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Abstract

Graft disease leads to graft failure. From the clinical point of view, graft failure could be acute, subacute, and chronic. Acute failure is related to thrombosis, and subacute and chronic to neointima development and atherosclerotic degeneration, respectively. Histological studies evaluating vessel wall morphological, functional, and regenerative integrity may, at least to some extent, predict the risk of graft failure. Transplantation of venous segments into the coronary arterial circulation initiates an inevitable process of arterialization with occluding atherosclerotic plaques as a net final result. Plaques in venous grafts differ from atherosclerotic lesions found in the native coronary arteries and these morphological differences have impact on their mechanical properties. More fragile vein graft atherosclerotic lesions are very prone to rupture and subsequent thrombosis and consequently acute coronary syndrome. Up to now, some preventative measures against atherosclerosis development have been proposed and their results in histological studies are presented in this chapter.

Keywords

Graft disease • Graft failure • Histology • Coronary artery bypass grafting (CABG) • Vessel wall integrity • Atherosclerosis • Acute graft failure • Subacute graft failure • Chronic graft failure • Thrombosis • Intimal hyperplasia • Morphological integrity • Functional integrity • Regenerative integrity

Graft Disease and Graft Failure

The graft disease in aortocoronary bypass conduits is defined as the irreversible reconstruction of the vessel wall eventually leading to graft failure. The appropriate diagnosis of graft failure, although of clinical importance, is not easy in many cases.

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From the clinical point of view, a graft failure could be acute, subacute, and chronic. Acute graft failure usually follows the process of thrombosis and in many cases clinically manifests as sharp chest pain and/or impaired hemodynamic myocardial performance within 1 month (or less) after coronary artery bypass grafting (CABG). However, in the early postoperative period following surgery, the clinical symptoms of graft failure may be subtle or even silent because patients are sedated and pain killers are routinely administered. Moreover, significant reduction in exercise capacity secondary to perioperative myocardial ischemia due to in-graft thrombosis is usually seen after discharge; i.e., after return to normal physical activity. Thus, a real incidence of early graft failure may be underestimated.

Subacute and chronic graft failures are related to intimal hyperplasia and accelerated atherosclerosis, respectively. In

the late follow-up period, 10–15 years after CABG, the complete obliteration of transplanted graft in the coronary circulation is observed in more than 50 % of patients. The majority of these lesions involves saphenous vein grafts. Both subacute and chronic graft failures are predominantly silent. Some patients may manifest progressive clinical deterioration but typically the rate of progression is slow enough for the patient to get accustomed to reduced coronary perfusion. A lack of acute clinical symptoms in these cases makes the early recognition of the lesions difficult. Simply, the patients who do not feel acute coronary pain and do not experience a significant reduction in exercise capacity do not undergo follow-up visits. For this reason, a diagnosis of late total graft obliteration, if any, is markedly delayed or even established postmortem. In developing this observation, it is clear that we do not have, so far, a good human model for graft failure study in CABG. Most of the cited models have been developed on experimental animals. One must be aware that animals in experimental models are usually healthy, thus these models do not mimic real clinical scenarios. Naturally, the final lesions present in grafts in humans and animals could be similar. But the rate of change in humans is completely different than in animals. Thus, we cannot strictly extrapolate the results obtained in animal models into the clinical practice.

The CABG procedure is still considered as the most effective treatment strategy to relieve signs and symptoms of advanced forms of coronary artery disease (CAD). The life expectancy is, however, limited to the saphenous vein grafts' late patency and to a lesser degree to progression of atherosclerosis in the native coronaries. The gold standard in CABG is implantation of the left internal thoracic artery (LITA) to the left descending artery (LAD). Currently, there is no common consensus to routine application of both internal thoracic arteries. The use of the right internal thoracic artery (RITA) together with LITA may significantly increase the risk of sternum dehiscence and delayed healing, particularly in high risk groups such as diabetic, obese, and elderly patients. Unfortunately, these aforementioned groups of patients constitute an increasing number of CABG patients. The saphenous vein is used as an aortocoronary bypass conduit in the majority of cases (even up to 75 %). What is the reason the saphenous vein is still commonly used in CABG? The answer is relatively easy. The simplicity of its obtaining, the length of harvested graft, and, quite often, the necessity for grafting more than one coronary artery. The transplanted graft, no matter whether it is vein or artery, is to perform only one function: to supply blood to the area of ischemic myocardium. So, it is to be a conduit. Nothing else. Unfortunately, venous grafts are prone to vascular remodeling.

