Narrative Review

Pathophysiology and Therapeutic Control of Coronary Artery Restenosis

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SUMMARY

Coronary artery restenosis is an occlusive vascular response to arterial injury and inflammation that often occurs after surgical interventions for cardiovascular disease. Extensive studies have been done to understand the pathophysiology of restenosis and therefore figure out the solutions to this problem. In this review, we attempt to summarize the physiological roles of the main constituents in coronary artery and their relation to the pathophysiology of restenosis. We also discuss current clinical therapeutic measures to prevent restenosis and the efforts made in the research to improve the performance of implanted devices for cardiovascular disease treatment.

KEYWORDS  Coronary Arteries; Restenosis; Vascular Tissue Engineering; Cardiovascular Diseases; Vascular Endothelial Cells

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Coronary heart disease (CHD) is the most common type of cardiovascular diseases (CVDs), and is responsible for almost half of the total deaths from CVDs (1). In CHD, coronary arteries are narrowed or blocked, usually by the slow build-up of plaques (cholesterol and fatty deposits) on their inner walls (atherosclerosis). The resultant reduced blood circulation fails to supply enough oxygen to the cardiac muscles, and thus leads to heart deficiency and death.

Conventional therapeutic strategies for CHD include angioplasty with or without stent application. When the occlusion condition of the vessel is more severe, reconstructive surgery such as coronary artery bypass graft surgery (CABG) is applied to substitute the diseased vessels or generate bypass to improve the blood supply downstream of the stenosed vessels (2). Every year, more than half a million coronary artery bypass grafts are implanted (3). Autologous vascular grafts such as mammary artery and saphenous vein segments harvested from the patient, are considered as the first choice of substitutes for coronary and peripheral bypass procedures (2, 4–6). However, the supply of autologous grafts is not readily available due to size mismatch, previous surgical intervention or the pre-existence of pathological conditions (3, 7–8). When a venous graft is used in the arterial circulation, it often undergoes vessel remodeling with neointimal thickening, leading to vessel stenosis or aneurysm formation (2, 9). Elderly patients are especially prone to this problem when the graft from their saphenous vein is transplanted into high-pressure arterial sites (5, 10). Moreover, the necessity of two surgeries on the same patient increases the risk of infection and costs.

To supplement the limited supply of autologous graft, synthetic vascular prostheses such as expanded polytetrafluoroethylene (ePTFE) and Dacron™ fabric grafts have been developed and used conventionally (2–5). Although they perform satisfactorily in high-flow, low-resistance conditions such as the large peripheral arteries, they have low patency for small diameter (less than 6 mm) arterial reconstruction (11–12). A major problem with synthetic vascular grafts is early and mid-term restenosis. Restenosis refers to the post-surgery reoccurrence of vascular stenosis, which is the narrowing of the graft characterized by the early formation of thrombosis, which is the aggregation of platelets and fibrin, and neointimal hyperplasia developed at longer time, which is the abnormal migration and proliferation of vascular smooth muscle cells (vSMCs) with associated deposition of extracellular connective tissue matrix (13–14). Pathological studies of restenosis showed that the removal or mechanical injury of endothelial cells (ECs) of the blood vessel either by surgical operation or flow turbulence generated due to compliance mismatch between the graft and native artery, is a major trigger for the thrombotic events and hyperplasia formation (15–16). A mismatch of mechanical compliance between the graft and host tissue also induces vSMCs at the anastomotic sites to hyperproliferates and secretes excessive extracellular matrix (ECM) (17).

In this review, we aim to give a comprehensive summary of the physiological structure and functions of coronary artery, and how the main constituents are involved in the pathophysiology of restenosis development. The clinical approaches to control restenosis and the state-of-the-art research in restenosis prevention are also addressed.

Coronary Artery Structure and Physiological Functions

The vascular system is a network of blood vessels. It is one of the most important and complex organs in the mammalian body. As part of the circulatory system, the vascular system is responsible for blood circulation throughout the body (18). The oxygen and nutrients in the blood diffuse across the blood vessel walls to the interstitial fluid and then to the target cells, while carbon dioxide and wastes from the target cells diffuse in the opposite direction and are sent back to the heart. In the vascular system, there are three major types of blood vessels: (1) the arteries which transport the blood away from the heart, (2) the capillaries which realize the exchange of gas and chemicals between the blood and the tissues, and (3) the veins which return the blood from the capillaries back to the heart (19).

