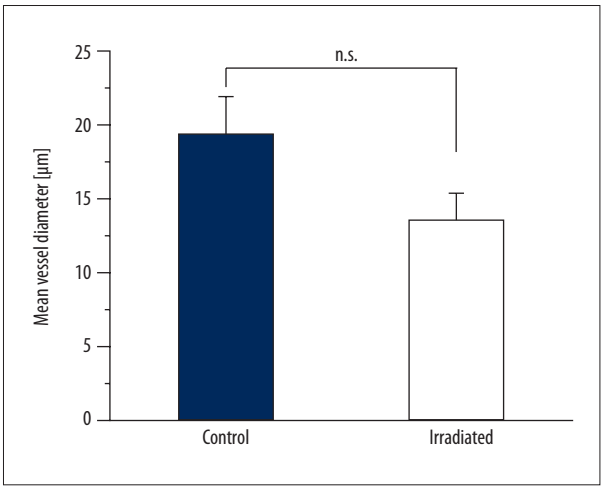

Figure 5. Gross morphometrical analysis of radiation-sensitive neovessel formation in the AV loop. Mean vessel diameter calculated for the total cross section area (no significant differences between total cross section area of control and radiation group) was not significantly reduced after graft irradiation compared to non-treated controls, although it tends to be lower.

Local graft irradiation and neovessel formation in the AV loop

Although luminal sprouting was strongly impaired, the formation of new vessels from the luminal graft was not thoroughly abolished. Single-dose irradiation of 2 Gy reduced the mean number of vessel sprouts to 311±73 (n=7) and was significantly different from the control (Figure 4A). Irradiation reduced mean vessel area to 43,137±10,224 µm² (n=7) compared to 238,924±63,126 µm² (n=7) in corresponding control grafts (Figure 4B). Similar data were obtained when analyzing inflow and outflow segments. The mean number of vessels and area of irradiated grafts were significantly lower when compared to non-irradiated control grafts (Figure 4A and 4B). While not statistical significant, mean vessel diameter after graft irradiation (14±1.8 µm, n=7) tended to be lower compared to control grafts (19±2.6 µm, n=7, Figure 5). Densities of neovessels were differently distributed when comparing AV loop constructs with control and irradiated grafts. Vessel formation aroused primarily within a peripheral radius of 401–600 µm when normalized to the main vessel axis. Irradiation homogeneously decreased vessel densities (Figure 6A). In contrast, mean vessel diameter began to differ at radii greater than 400 µm, suggesting that radiation may have negative effects on peripheral maturation of newly formed vessels (Figure 6B).

Discussion

To the best of our knowledge, this is the first study investigating the effects of ionizing radiation within an AV loop-based neovascular system. Grafts were exposed intraoperatively to a single dose of 2 Gy ionizing radiation prior to loop construction in order to elucidate whether they provide angio-inductive features under conditions of high flow that are mandatory for AV loop associated angiogenesis. As assessed by three-dimensional micro-CT analysis and two-dimensional quantification of mean vessel number and area, we demonstrated that vessel sprouting was significantly impaired when irradiated vein grafts were used for AV loop construction. Further dissecting this angio-inductive phenomenon histologically, we provided