The basic graft remodeling follows vascular endothelium loss, which might be secondary to the transplanted vessel operation prior to grafting. It is well established that inap-

propriate handling of blood vessel between its harvesting and implantation to the coronary circulation is an important reason for endothelial exfoliation. The transplanted graft that lacks endothelial cells might be the trigger mechanism for conduit thrombosis and acute graft failure. Naturally, all the “no touch” operating techniques enabling graft harvesting, saving a vascular adventitia and avoidance of any vessel injuries during graft tightness assays, increase the endothelium integrity and significantly decrease the risk of acute thrombosis (the presence of integral endothelial cells in the conduit lumen is the best way to avoid the adhesion of blood cells to the walls of bypass and thrombus formation). Moreover, the preserved vessel adventitia increases its tensile strength, which is crucial during performance of all tightness tests prior to graft implantation.

The preservation of adventitia in saphenous vein grafts might be, however, a pronounced risk factor for the subsequent intimal hyperplasia. It is well proven that adventitia is the second most important source of nitric oxide (NO) in the vein vessel. NO is not only crucial for the relaxation of smooth muscles in the median layer of the vessel, it is also the factor that increases the migration of smooth muscle cells into the internal layer of the vessel, which causes intimal hyperplasia. Thus, the preservation of adventitia in the vein graft during CABG decreases the risk of acute graft failure, but on the other hand increases the possibility of intimal hyperplasia, which might be followed by subacute graft failure. Nobody really knows how to handle the graft to avoid both complications.

Taken together, all the aforementioned information seems to support the statement that venous aortocoronary graft reconstruction is an inevitable biological process. If so, do we have any tools to predict the previously mentioned complications? Can we then separate higher risk patients?

In 2010, Sarzaeem et al. proposed a scoring system for predicting saphenous vein graft patency that takes into account not only morphology of venous segments—such as number of branches, degree of varicosity, diameter, and wall thickness—but also angiographic appearance of the recipient coronary arteries [1]. Data presented below suggest that actually we can attempt to define such factors.

Baseline Histological Integrity of Harvested Grafts

The easiest and also the most convincing method of graft integrity evaluation is histological examination of both artery and vein grafts prior to their implementation into the coronary circulation. The harvested vascular segments of 5–10 mm in length are generally long enough for such assessment. They might be collected during surgery without any adverse impact on CABG outcome, then stained and

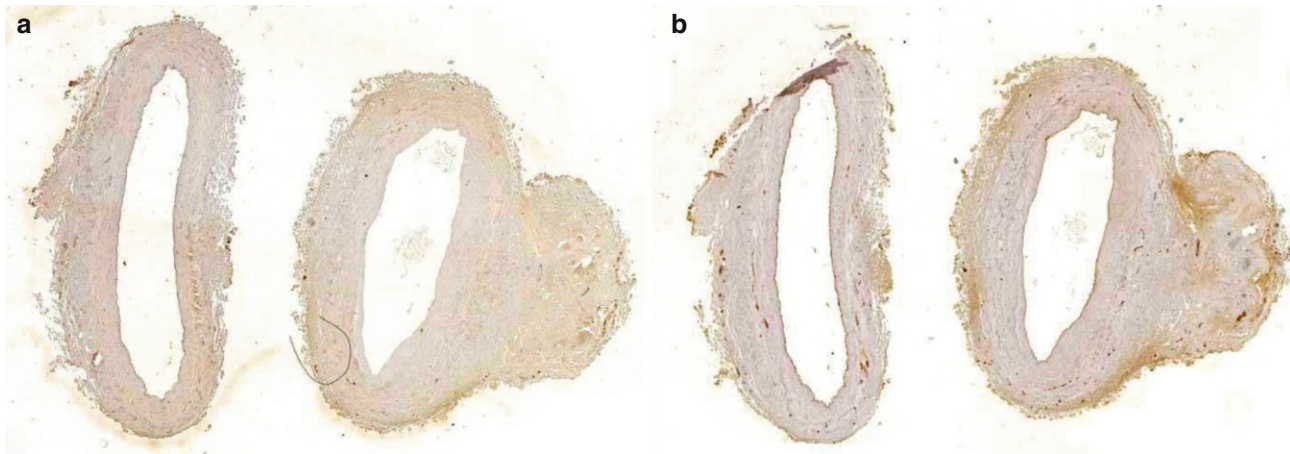


Fig. 21.1 (a) CD31 antigen expression in the wall of saphenous vein segment used as aorto-coronary bypass graft. Note, expression of CD31 antigen in endothelial cells and in *vasa vasorum* in the tunica adventitia. <http://caom.pl/preparat/bp-34cd31.html>. (b) Expression of von Willebrand factor in venous segment prior to implantation in the coro-

