

ORIGINAL RESEARCH

Ex-vivo Mechanical Augmentation of Human Saphenous Vein Graft By UV-A Irradiation in Emergency Vascular Reconstruction – Preliminary Results

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ABSTRACT

Introduction: In vascular reconstruction in arterial trauma, ruptured abdominal aortic aneurysm or ruptured aneurysmal arteriovenous fistula, the challenge no longer lies in the surgical procedure itself, but rather the prevention of intimal hyperplasia, thrombosis and aneurysm formation, in parallel with extending as long as possible the patency of the grafts. The **aim** of this study is to present the preliminary findings of a novel non-ionizing radiation-based therapeutic method for stabilizing and strengthening the extracellular matrix of the venous wall, improving the biomechanical profile of the autologous graft used in myocardial and lower limb revascularization. **Material and methods:** We developed the protocol and method for

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UV-A irradiation as a new method of mechanical augmentation of the resistance structure of the venous graft. Samples of the superficial femoral artery, superficial femoral vein, and great saphenous vein (GSV) were extracted from a 58-year-old patient who underwent above-the-knee amputation, and were prepared in $5 \times 5 \text{ cm}^2$ patches. Additionally, we analyzed the samples biomechanically biaxially with the BioTester® 5000, in which we established a 25% equibiaxial stretch. The GSV sample was also treated by UV-A irradiation after being kept in riboflavin 5'-phosphate monosodium salt for 30 min. **Results:** After UV-A treatment of the GSV wall, we observed an important increase of Cauchy stress from 82 kPa to 131 kPa in the longitudinal axis and from 66 kPa to 115 kPa in the circumferential axis. Young's modulus also changed after treating the GSV wall from 0.564 MPa to 1.218 MPa (longitudinal) and from 0.397 MPa to 0.709 MPa (circumferential). As a result of the therapy, we observed a considerable similarity of the mechanical behavior of the GSV wall to that of the artery wall. **Conclusion:** The photocrosslinking of collagen fibers at the vein graft adventitia hardens and stiffens the venous wall, making it behave like the arterial wall after treatment. These preliminary ex vivo results on human vascular tissue may serve as the foundation for the development of new treatment approaches utilizing mechanical augmentation of the vein grafts.

Keywords: mechanical augmentation, UV-A irradiation, biomechanical, therapy, photocrosslinking

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INTRODUCTION

In vascular reconstruction in arterial trauma, ruptured abdominal aortic aneurysm, or ruptured aneurysmal arteriovenous fistula, the challenge no longer lies in the surgical procedure itself, but rather the prevention of intimal hyperplasia, thrombosis, and aneurysm formation, in parallel with extending the patency of the grafts for as long as possible.^{1–4} Vein grafts continue to have a significant failure rate of 25–50% at 5 years.^{5,6}

The most common cause of vein graft failure is neointimal hyperplasia. This complex process begins intraoperatively and continues when the artery is exposed to significantly higher pressure,^{7,8} resulting in mechanical strain that upregulates insulin-like growth factor-1 (IGF-1) and its receptors, inducing vascular smooth muscle cells proliferation or intimal hyperplasia.^{9,10} Moreover, the wall stretch induces cellular apoptosis with subsequent cell proliferation,¹¹ but this could be reduced with a vascular external sheath, which diminishes the force exerted on the arterial wall.^{12–15}

A current approach to this issue is that of photochemical crosslinking, during which the tissues are crosslinked by riboflavin photosensitization to increase their stiffness or reduce enzymatic degradation. Crosslinking occurs during the irradiation and is followed by healing processes. The light-induced effects appear to result largely from crosslinks in collagen, the major connective tissue protein, with possible crosslinking to the proteoglycans that surround collagen fibrils and to other proteins.¹⁶

In two recent studies, Chirila et al.^{17,18} demonstrated that the photocrosslinking of fibers from the adventitia of

the porcine aorta by UV-A irradiation increases resistance and strengthens the wall, being proposed as a new therapeutic method to inhibit the progression and lower the risk of rupture of abdominal aortic aneurysms.

