POLYMER- AND NANOPARTICLE-BASED SURFACE MODIFICATION OF ARTIFICIAL VASCULAR GRAFTS

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10.1. INTRODUCTION

Vascular disease, or atherosclerosis, is a widespread disease of civilization and the number of patients with this disease is constantly increasing. Atherosclerosis is a degenerative disease of the vascular wall. Its development is a long process that involves solidification of the artery wall and narrowing of its lumen to form so-called atherosclerotic plaques. As a result of this narrowing, there is insufficient blood circulation to the organ supplied by a vessel. The main cause of atherosclerosis is an increased level of cholesterol in the blood. A higher risk of its occurrence is associated with diabetes, high blood pressure, obesity and cigarette smoking [1,2].

In most developed countries, atherosclerosis is the most common cause of death since it is a contributing factor to most heart attacks and strokes, yet a hundred years ago it belonged to the rare and unstudied diseases. Diet and lifestyle change (to consume more fruits, vegetables and fish; to reduce excess weight; to eliminate smoking and alcohol intake and to increase the frequency of physical activity) can provide possible protection [1].

Vascular surgery undoubtedly belongs among the highly specialized branches of medicine. Close cooperation between angiologists and diabetologists plays an important role in the diagnosis and in solving of both acute and chronic vascular diseases. The area of vascular surgery involves venous issues (varicose veins, oedema and venous insufficiency) and arterial issues (signs of limb ischemia, narrowing of the arteries or their closures).

The use of autologous *vena saphena magna* as a patch or bypass is always a priority for the reconstruction of peripheral vessels. In cases where it is impossible to perform autologous reconstruction, alternative vascular grafts (graft knitted from polyester yarn, graft made from expanded poly(tetrafluoroethylene) (PTFE) and others) are used. Implantation of artificial vascular grafts is associated with a higher risk of infection [1].

Infection is the most serious complication of vascular surgery, which significantly increases the morbidity and mortality of patients in this field. Infection is closely related to the increased use of artificial vascular grafts, which started during the second half of the 20th century. According to literature sources, infection affects 2–3 % of patients undergoing vascular surgery [1,2,8,9].

10.2. VASCULAR GRAFTS

The implanted vascular graft must heal well into the arterial system and should fully take over the function of the original arteries. The vascular graft healing process is quite specific for the system and should be completed within all layers of the arterial wall. Vascular graft should ensure a permanent mechanical support and its properties must not negatively affect the formation of a new blood vessel wall [1,2,10].

10.2.1. Properties of vascular grafts

The main criterion for introduction of implants into the practice is their biosecurity. There are strictly defined tests that evaluate the safety of the materials that come into contact with a patient's tissues and blood. Most of the tests are performed *in vitro*, i.e., in cell culture, but tests using laboratory animals are irreplaceable. It is impossible to create conditions in tissue culture that imitate the behaviour within the organism. However, the development of technologies for *in vitro* testing is advancing, and therefore, the number of laboratory animals needed is decreasing. However, vascular grafts must still meet a number of criteria, which can be divided into biological, surgical and technical properties [1,2,10].

10.3. TYPES OF VASCULAR GRAFTS

Vascular grafts are divided into three groups. The first is the biological substitutes: biological origin, regardless of further modification and adaptation. Another kind of graft is artificial: they are made from non-biological material. A third group of grafts are designated as special types. In these grafts, basic characteristics are amended by modifications, the parameters of which are not possible to define as in the previous two basic groups [1]. Individual groups of artificial vascular grafts are further subdivided according to the manufacturing technologies used (Figure 1).

Figure 1. Basic sorting of commercially produced types of vascular grafts. Dividing them into separate subgroups is according to natural or artificial origin of recovered material and according to techniques of vascular grafts industrial production.

10.3.1. Biological vascular grafts

Biological, autologous grafts are selected in cases where a low flow rate or an increased risk of infection is expected. However, the limitation of their use depends on the availability and also on the dimensions (length, lumen). Biological grafts can be further subdivided into the following categories:

- vascular autograft – graft is removed and implanted in the same organism
- vascular allograft – graft is removed from one organism and implanted into another organism of the same species
- vascular xenograft – graft removed from one organism and implanted into another organism of a different species

It should be noted that even autologous transplantation has its drawbacks and obstacles. The main challenge is obtaining a graft with the required dimensions. Every large artery is critical for supplying an organ and cannot be removed without sequelae. Atherosclerosis does not only affect particular parts of the body but also the disease overall, and therefore, the same implanted tissue will tend to develop the same pathological changes [1,2].