nary circulation. <http://caom.pl/preparat/bp-341vw.html> (Note: Figures may be viewed under magnification using included URLs for Center for Documentation of Morphological Images and Digital Database of Microscope Images, Poznań University of Medical Science.)

eventually examined by histologists. The basic task histologists have to perform is to evaluate the degree of integrity of the transplanted grafts. Such integrity can be defined on three levels: morphological, functional, and regenerative.

The morphological integrity is defined as the ratio of endothelial cells expressing the presence of CD31 and von Willebrand protein to the total number of endothelial cells (the percentage of CD31-positive or von Willebrand-positive cells can be easily calculated by the standard morphometric software as the length of positive endothelial cells to the endothelium circumference). Figure 21.1a, b present pictures of histological study to assess morphological integrity. Figure 21.1a documents the immunohistochemical presence of CD31 antigen in endothelial cells of saphenous vein present in tunica intima (continuous expression) and individual *vasa vasorum* localized in adventitia. Expression of von Willebrand factor is captured on Fig. 21.1b. Similarly to CD31 antigen, it is present within endothelial cells of tunica intima and adventitia.

CD31 is a transmembrane protein with a molecular weight of 130 kD, serving for adhesive interactions between neighboring endothelial cells as well as leukocytes and endothelium. The protein belongs to the immunoglobulin superfamily known as PECAM (platelet endothelial cell adhesion molecule). CD31 is present in all types of blood vessels (arteries, arterioles, veins, venules, and capillaries) and in the endothelium of lymphatic vessels. What is interesting is that CD31 and von Willebrand factor (vWF) are the marker proteins of mature endothelial cells. vWF is a glycoprotein composed of several subunits of molecular weight in the range of 500–10,000 kDa. It is found in Weibel-Palade corpuscles and Golgi complex of endothelial cells. The protein mediates the adhesion of platelets to the damaged endothelial cells,

and it is a plasma transporter of factor VIII, protecting it from the effects of proteolytic enzymes.

The functional integrity of graft endothelium can be studied by the expression of endothelial nitric oxide synthase (eNOS) [2, 3]. It is an enzyme responsible for the synthesis of nitric oxide (NO) in endothelial cells. NO is the most important factor of vasodilatation in blood vessels, thus eNOS expression is a direct prove for functional capacity of transplanted graft. Figure 21.2a, b show histological assessment of functional integrity of venous (Fig. 21.2a) and arterial (Fig. 21.2b) conduits prior to implantation into the coronary circulation. In both cases, eNOS is present in endothelial cells of tunica intima, as well as number of capillaries localized in subendothelial meshwork, tunica media, and adventitia. Interestingly, eNOS is also present in smooth muscle cells present in arterial tunica media (Fig. 21.2b).

As already mentioned, CD31 and vWF are the marker proteins of mature endothelial cells. The physiological endothelium, however, is composed not only of mature, but also immature, progenitor cells. The presence of progenitor cells is regarded as the index of the endothelial regenerative capacity. The marker proteins indicating regeneration integrity are CD133 and CD34. CD133 is a transmembrane glycoprotein with a molecular weight of 120 kDa. This antigen takes part in the regulation of growth and differentiation of stem cells. Its expression occurs in immature hematopoietic cell lines, and rapidly decreases during the process of their differentiation. Therefore, the presence of CD133 antigen is not observed in mature cells derived from bone marrow and mature endothelial cells. CD34 is one of the most commonly used of endothelial markers in general histological practice. It is a transmembrane protein with molecular weight of 115 kD, involved in adhesion of endothelial cells and their