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

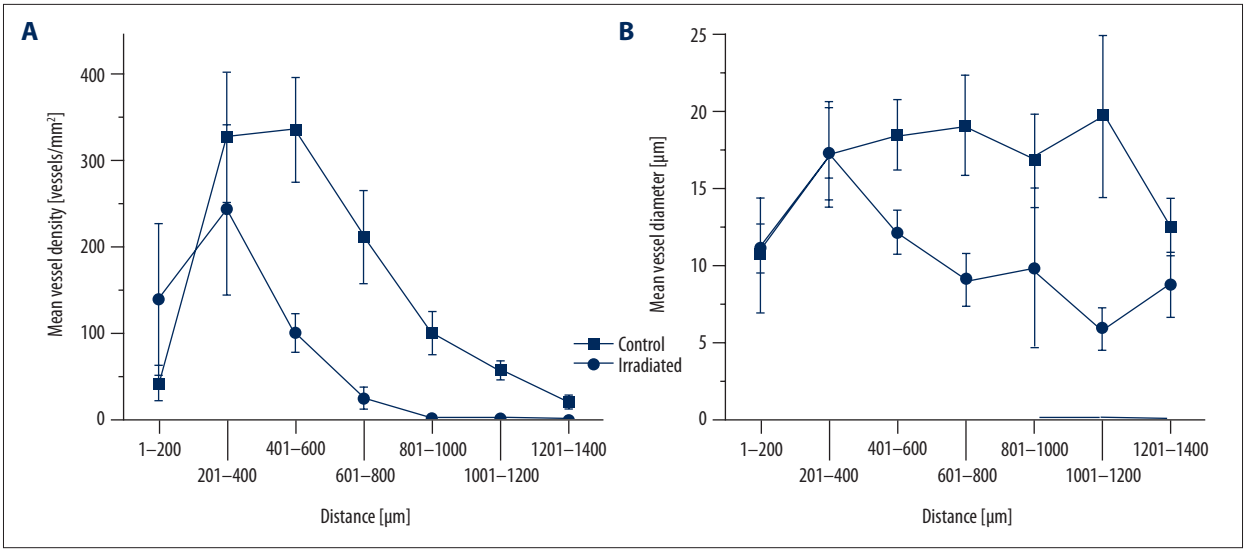

Figure 6. Spatial distribution of mean vessel density and diameter after local graft irradiation. Mean vessel density and mean vessel diameter were assessed separately in the stated areas. Graft-irradiation homogeneously reduced mean vessel density with increasing distance from the main vessel axis within the 3-dimensional construct when compared to non-irradiated controls (**A**). Interestingly mean vessel diameter was primarily diminished in more peripheral regions of the construct (**B**).

evidence that the emerging microcirculatory system was homogeneously distributed along the main vessel axis. The vessel amount was significantly reduced when an irradiated venous graft was used. Additionally, we illustrated that radiation compromises neovessel stabilization and maturation as mean vessel diameter primarily decreased in more peripheral regions of constructs containing irradiated vein grafts. Mean vessel density, however, was reduced evenly throughout the formed construct.

Changes in mechanical load following vein grafting into an arteriovenous shunt flow have been shown to foster angio-inductive properties in the graft endothelium [12]. However, it has also been demonstrated that vascular grafts are exposed to a significant amount of endothelial denudation [13,14,20]. Notably, even decelluarized and avital vascular allografts seem sufficient to provide angiogenesis in the AV loop [15]. Thus, it has been proposed that the venous graft merely serves as a connecting prosthesis providing sufficient length to ensure a tension-free AV anastomosis. Vessel sprouting was assumed to originate exclusively from the recipient artery and vein respectively [15]. Contrary to this believe, we repeatedly observed luminal sprouts emerging from the grafted vein. Polykandriotis et al. observed by means of scanning electron microscopy (SEM) of corrosion casts, that luminal sprouting was present in the vein and the grafted vein but not in the arterial segment between day 10 and 14 after AV loop creation [6]. We previously pointed out that, in contrast to pulsatile flow, increased vascular shear rate induced specific cellular responses linked to angio-inductive behavior [12]. Notably, intercellular gap junction protein connexin (Cx) 43 is specifically upregulated in vein graft endothelium, and it has been suggested that it is vital for angiogenic signaling and vessel growth [21,22]. Thus, we believe that the interposed graft transduces mechanosensitive stimuli after exposure to high flow.

The detrimental effects of ionizing radiation have long been recognized and are widely used in adjuvant and neoadjuvant treatment options worldwide. Hydroxyl radicals (OH) alter cellular lipid hemostasis, membrane transport, and constitute significant genotoxic stressors on vascular cells [23,24]. Double-strand DNA breaks ultimately activate proto-oncogenes and propel malignant cell transformation. The molecular mechanisms behind radiation-induced genetic alterations are for the time being largely unidentified but may, among others factors, be linked to dysfunctional expression and nuclear translocation of cytoplasmic transcription factors [25]. Within blood vessels, endothelial cells are the most vulnerable to irradiation [26]. Radiation-induced vascular injury correlates histologically with desquamated endothelial cells, which leads to small vessel obstruction and subsequent tissue ischemia [25]. It has been previously demonstrated that the acute effect of radiation-induced vascular injury depends on vessel diameter. Severity of adventitial fibrosis and medial hyalinization are most distinctively recognized in vessels <500 µm [25].