10.3.2. Artificial vascular grafts

Currently, there are three basic types of artificial vascular grafts. They differ in their properties, which are given by production technology. The first group consists of the so-called knitted grafts. These grafts are prepared by knitting

synthetic fibres, typically polyester fibres. The walls of these grafts are porous. Blood loss is minimized by impregnation with collagen or gelatin. The second group includes woven grafts. These grafts have walls with very low porosity. Their disadvantage is insufficient anchorage of the internal fibrin layer. Grafts of expanded poly(tetrafluoroethylene) (ePTFE) are so-called casted artificial grafts and form the third group. They are non-porous and their water-repellent walls are suitable for the smaller flow rates. Predominantly grafts with small diameter are used due to their higher price. Textile grafts can be used in the chest, abdomen and pelvic areas. PTFE grafts cannot be used in peripheral reconstructions, but can be used for arteriovenous connections, *e.g.*, locations on the forearms, arms and legs. The selection of materials for vascular grafts is nowadays subject to rigorous quality control, frequently regarding biocompatibility. Polyester fibres, PTFE and most polyurethanes fulfil these requirements. The synthetic materials dacron and teflon are currently most commonly used for the production of fibres [1,2].

10.4. INFECTIONS OF VASCULAR RECONSTRUCTIONS

Infection complicates approximately 2–3 % of angiosurgical operations [10,11]. Complications that follow a surgical operation may occur immediately after surgery or even several days or weeks later (Table 1). The emergence of infection is influenced by many factors. Some of them can be eliminated, while others cannot be affected. Often, the infection is associated with a risk of loss of a limb or even death. Complications are associated with high morbidity and mortality due to the accompanying sepsis [12]. Infection may occur because of nonspecific complications. Timely and correct anamnesis and clinical examination of the patient is important. For these reasons, it is important to transfer vascular surgery patients with incidence of infection to specialist centres, which have excellent diagnostic and therapeutic options and particularly experienced, professional staff [1,8,11,13].

10.4.1. Classification of infection

The following modified classification by Szilagyi is the most commonly used in practice [14]. A first-degree infection skin involves only the skin, a second-degree infection extends into the subcutaneous tissue above the reconstruction and a third-degree infection affects vascular reconstruction. Within 30 days after the primary vascular operation, the infection is referred to as an early, and 1 month after implantation, a late infection. Infection of reconstruction can even occur 10 or more years after the primary operation [14].

10.4.2. Possible risks of infection

The presence of defective limbs during the vascular reconstruction increases the risk of late infection and the necessity of reoperation. This risk also increases in the case where a patient suffers from multiple diseases, such as diabetes mellitus, renal insufficiency, nicotine abuse or infectious fungal aneurysm, *etc.* Before the operation, it is important to eliminate an eventual chronic infection [15,16].

The incidence of infection increases with reoperation in the same area. In the case of artery operation, the duration of the reconstruction plays a role (artery exposure time). In order to prevent colonization of the reconstruction immediately after implantation, it is necessary to operate in surroundings protected by coagulum [1,17,18].

New procedures and technologies have been introduced into clinical practice for the reduction of infections by implanted vascular grafts. These are laparoscopic and robot-assisted surgery.

Table 1. List of early and late complications during operations in transplant surgery and percentage risk of emergence of complications. Adapted from [19].

Early complications	Risk (%)	Late complications	Risk (%)
Infections of the skin and subcutaneous tissue	15	Infections of the skin and subcutaneous tissue	2
Other reoperation	13	Prosthetic infection	3
Vascular reoperation	2	Pseudoaneurysms	4
Thrombosis	2	Vascular reoperation	18
Embolism	1	Thrombosis	11
Haemorrhage	1	Embolism	0
Amputation	2	Amputation	5

10.4.3. Bacteria causing postoperative infection

The causative agents of infection are usually *Staphylococcus aureus*, *Staphylococcus epidermidis*, and Gram-negative bacteria, especially *Escherichia coli*. Information about infection by MRSA (methicillin-resistant *Staphylococcus aureus*) can be also found in the literature. In most cases, results of cultivation

are negative, despite a macroscopically obvious infection of reconstruction [1,12,14,15,17,18].

10.4.4. Treatment

There are many recommended practices for the treatment of infections after vascular reconstruction. Some papers report options for infection treatment in the affected area by the long-term lavage (by disinfectant or antibiotics). In most cases, experts are inclined to agree that it is impossible to get rid of the infection in the already infected graft. The infection is only suppressed by the use of antibiotics or disinfectant. Usually, reoperation is necessary [1,20]. Despite the efforts of manufacturers of vascular grafts, none of them are 100 % resistant to infection.