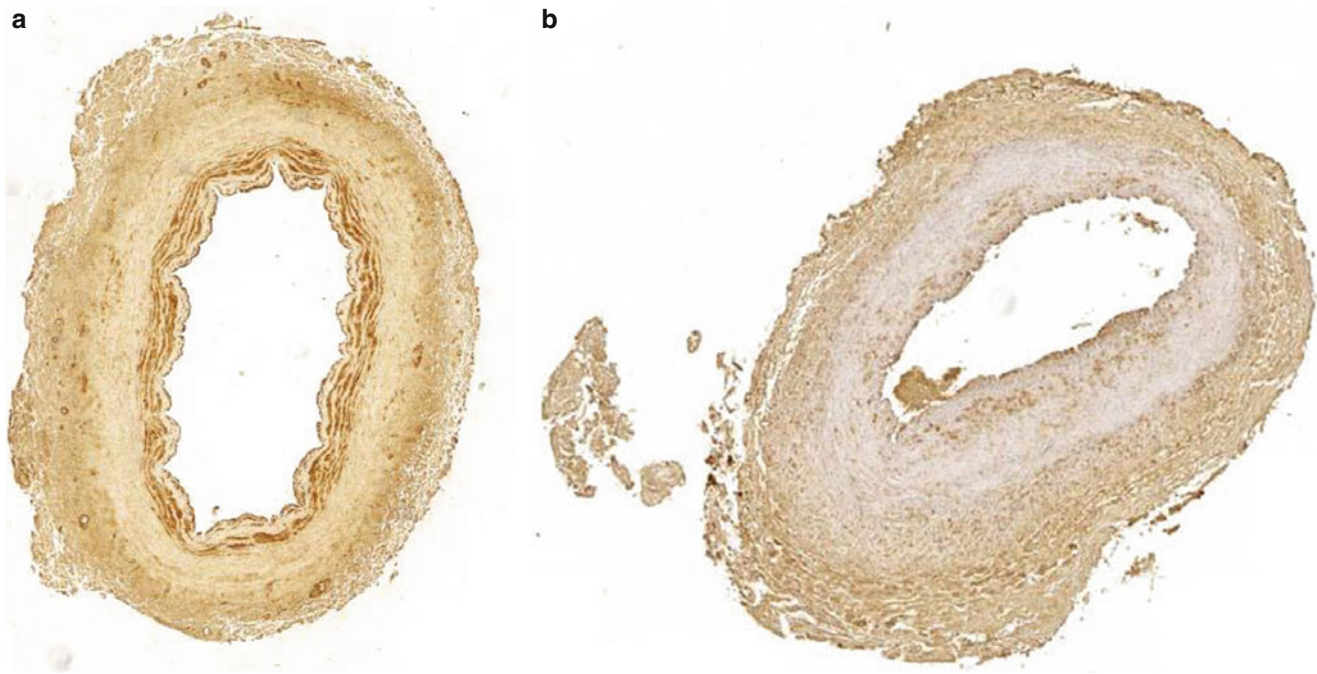


Fig. 21.2 (a) Expression of endothelial nitric oxide synthase (eNOS) in the venous wall. Note, eNOS expression in endothelial cells of the tunica intima, as well as number of capillaries localized in subendothelial meshwork, tunica media and in the tunica adventitia. <http://caom.pl/preparat/bp-3010-03enos.html>. (b) Expression of endothelial nitric oxide synthase (eNOS) in the wall of left internal mammary artery

(LIMA). Interestingly, eNOS is also present in smooth muscle cells present in arterial tunica media. <http://caom.pl/preparat/lima-21enosgora.html> (Note: Figures may be viewed under magnification using included URLs for Center for Documentation of Morphological Images and Digital Database of Microscope Images, Poznań University of Medical Science)

migration during angiogenesis. CD34 antigen is present in the majority of bone marrow hematopoietic cells and endothelial cells of all blood and lymph vessels. CD34 expression is observed in the early stages of endothelial progenitor cells (EPCs). With the maturation of EPCs the expression of CD34 on the surface of endothelial cells decreases. It is stated the adult, mature endothelial cells do not express CD34.

Taken this information together, and based on the scientific reports, the risk of graft failure (acute thrombosis, intimal hyperplasia, and accelerated atherosclerosis) can be (to some extent) predicted by the studies of endothelial integrity estimated prior to grafting [2, 3].

The ratio of CD31-positive endothelial cells to the total number of endothelial cells <80 % and the percentage of vWF-positive cells in endothelium <85 % is correlated to the increased risk of acute graft failure and thrombosis. The ratio of CD31-positive cells and vWF-positive cells in graft endothelium depends on the graft operation procedure. It was proved that all the “no-touch” techniques as well as the preservation of adventitia in transplanted graft increased the number of CD31 and vWF-positive cells in endothelium (both transplanted arteries and veins). Thus, the aforementioned techniques significantly reduce the risk of acute thrombosis. What is also interesting, the ratio of CD133-

positive cells >20 % in transplanted graft endothelium as well as the percentage of CD34-positive cells >90 % percent are factors of favorable prognosis (they protect against the development of acute thrombosis).