The aim of this study is to present preliminary findings from a new non-ionizing radiation-based therapeutic method for stabilizing and strengthening the extracellular matrix of the venous wall, improving the biomechanical profile of the autologous graft used in myocardial and lower limb revascularization. The study also aims to provide a thorough explanation of the methodology employed.

MATERIAL AND METHODS

HUMAN ARTERIAL AND VENOUS TISSUE SAMPLING PROTOCOL

Starting from the results and the method proposed by Chirila et al.,^{17,18} we developed the protocol and method for the histological structure of the venous wall, and we proposed UV-A irradiation as a new method of mechanical augmentation of the resistance structure of the venous graft, with preliminary results presented in this paper.

The investigations were carried out in a 58-year-old patient with Rutherford III acute limb ischemia and indication of amputation above the knee. Intraoperative tubular samples of the superficial femoral artery (SFA), superficial femoral vein (SFV), and great saphenous vein (GSV) were taken across a length of 3 cm. The samples were then immersed in phosphate-buffered saline (PBS) and dried at 4 °C, before being analyzed in the Laboratory of Re-

generative Medicine of the Advanced Centre of Medico-Pharmaceutical Research within the “George Emil Palade” University of Medicine, Pharmacy, Science and Technology of Târgu Mureș, Romania, in less than 12 h.

Each tubular sample was sectioned along the longitudinal axis, and with the help of a scalpel, 5 x 5 mm² samples were formed to be analyzed biomechanically. The thickness of the samples was measured with a digital caliper in the middle of each side, and the average of the four measurements was considered the average thickness of the sample. To reduce any risk of bias, the measurements were made by the same person.

BIOMECHANICAL BIAXIAL TESTING PROTOCOL

We used the best-performing biotester on the market for the biaxial biomechanical analysis of human vascular tissue: the BioTester® 5000 (CellScale, Waterloo, ON, Canada), which was outfitted with four actuators of 23 N and four special rakes for vascular tissue of 5 mm, which were attached to the actuators in the precise location using the built-in magnet. In addition, the biotester included a plastic container filled with PBS and heated to 37 °C, to mimic the body's homeostasis.

Regarding the analysis protocol, we set an initial working area of 5 mm², and after the sample was fixed in the active part of the four rackets, we initiated a preconditioning cycle of stretching the sample until reaching the force of 50 mN, followed by 10 cycles of 50 s each (25 s of stretch and 25 s of recovery) until reaching 125% of the initial size. The data generated by the device at the last cycle were used for analysis and graphic representation. This protocol was used for all types of human vascular tissue analyzed.

In addition, the wall of the internal saphenous vein was exposed to UV-A irradiation (protocol provided later) after being treated for 30 min with a photosensitizer 0.1% riboflavin 5'-phosphate monosodium salt (henceforth, RF). Following the irradiation procedure, the sample was placed in PBS solution and allowed to rest for 1 h at room

temperature. Another measurement was done on the GSV sample treated with UV-A using the previously indicated methodology.

IRRADIATION PROTOCOL

After 30 min in the RF solution, the internal saphenous vein sample was treated with UV-A radiation, at a wavelength of 365 nm, using the UV Curing System OmniCure 1500 device (Excelitas Technologies Corp., Waltham, MA, USA), with a 180-s exposure at the level of the adventitia with an intensity of 50 mW/cm², determined using the Dymax ACCU-CAL 50 radiometer (Dymax Corp., Torrington, CT, USA).

All study procedures were performed in accordance with the Declaration of Helsinki, and the study was approved by the ethics committee of the medical institution.