10.5. SURFACE MODIFICATION OF VASCULAR GRAFTS

Vascular grafts are an essential part of contemporary medicine and find their application in clinical practice. They are prepared from fabric, the nanostructuralized network of which resembles the extracellular matrix in natural blood vessels and improves the biocompatibility of artificial blood vessels [21]. Vascular grafts have to be further modified to increase their biocompatibility, leading to acceptance by an immune system. The graft must also be modified to maintain flow and anticlotting properties.

Bacterial infections (especially those caused by *Staphylococcus aureus*) belong to the most serious complications of operations associated with the use of vascular implants [22-24]. Vascular grafts may therefore be modified by antibiotics that are bound to collagen or gelatin [22,25]. The drawback of antibiotic use is a rapid development of bacteria that are resistant to these drugs. Another possibility for antibacterial modification is the use of silver ions and the more effective silver nanoparticles [26-29]. Additionally, silver nanoparticles can be used in combination with palladium nanoparticles and other antibacterial agents [30,31]. Antimicrobial biopolymers, such as hyaluronic acid and chitosan, are also known. Both substances are highly biodegradable and biocompatible with the human organism [32,33].

The most common technique for the assessment of antimicrobial activity of the test component is a disc dilution method, which uses impregnation of discs (from woven vascular grafts with a diameter of 1 cm) by the test substance. After their attachment to the agar in Petri dishes coated by bacterial culture, the formation of inhibition zones is monitored, indicating the degree of antimicrobial activity (Figure 2).

Figure 2. Testing the antimicrobial activity of vascular grafts by the dilution method.

Squares from the knitted vascular graft (made by VUP Medical, a.s., Brno, Czech Republic) are soaked with the antimicrobial compound and placed into a Petri dish covered with the bacterial culture diluted to an absorbance of 0.1 AU. After 24 h of incubation (37 °C) the resulting inhibition zones are measured. An increasing size of the inhibition zones indicates increasing antimicrobial activity of the test substance.

10.5.1. Modification by metal or semimetal nanoparticles

Nanotechnologies are one of the most advanced scientific disciplines today. The aim is to form nanoparticles that are in compliance with the size, shape, structure and distribution in the organism. Metal nanoparticles with a size of 1–200 nm may be modified by layers of different compounds, such as biopolymers [34]. Nanoparticles prepared by this method show altered physical and chemical properties compared with nanoparticles based only on metals [35,36].

Due to specific properties of the nanoparticles, they are used as catalysts and important components of sensors based on optical or electrochemical methods. They have also found wide use in biomedical applications that require bacterial sterility [37]. A high catalytic ability, given by the large surface of the nanoparticles, and the ability to generate reactive oxygen species causes a high reactivity of nanoparticles, which thus become more toxic to bacteria [38].

Using modern techniques, metal nanoparticles can be functionalized, and thus it is possible to increase their antimicrobial activity [39]. Due to their properties, nanoparticles exhibit greater retention in the body, so it is easier to achieve the desired distribution effect [40].

One potential solution that can reduce the number of infections in operations is the application of silver nanoparticles (micrograph depicted in Figure 3) [4,5]. Silver nanoparticles are commonly used in the textile industry and in medicine [41]. Until recently, silver nanoparticles were commonly used for implant materials that reduced the incidence of postoperative complications. However, over time these applications were associated with a potential risk of toxicity [42].

Figure 3. AgNPs characterized by scanning electron microscope (SEM). Micrographs: a) SEM HV: 15 kV, view field: 1.445 μm , WD: 3.761 mm, det: InBeam, b) SEM HV: 15 kV, view field: 8.668 μm , WD: 2.984 mm, det: InBeam.

Silver ions affect the process of cell proliferation and silver ions and nanoparticles have thus found the use in dermatology for facilitating wound healing and in a wide spectrum of therapeutic use [43].

The mechanism of silver nanoparticle action is still unclear. However, the possible effects of silver nanoparticles are proposed on the basis of enzyme inhibition, alteration of membrane integrity, penetration into the bacterial cytoplasm and accumulation in the periplasmic space or in the formation of reactive oxygen species. It was confirmed that the composition of the cell wall and plasma membrane plays an important role in the penetration of

nanoparticles into cells. In G⁺ bacteria, transmittance is significantly slower than in case of G⁻ bacteria [44].