On the other hand, the subacute graft failure, which is related to the intimal hyperplasia, strictly follows the expression of eNOS in graft layers. Interestingly, eNOS might be present not only within endothelial cells but also in muscle cells present in the median vascular layer as well as *vasa vasorum* in adventitia. As it was already stated, eNOS is necessary for blood vessel dilatation. But it is also a factor stimulating muscle cells for proliferation and migration into endothelium. Thus, the preservation of adventitia in vessel grafts during standard operation procedures might be, from the one hand, the counteracting factor for acute thrombosis, but from the other hand, the strategy increasing the risk of intimal hyperplasia.

Venous Conduits Arterialization: Histological Characteristics

Data regarding venous graft arterialization were collected from the findings of the studies carried out on animal experimental models. However, we must be aware of

crucial difference between them and clinical studies on human beings. In animal model, venous grafts are not transplanted between ascending aorta and coronary artery as during CABG but usually jugular or other veins are interposed into the carotid artery circulation [4].

The veins are the vessels that normally work in low-pressure conditions, thus venous wall microstructure and function are optimally adapted to them. The native saphenous vein tunica intima is composed of a continuous layer of endothelial cells on a fenestrated basement membrane embedded with internal elastic lamina. Below there are three layers of smooth muscles with loose connective tissue and elastic fibers between them that comprise the tunica media. The outermost layer is tunica adventitia that is composed of longitudinally arranged smooth muscle cells (SMCs), elastin, and collagen fibers. Nerves and vasa vasorum of the tunica adventitia connect vein wall with surrounding tissues [5–7]. There are some specific features of saphenous veins compared to other veins. They are more muscular than a typical vein wall [8]. Untouched native saphenous veins may manifest mild intimal and/or medial fibrosis even before grafting. Moreover, sparse SMCs may be seen in the tunica intima of saphenous vein but not in typical veins. Harvesting saphenous vein segment means exposure of vein to abnormal conditions both inside and outside of vessel. Primary changes are observed in endothelium even just after surgical procurement of the venous segment. Disruption of endothelial layer of saphenous vein, seen in scanning electron microscopy, results from mechanical trauma and manual excessive distension to overcome vessel spasm typically observed after its dissection free from the surrounding tissues. A degree of injury is proportional to pressure applied during graft preparation. Vein transplantation into the arterial circulation dramatically changes the physical condition of vein functioning. The transplanted graft is exposed to high blood pressure that causes the reconstruction of the graft. It aims to strengthen the graft wall and is related to the proliferation of smooth muscle cells (SMCs) in tunica media. It was proved that the process of SMCs proliferation was associated with their histological transformation (from contractile to synthetic phenotype) that involved both cells microstructure and function. SMCs themselves and their nuclei change their shape from elongated (spindle-shaped) to epithelioid-shaped. The shape of nuclei and cells was shown to be an important indicator of biological activity [9]. In *in vitro* models, SMCs exhibiting more elongated nuclei were associated with significantly lower proliferation rate. Moreover, expression of cytoskeletal proteins is shifted from these typical for mature and differentiated cells (calponin, smooth muscle – myosin heavy chains) into immature (cytokeratin-8) proliferating cells. These synthetic cells are able to produce and release metalloproteinases that enable them to migrate in the direction of

the lumen. Higher preexisting expression of both cytokeratin-8 and matrix metalloproteinase-2 (MMP-2) were shown to be independent risk factors for accelerated venous graft failure [10, 11]. This process does not affect the function and morphology of endothelial cells until muscle cells begin to migrate into the intima through digested internal elastic lamina. This process accelerates intimal hyperplasia and formation of neointima. Contrary to normal intima, neointima contains many active, proliferating SMCs and fibroblasts both. Size mismatch between the graft and the target vessel that creates turbulent flow and graft ischemia are factors that may additionally promote denudation of endothelial cells from the luminal surface of venous wall. In practice, neointima itself does not obstruct totally the graft lumen but is considered as a prerequisite for superimposed atherosclerosis. However, this process decreases not only the graft lumen but also affects production of NO, which is crucial for stiffening and assists construction of graft wall.