In human vascular system, the arteries that deliver oxygen-rich blood to the myocardium are called coronary arteries. They originate from the left side of the heart at the root of the aorta, and are divided into right and left coronary arteries on the surface of the heart, which further branch into segments and capillary networks that penetrate into the tissue (Figure 1). Generally, the right coronary artery supplies the right ventricle and atrium, while the left coronary artery supplies the...
Figure 1. Anatomy of the Coronary Arteries of the Heart

left ventricle and atrium as well as the intraventricular septum. Coronary arteries are very important in vascular system as they participate in the coronary circulation, and are the only source of blood supply to the myocardium. They are subjected to cyclic pulsatile force from the heart, and the blood flow through coronary arteries depends on both the perfusion pressure in the aorta and the extravascular compression from the myocardial contraction (20-21).

Same as the other arteries, coronary arteries are composed of three histologically distinct layers (Figure 2) (22). The innermost layer in contact with the blood stream is called the intima, which is a monolayer of vascular endothelial cells (ECs) mounted on a basement membrane (or basal lamina) composed of laminin, fibronectin, type IV collagen and some other extracellular matrix (ECM) constituents (23). Separated by the internal elastic membrane from the intima, the middle layer named as tunic media, is composed of vascular smooth muscle cells (vSMCs) and elastin fibers. This layer is located between the internal and external elastic membranes. The outermost layer, the adventitia consists almost entirely of connective tissue with nerve fibers, small blood vessels, and fat in loose interstitial matrix (24).

Cells in Coronary Arteries

For a coronary artery, the elastic components such as elastin and collagen determine the mechanical characteristics including elasticity and strength, while the cells play active and regulatory roles in physiology, local responses to injury and the pathogenesis of vascular diseases. The principal cells of the coronary artery wall are ECs and vSMCs.

Endothelial Cells (ECs)

ECs are the cells that form the endothelium, which is the thin layer that lines the entire vascular system, from the heart to the smallest capillary (25). These cells usually have a characteristic squamous morphology in situ (26). A fundamental ability of vascular ECs is to proliferate and form a network of capillaries, which is known as “angiogenesis” (27). They are also involved in various physiological processes of the blood vessels via cell-cell interaction and the release of vasoactive or growth regulating agents. ECs can regulate the growth and development of the vSMCs and connective tissue cells through signalling pathways. As ECs are in direct contact with the blood, they perform a critical role in all aspects of tissue homeostasis (19). Quiescent ECs can generate an active antithrombotic and anti-platelet surface to facilitate the transit of plasma and cellular constituents throughout the vasculature by secreting factors such as tissue plasminogen activator and expressing membrane thrombomodulin (28-29). In inflammatory and immunological processes, ECs are induced by the perturbations to create a prothrombotic and antifibrinolytic microenvironment (30). ECs can also mediate vascular tone in both a paracrine and cell-cell gap junction manner through rapid response to the signal from the peripheral nervous system and the release of endothelium-derived relaxing or contracting factors such as nitric oxide (NO), prostanoids and endothelin to make smooth muscle relax or contract in the vessel wall (31).

In addition, ECs can regulate the flow of nutrient substances, biologically active molecules, and the blood cells through the presence of membrane-bound receptors on their surface. For instance, ECs can sense the shear stress from the blood flow via the mechanoreceptors on their surface, and signal the surrounding cells to adapt the vascular diameter and wall thickness to suit the blood flow (22). The EC surface also consists of a layer of surface glycoprotein (glycocalyx), which not only provides a local charged barrier to the transendothelial migration of blood cells and plasma proteins under normal physiological conditions but is also very metabolically active (32). Because of the numerous functions of ECs, the integrity of endothelium is significant in maintaining the structure and functions of the blood vessels.
Figure 2. Layered Structure of A Coronary Artery

Vascular Smooth Muscle Cells (vSMCs)

In a typical blood vessel, the vSMCs of the tunica media are elongated bipolar cells containing both thin actin filaments and thick myosin filaments (33). They are highly oriented in a circumferential direction of the blood vessel wall (34). There is a marked heterogeneity in vSMCs in different types or locations of blood vessels due to their different embryonic origins (35). In large elastic (or conducting) arteries such as the aorta, layers of vSMCs are sandwiched between lamellae of connective tissue in a highly organized structure so that they can tolerate the pressure coming from the heart. In muscular (or distributing) arteries such as the coronary arteries, the layers of vSMCs are less demarcated and overlapping (22, 36). The vSMC layers near the vessel lumen receive oxygen and nutrients via direct diffusion from the vessel lumen through the holes in the internal elastic membrane, while the outer portions of vSMC layers in medium and large sized arteries are nourished by small arterioles arising from outside the vessel coursing into the outer one half to two thirds of the media (37).

vSMCs are very important for the maintenance of vascular tone and the regulation of blood circulation due to their contractile characteristics. They also participate in the morphogenesis and maintenance of the normal architecture of the blood vessel wall (38). vSMCs can secrete ECM proteins that are major components of the vascular media (39), and synthesise type I and III collagens that are components of the vascular interstitial matrix (40). They can also synthesise elastin that provides mechanical properties for the normal function of the elastic arteries (41).