Another, newer option in terms of nanotechnology and bacterial infections, is selenium nanoparticles. Selenium nanoparticles were investigated for various medical applications and as a potential material for orthopaedic implants [45]. Studies that point out the ability of the selenium compounds to inhibit the growth of bacteria and the formation of bacterial biofilms are now available [45]. In a number of selenium compounds (such as 2,4,6-tri-*para*-methoxyphenylselenopyrylium chloride, 9-*para*-chlorophenyloctahydro-selenoxanthen, and perhydroselenoxanthene) *in vitro* antibacterial activity, particularly against *Staphylococcus aureus* has been demonstrated. However, the effects of elemental selenium nanoparticles remain largely unknown [46,47].

10.5.2. Modification by nonpolymeric or polymeric substances

Application of metal ions or nanoparticles in combination with other substances having antimicrobial properties is another way to increase antimicrobial activity [30,31]. The combination of nanoparticles with certain polymeric substances was confirmed to be very effective at minimizing the risk of bacterial infection [6,7]. Complexes of polymer materials with silver nanoparticles are widely used as growth inhibitors of G⁺ and G⁻ bacteria [48] and also as the substances used for the treatment of post-traumatic and postoperative tissues [49,50].

The first of the most commonly used polymeric substances, chitosan, possessed a number of useful properties [7]. Chitosan is a linear polysaccharide composed of 2-amino-2-deoxy-D-glucopyranose and 2-acetamido-2-deoxy-D-glucopyranose units [51]. It is synthesized either in an animal body or artificially by partial deacetylation of chitin, which is the main component of crustacean shells [52]. It is the most widespread biopolymer material [53]. Due to its nontoxic properties, biocompatibility and biodegradability, chitosan is used in targeted drug delivery [54] and also stands out for its antimicrobial properties. For this reason, it is commonly used as a substance to maintain the sterility of artificial implants [55,56]. Due to the polymeric structure of chitosan, it easily forms complexes with metals [56-58].

Another equally important polymeric material that is utilized in the formation of complexes with metals is hyaluronic acid. Hyaluronic acid, or its salt, is a linear polysaccharide of disaccharide units of D-glucuronic acid and N-acetylglucosamine [59]. It is naturally present in mammals, extracellular matrix, muscle and nerve tissues in the vitreous humor and on the skin [60]. It is compatible with the human organism and biodegradable [61-64]. Hyaluronic acid synthesis occurs in the plasma membrane of fibroblasts [65]. Hyaluronic acid in the intracellular matrix binds water to proteins, is involved in the construction of extracellular matrix and also plays an important role in the

organization of proteoglycan complexes in soft connective tissues [32,33]. The greatest incidence of this polymer in humans is in areas with proliferating cells. Due to its organization, hyaluronic acid is used in eye surgery, tissue engineering and cosmetics, which are used to accelerate tissue regeneration [66-70].

Interactions of biopolymer materials with metals are based on the binding of metal ions to the amino groups of chitosan *via* chelating mechanisms [71]. Similar mechanisms have also been described in connection with the binding of metal ions or nanoparticles to hyaluronic acid [72].

One of the other biopolymer materials is adiponectin. Adiponectin is a protein produced mainly by mature adipocytes and is present in a surprisingly large amount in human plasma [73]. It was found that adiponectin significantly regulates the metabolism of carbohydrates and lipids, and increases the utilization and transport of glucose and free fatty acids into muscle, liver and fat cells [74]. Adiponectin in human plasma occurs in the form of several polymeric isoforms. Adiponectin accumulates subendothelially in damaged areas of vessel walls and increases in adiponectin significantly reduce the progression of atherosclerotic lesions in animal models of atherosclerosis. Hypoadiponectinemia was found to be an independent predictor of cardiovascular disease in patients with advanced stage renal insufficiency. In further work, high plasma levels of adiponectin was associated with a low risk of myocardial infarction in men [75].

10.5.3. *In vivo* application of surface modified vascular grafts

Finally, the application of modified vascular grafts in animal cell cultures was used. In our experiments *in vivo*, three different cell lines (quail and human fibroblasts and endothelial cells) were studied. All lines were stabilized at the beginning of experiment in two independent passages, while ensuring optimal conditions of cultivated media. Cultivation was carried out at 37 °C and 5 % CO₂. In addition, we designed a simple simulation of blood flow through oscillation. For the assessment of biological properties, we found that the cells exhibited very good morphological properties. The cell lines were further characterized based on other indicators, such as the structure of the nucleus and nucleolus, the activity of mitochondria and RNA. During each experiment, there were no significant differences in the input morphological indicators (Figure 4).