Although occluding atherosclerotic plaques, which are responsible for total occlusion of venous grafts many years after surgery, share most of the pathologic features of native coronary arteriosclerosis, they also may differ from them. These differences may be of importance in the pathogenesis of acute coronary syndromes throughout the late follow-up period. The venous plaques are established in the ground of intimal hyperplastic tissue. They consist of a thin fragile fibrous cap, cellular components such as proliferating SMCs, foam cells, macrophages, lymphocytes, giant cells, dendritic cells, extracellular lipid droplets, neovasculature, and modified matrix components such as glycosaminoglycans and proteoglycans [12–14]. Calcification may be superimposed onto venous atherosclerotic plaques but they are less extensive than in arteries [15]. In contrast to arterial atheroma, venous plaques are more concentric and diffuse, and the fibrous cap is less fully developed, making them more vulnerable to rupture or disruption and ensuing thrombus formation [16, 17]. Late thrombosis is considered to be a dominating pathogenic factor of acute coronary incidences in the late follow-up period of CABG patients. In addition, in veins, lipolysis is impaired, whereas lipid biosynthesis and uptake is more effective compared to arteries, thus optimal conditions exist for the accelerated formation of venous graft atherosclerosis [18]. As time passes, dense fibrous tissue is increased proportionally, so many years after surgery venous lesions resemble those of native coronary atherosclerosis [19].

Taking this information together, one can presume the process of vein obliteration following the intimal hyperplasia and atherosclerotic degeneration is unavoidable in vein grafts. According to current knowledge, applying preventative measures, venous graft arterialization may be slowed down but not completely inhibited. Some strategies with potentially favorable impact on late graft performance are presented as follows.

Histological Evidence of Efficacy of Preventative Strategies

In the studies that compared different storage media (blood, saline, or buffered saline solutions) and optimal temperature, histological evaluation is the most important measure to assess safety of venous graft storage. Moreover, histological analysis enabled to evaluate the degree of intimal thickening after a given period of time in the groups of different storage solutions. Applying histological techniques, the negative effects of saline on vascular endothelial ultrastructure (luminal surface assessed in scanning electron microscopy) and function (e.g., endothelium-derived vasodilatory function) were proved [20, 21]. A lower temperature of medium provides better conditions to preserve endothelial integrity. Saphenous vein segments immersed in warm saline solution showed massive endothelial cell loss, whereas when immersed in warm blood, they showed only moderate endothelial damage. Meanwhile, at 4 °C, although both cold blood and cold saline immersion fully preserved endothelium, cold saline immersion produced mural oedema [22]. In another study, the best angiographic results 1 year after surgery were obtained if venous segments were kept the buffered saline solutions [23].

A promising area of research is development of gene therapy to prevent the cellular proliferation that leads to intimal hyperplasia and, ultimately, vein graft failure. In animal models, locally delivered gene therapy has prevented intimal hyperplasia in models of arterial balloon injury and venous grafts placed in arterial circulation. It was found out, the area of interest in modification of gene expression leading to intimal hyperplasia involves inhibition of metalloproteinases and growth factors for vascular smooth muscle cells, interfering with the transcription of factors involved in cell cycle regulation as well as apoptosis and functioning of serine/threonine protein kinase.

In the Project of Ex-vivo Vein graft Engineering via Transfection (PREVENT) trial, 41 patients were undergoing infrainguinal arterial bypass using vein grafts [24]. They were subsequently randomly assigned to receive grafts treated ex vivo with an oligodeoxynucleotide (ODN) decoy for E2F, a scrambled ODN or saline solution. E2F is an important transcription factor involved in regulation of the cell cycle. Fluorescent microscopy confirmed the successful delivery of ODNs. The subjects were followed for a median of 53 weeks with serial duplex ultrasonography. At 12 months, there were fewer graft occlusions, critical stenoses and revisions in the group treated with the E2F decoy.

What is also interesting, the reduction of the tangential stress on the vessel wall and, therefore, prevention of disruption of the endothelium and intimal hyperplasia in vein grafts transplanted to arterial circulation, may follow implementation of

stents or rigid grafts placed externally around a vein graft at the time of grafting. These observations were noticed on animal models involving pig saphenous veins anastomosed into the animals' carotid arteries or jugular vein – carotid artery in mongrel dog model [4].

Acknowledgements We would like to thank Agnieszka Malinska, PhD for preparation of the histological figures.

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