In the presented study, we showed that single-dose graft irradiation with 2 Gy impaired the development of a functional microcirculatory system likely due to dysfunctional graft-specific and angio-inductive properties.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

The clinical management of head and neck tumors and extended extremity sarcomas continue to challenge reconstructive surgeons worldwide; AV loop-based tissue transfer may provide an essential adjunct for vascularized tissue transfer after oncologic resection. As the majority of these patients regularly require adjuvant radiotherapy, the effect of radiation on AV loop vascularization is of great clinical importance. Although strongly decreased, vessel sprouting was not fully abolished after irradiation in our study. Hence, vascular shear rate might constitute a fairly sturdy and vital determinant for loop-associated neoangiogenesis even after isolated and high-dose radiation of the mediating vein. Nevertheless, we only examined short-term effects of radiation on AV loop associated angiogenesis. As vascular tissue mainly consists of slowly proliferating cells [23], single-dose local graft irradiation may impede vascular hemostasis of bioengineered constructs primarily in the long term. Further analysis of radiation-mediated ramifications of the AV loop model may contribute to safe clinical implementation and adequate patient selection.

The isolated and homogeneous chamber environment, as well as the established quantification algorithms, render the AV loop model eligible for compartment-specific characterization of newly developing vascular networks. As various malignant microenvironments induce different dynamic and angioinductive behavior, the AV loop model may conceivably be a useful tool for further *in vivo* characterization of anti-angiogenic treatment options [27]. Dynamic and molecular interactions of various cancer stem cells with a defined vascular supply, as well as their intrinsic susceptibility to radiotherapy and other novel and locally restricted treatment options, may conveniently and separately be studied within the AV loop chamber. In the presented study, we investigated the effects of radiation in a benign angiogenesis rat model. These findings provide a useful tool for further molecular characterization of tumor growth; especially as various tumor cells differently interact with vascular structures thereby altering radiation sensitivity [28].

Conclusions

Taken together, we provided evidence that the venous graft constitutes a vital part of the AV loop model and is essential to initiate vascular luminal sprouts. It remains speculative whether reduced graft viability or cellular alterations are responsible, as irradiation induces a plethora of vascular and metabolic changes within the vascular tissue. Although not completely impaired, graft-specific irradiation negatively influences AV loop associated vascularization. Radiation compromises neovessel stabilization and maturation primarily in peripheral regions of AV loop-based constructs. These results add to the current understanding of AV loop-based neovascularization and suggest clinical implications for patients relying on combined AV loop-based tissue transfer and adjuvant radiotherapy.

Acknowledgements

The excellent technical assistance of Ilse Arnold, Marina Milde and Stefan Fleischer is gratefully acknowledged.

Conflicts of Interest

None.

References:

1. Kneser U, Schaefer DJ, Polykandriotis E, Horch RE: Tissue engineering of bone: the reconstructive surgeon's point of view. J Cell Mol Med, 2006; 10(1): 7–19
2. Erol OO, Spira M: New capillary bed formation with a surgically constructed arteriovenous fistula. Surg Forum, 1979; 30: 530–31
3. Murphy WL, Peters MC, Kohn DH, Mooney DJ: Sustained release of vascular endothelial growth factor from mineralized poly(lactide-co-glycolide) scaffolds for tissue engineering. Biomaterials, 2000; 21(24): 2521–27
4. Tanaka Y, Sung K-C, Tsutsumi A et al: Tissue engineering skin flaps: which vascular carrier, arteriovenous shunt loop or arteriovenous bundle, has more potential for angiogenesis and tissue generation? Plast Reconstr Surg, 2003; 112(6): 1636–44
5. Arkudas A, Pryymachuk G, Hoereth T et al: Composition of fibrin glues significantly influences axial vascularization and degradation in isolation chamber model. Blood Coagul Fibrinolysis, 2012; 23(5): 419–27
6. Polykandriotis E, Tjiawi J, Euler S et al: The venous graft as an effector of early angiogenesis in a fibrin matrix. Microvasc Res, 2008; 75(1): 25–33
7. Tanaka Y, Tsutsumi A, Crowe DM et al: Generation of an autologous tissue (matrix) flap by combining an arteriovenous shunt loop with artificial skin in rats: Preliminary report. Br J Plast Surg, 2000; 53(1): 51–57
8. Horch RE, Beier JP, Kneser U, Arkudas A: Successful human long-term application of *in situ* bone tissue engineering. J Cell Mol Med, 2014; 18(7): 1478–85
9. Weigand A, Beier JP, Hess A et al: Acceleration of vascularized bone tissue-engineered constructs in a large animal model combining intrinsic and extrinsic vascularization. Tissue Eng Part A, 2015; 21(9–10): 1680–94
10. Semenza GL: Vasculogenesis, angiogenesis, and arteriogenesis: Mechanisms of blood vessel formation and remodeling. J Cell Biochem, 2007; 102(4): 840–47
11. Watson O, Novodvorsky P, Gray C et al: Blood flow suppresses vascular Notch signalling via dll4 and is required for angiogenesis in response to hypoxic signalling. Cardiovasc Res, 2013; 100(2): 252–61
12. Schmidt VJ, Hilgert JG, Covi JM et al: Flow increase is decisive to initiate angiogenesis in veins exposed to altered hemodynamics. PloS One, 2015; 10(1): e0117407
13. Polykandriotis E, Tjiawi J, Euler S et al: The venous graft as an effector of early angiogenesis in a fibrin matrix. Microvasc Res, 2008; 75(1): 25–33
14. Westerband A, Crouse D, Richter LC et al: Vein adaptation to arterialization in an experimental model. J Vasc Surg, 2001; 33(3): 561–69
15. Zdolsek JM, Morrison WA, Dingle AM et al: An "off the shelf" vascular allograft supports angiogenic growth in three-dimensional tissue engineering. J Vasc Surg, 2011; 53(2): 435–44
16. Moulder JE, Fish BL, Wilson JF: Tumor and normal tissue tolerance for fractionated low-dose-rate radiotherapy. Int J Radiat Oncol Biol Phys, 1990; 19(2): 341–48

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

17. Weis C, Covi JM, Hilgert JG et al: Automatic quantification of angiogenesis in 2D sections: A precise and timesaving approach. J Microsc, 2015; 259(3): 185–96
18. Arkudas A, Tjiawi J, Bleiziffer O et al: Fibrin gel-immobilized VEGF and bFGF efficiently stimulate angiogenesis in the AV loop model. Mol Med, 2007; 13(9–10): 480–87
19. Arkudas A, Beier JP, Pryymachuk G et al: Automatic quantitative micro-computed tomography evaluation of angiogenesis in an axially vascularized tissue-engineered bone construct. Tissue Eng Part C Methods, 2010; 16(6): 1503–14
20. Qin F, Dardik H, Pangilinan A et al: Remodeling and suppression of intimal hyperplasia of vascular grafts with a distal arteriovenous fistula in a rat model. J Vasc Surg, 2001; 34(4): 701–6
21. Wang H-H, Su C-H, Wu Y-J et al: Reduction of connexin43 in human endothelial progenitor cells impairs the angiogenic potential. Angiogenesis, 2013; 16(3): 553–60
22. Schmidt VJ, Hilgert JG, Covi JM et al: High flow conditions increase connexin43 expression in a rat arteriovenous and angioinductive loop model. PloS One, 2013; 8(11): e78782
23. Menendez JC, Casanova D, Amado JA et al: Effects of radiation on endothelial function. Int J Radiat Oncol Biol Phys, 1998; 41(4): 905–13
24. Soloviev AI, Tishkin SM, Parshikov AV et al: Mechanisms of endothelial dysfunction after ionized radiation: selective impairment of the nitric oxide component of endothelium-dependent vasodilation. Br J Pharmacol, 2003; 138(5): 837–44
25. O'Connor MM, Mayberg MR: Effects of radiation on cerebral vasculature: A review. Neurosurgery, 2000; 46(1): 138–49; discussion 150–51
26. Dimitrievich GS, Fischer-Dzoga K, Griem ML: Radiosensitivity of vascular tissue. I. Differential radiosensitivity of capillaries: A quantitative *in vivo* study. Radiat Res, 1984; 99(3): 511–35
27. Folkman J: Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med, 1995; 1(1): 27–31
28. Moeller BJ, Richardson RA, Dewhirst MW: Hypoxia and radiotherapy: Opportunities for improved outcomes in cancer treatment. Cancer Metastasis Rev, 2007; 26(2): 241–48

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]