Figure 4. Morphological indicators of used cell cultures

Cell cultures were grown on various types of cultivation surfaces (various sizes of specialized Petri dishes, cultivation bottles or cultivation tubes). We did not observe any major differences between cultivation on various types of surfaces. Therefore, it was possible to interchange the individual cultivation methods as necessary.

Figure 4 shows a massive growth of fibroblasts in the central portion of the prosthesis after the application of 25 ng mL^{-1} of adiponectin. Figure 5 shows the cultivation of fibroblast cells in a culture tube, which was subsequently placed on a thermomixer. To these tubes, it was possible to place the vascular prosthesis and subsequently add a fibroblast cell culture. Moreover, it was possible to apply the adiponectin to the different parts of the graft.

Cultivation of cells

Visualization of metabolic activity on vascular graft with adiponectin

Figure 5. Possible methods of cell cultivation

If the vascular prostheses were placed on a cycler (simulation of blood flow), it was possible to observe a better growth of the cell culture on the surface of the graft. The amount of cells captured on the vascular graft has increased about 30 % compared with the variant without oscillation. However, the intensity of oscillation could not exceed 20 Hz or else the coming cells would grow less rapidly. Figure 6 shows photographs of the cultivation of vascular grafts in a cultivation bottle. There the cells also grow and colonize the graft.

Figure 6. Cultivation of fibroblasts on grafts in a cultivation bottle

Cell lines derived from endothelium showed similar behaviour to that of fibroblast cells. If the cell lines were cultivated on the graft in the presence of adiponectin, a gradual increase in cell amount was observed (Figure 7). The amount of cells increased by an average of 20–30 % in comparison with vascular grafts without the application of adiponectin. In the experiments, the adiponectin concentrations in the range of 5–15 ng mL⁻¹ were monitored. However, the increase in cell amount in these experimental variants was similar.

Figure 7. Cultivation of endothelial culture on the fabric after application of adiponectin

The aim of this prospective study was to verify the properties of the new species of vascular grafts with adiponectin in the experiment. Before conducting clinical trials of a new kind of vascular graft on humans, it is necessary to evaluate the rheological and immunological response of this vascular graft in live animals *in vivo*. The experiment cannot be replaced by alternative methods *in vitro*. The number of animals is chosen so that the study is informative, while being feasible under the given conditions.

A Sheep model was selected as an experimental animal due to the similar behaviour of the arterial vessels in sheep to those in humans. According to literal data, for the transplantation of vascular grafts of the required size, only sheep are suitable for experimentation with vascular grafts in the carotid artery, due to the size of the carotid artery and the long and easily accessible neck [76–80]. Another advantage is that in the postoperative period, sheep require minimal care and monitoring is not so expensive.

Nine sheep were observed from 30–100 days (3 sheep every 30 days). All implanted grafts (vascular prostheses in the control group and vascular prostheses with adiponectin) remained feasible during the experiment. Favourable macroscopic images of the implanted grafts with adiponectin in the sheep body have demonstrated excellent healing of grafts due to their good rheological properties. This image was more favourable than those of the grafts in the control groups, both in the terms of the reaction of the environment (the control group had a prosthesis with a more pronounced reaction) and in terms of the subtleties of the inner surface of the graft (the control group had a rough inner surface). Histological findings demonstrated the high quality and speed of healing of the prostheses with adiponectin.

Dissection of carotid artery in sheep

Implantation of vascular graft with adiponectin

Figure 8. Transplantation of artificial vascular graft with adiponectin into the sheep body as a replacement for carotid artery

10.6. CONCLUSION

The material used, preoperative preparation, the actual surgical procedure and postoperative care play important roles in vascular surgery during vascular reconstructions.

The selection of vascular grafts is usually determined by surgeons. Traditionally, they used to favour the use of artificial vascular grafts knitted or woven, which were coated with a compact layer of either collagen or collagen with subsequent treatment. This is a modification of the antibacterial effect.

Nowadays, the selection of artificial vascular grafts is very diverse. But in spite of such a wide range of materials, surgeons struggle with the emergence of sepsis at the reconstruction site. There are many different opinions on how to proceed in the treatment of infections: whether to treat the infection or to reoperate. Time plays a particularly important role in this case. Early diagnosis

and detection of advancing infection can rescue the patient's limb, or even their life.

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