RESULTS

The biomechanical properties of the samples, including Cauchy stress, Young's modulus, and thickness are presented in Table 1. As it can be seen both in the longitudinal and circumferential planes, the arterial wall registered higher values of Cauchy stress and Young's modulus, compared to SFV and GSV. Furthermore, after the irradiation of the GSV wall, we observed an important increase of Cauchy stress from 82 kPa to 131 kPa in the longitudinal axis and from 66 kPa to 115 kPa in the circumferential axis. Young's modulus also changed after treating the GSV wall from 0.564 MPa to 1.218 MPa (longitudinal) and from 0.397 MPa to 0.709 MPa (circumferential). As a result of the therapy, we observed a considerable similarity of the mechanical behavior of the wall of the GSV to that of the wall of the SFA (Table 1). To emphasize the mechanical strengthening of the venous sample following UV-A irradiation, we plotted the force-displacement plots of the four samples (Figure 1). Furthermore, in Table 2, we highlighted the forces measured at different stretch ratio values (1.05, 1.10, 1.15, 1.20, and 1.25) to demonstrate the dy-

TABLE 1. Thickness and biomechanical characteristics of the analyzed samples

Sample	Cauchy stress (kPa)		Young's modulus (MPa)		Thickness (mm)
	Longitudinal	Circumferential	Longitudinal	Circumferential	
SFA	146	134	1.505	0.995	0.749
SFV	93	73	0.604	0.455	0.431
GSV	81	66	0.564	0.397	0.266
GSV-UV	131	115	1.218	0.709	0.321

TABLE 2. The forces created by the biotester during the bi-axial study of the four samples while the stretch ratio was gradually increased

Axis	Stretch ratio	Force (mN)			
		SFA	SFV	GSV	GSV-UV
Longitudinal	1.05	71	25	11	23
Longitudinal	1.10	135	67	32	54
Longitudinal	1.15	178	88	51	107
Longitudinal	1.20	263	109	74	192
Longitudinal	1.25	349	121	95	275
Circumferential	1.05	77	32	23	44
Circumferential	1.10	161	75	54	88
Circumferential	1.15	245	96	69	147
Circumferential	1.20	414	118	96	382
Circumferential	1.25	624	160	139	681

namic mechanical augmentation of the UV-A-irradiated GSV sample compared to the non-irradiated sample and the SFA sample.

DISCUSSION

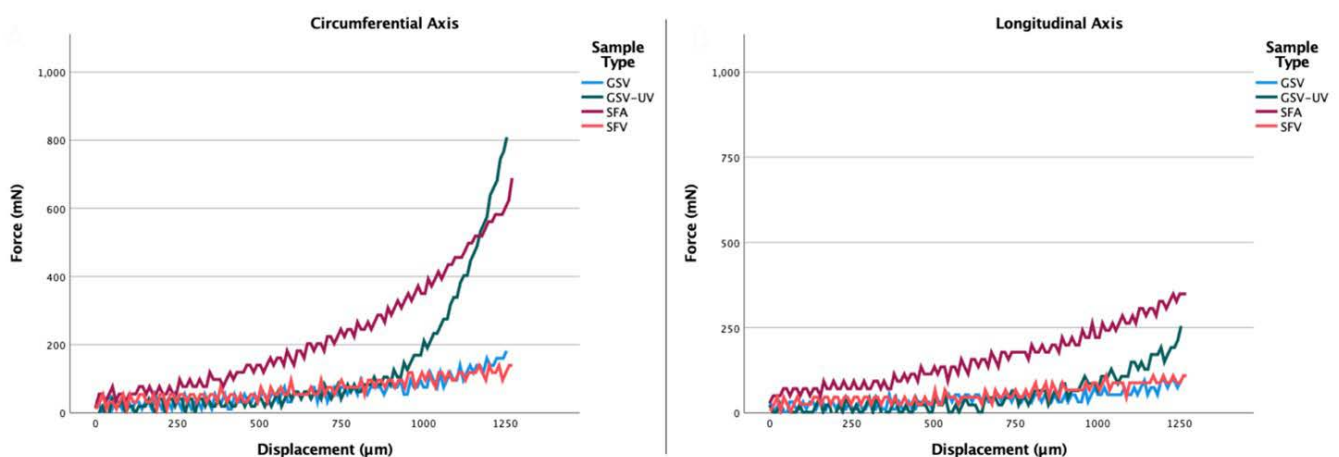
The main result of this study is the demonstration of the mechanical augmentation of the venous wall of the GSV through the photocrosslinking of the collagen fibers at the level of the adventitia, as well as the similarity to the mechanical behavior of the arterial wall at the level of the SFA.

In the case of patients with critical limb ischemia, when endovascular revascularization cannot be used, surgical revascularization remains the only option, and vein graft from the GSV is the first therapeutic option in the case

of patients with trophic disorders and peripheral ischemic skin infections. In addition, when direct arterial restoration is not possible due to substantial vascular damage, the implementation of a venous graft is the initial goal.¹⁹

Recent studies have proposed various techniques for increasing the resistance and stiffness of the venous wall to prevent remodeling and lower the chance of developing neo-intimal hyperplasia, hence enhancing patency.^{20,21} However, the findings are varied, and no approach with excellent outcomes has been identified. Furthermore, most studies are based on animal models.

Goldstone et al.²⁰ used photochemical tissue passivation (PTP) to strengthen the porcine venous adventitia, reducing compliance without affecting the endothelium. Their test revealed a substantial increase in stiffness of the PTP-treated veins compared to the control group

**FIGURE 1.** Graphic representation of the four thickness measurements

($p = 0.001$), similar to that of a porcine artery ($p = 0.2$), confirming that PTP decreases vein elasticity, reducing the degree of neointimal hyperplasia by 66% and medial hypertrophy by 49%. Moreover, in ex vivo testing, PTP increased Young's modulus from 99 kPa to 954 kPa, reducing the mismatch in compliance between the graft and the artery.

PTP therapy may also be beneficial in situations with arteriovenous fistulae. Goldstone et al. performed an end-to-end anastomosis between the distal femoral artery and the proximal epigastric vein in Sprague–Dawley rats. They observed that in the control group the vein grew in diameter by 78–241% (right after the restoration of blood flow to 4 weeks), while the PTP group registered an increase of only 10–69% in diameter. Similar results were obtained a year later, on six porcine models that received bilateral carotid artery interposition vein grafts, where neointima ($p = 0.045$), intima area ($p = 0.01$), and medial area ($p = 0.048$) were thicker in the control group.²⁰

The preliminary results of our study, in which we demonstrate that by photocrosslinking the collagen fibers at the level of the GSV adventitia, we mechanically augment the venous graft to behave similarly to the arterial one, are based on the results published by Chirila et al.^{17,18,22} In the first study,¹⁷ they demonstrated on 30 porcine aorta samples that treating the porcine aortic wall by UV-A irradiation at an intensity of 45 mW/cm² led to an increase in stiffness ($p = 0.001$) and resistance ($p = 0.001$). In other studies, they concluded that irradiation with UV-A mechanically strengthens the porcine aorta subjected to enzymatic degradation with collagenolysis¹⁸ and elastase.²²

Our study has not only validated the approach proposed by Chirila et al.^{17,18,22} on human subjects, but we also presented a new therapeutic method of stabilizing the extracellular matrix in the case of venous grafts. These preliminary results will be the basis for future in vivo studies on animal models, in which we will investigate the impact of photocrosslinking with UV-A at the level of the vein graft in the long term.

CONCLUSIONS

The photocrosslinking of collagen fibers in the vein graft adventitia hardens and stiffens the venous wall, making it behave like the arterial wall after treatment. The preliminary ex vivo results on human vascular tissue of this study will serve as the foundation for the development of new treatment approaches using mechanical augmentation of the vein graft in clinical settings. Our approach has practical relevance in arterial repair, such as vascular damage or

graft infection, as well as in the reconstruction of ruptured aneurysmal arteriovenous fistulas.

CONFLICT OF INTEREST

Nothing to declare.

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