Response to Injuries

For a healthy coronary artery, the EC and vSMCs are in a quiescent state that both types of cells seldom divide (38, 42). The ECs maintain themselves as a highly ordered monolayer at all times for the tissue hemostasis. The growth of vSMCs are controlled by both the growth factors released from blood cells and the endothelial cell-derived growth factor and inhibitor produced by the ECs and fibroblasts (FBs) of the vessel wall (43). However, the EC lining may be injured by mechanical stimuli evoked mainly by non-physiological high blood pressure or biochemical stimuli such as diabetes mellitus and dyslipidemia (37, 44). This damage of EC layer disrupts the endothelial integrity and leads to dysfunction of ECs, which will further result in compensatory responses that alter the normal homeostatic properties of the endothelium.

The initial responses of ECs to the injury include decreased production of NO and increased permeability to lipoprotein and other plasma constituents that do not occur inside the vessel under physiological conditions (35). The ECs are induced to become procoagulant instead of anticoagulant by the injury and form vasoactive substances, cytokines and growth factors. Moreover, the adhesiveness of the ECs is also increased with respect to the cells of immune system such as leukocytes (or platelets) due to the immune activation and synthesis of immunoglobulin and selectin adhesion molecules like endothelial-leukocyte adhesion molecule-1 (ELAM-1) (45).

As the platelets aggregate and degranulate at the lumen surface, they release a potent mitogen called platelet-derived growth factor (PDGF) that is chemotactic and mitogenic for vSMCs and certain other cells derived from mesenchyme such as FBs (22, 46). Inflammatory cells also penetrate into the vessel wall, release proteolytic enzymes that can disrupt and oxidise the ECM, and thus activate the migration and growth of vSMCs (47). In addition, the ECs, inflammatory cells or even vSMCs themselves can produce growth factors and cytokines similar as PDGF that induce the phenotypic change of vSMCs from the quiescent contractile state to
the active synthetic state, characterized by the loss of the differentiated state (37). The phenotypically changed vSMCs are prone to hyperplastic and hypertrophic cell growth and migration from the media into the intima, and increased production of ECM proteins such as collagen and osteopontin (44, 48), which can further result in many adverse changes that promote thrombosis, atherosclerosis and hypertension (29, 42). The abnormal migration and proliferation of vSMC become intermixed with the area of injury and form lesion and the basis for the complicated atherosclerotic plaque (22, 37). The accumulation of ECM material participates in the fibrogenesis in vascular pathology, and in the fixed structural alterations of the blood vessel that accompany hypertension and arteriosclerosis (49).

On the other hand, it is found that the endothelial cell growth ceases when they are in contact with vSMCs in co-culture system due to the activation of transforming growth factor (TGF)-β (50). The study using a rat aorta injury model also showed that the regeneration of endothelial layer after a scratch on the layer occurs by movement without cell replication unless the distance between the wound edges requires sufficiently long period of movement (51). Direct injury of the blood vessel also triggers sequential events involving adventitial FBs, which begin with apoptosis and lead to proliferation and differentiation of adventitial FBs into myofibroblasts that migrate to the site of injury (52-53).

**Pathophysiology of Restenosis**

The term ‘restenosis’ usually refers to the recurrence of stenosis, which is an abnormal narrowing of the blood vessel. In this review, ‘restenosis’ is defined as an occlusive vascular response to arterial injury and inflammation characterized by lumen narrowing that particularly appears after coronary artery bypass graft surgery (CABG) or percutaneous coronary intervention (PCI) such as angioplasty (54). It usually evolves over several months after the surgery (54). Statistics has shown that 15 to 25 % of the patients develop graft closure within one year following the CABG with saphenous vein graft (55). The immediate injuries caused by the surgical procedure include intimal tearing, endothelial cell damage, and exposure of subendothelial connective tissue to blood components (54, 56), which trigger a series of intravascular changes.

The most widely held theory in literature is that the pathophysiology of restenosis bears many relations to the process of wound healing (57). The vascular wall responds to the injury acutely with exuberant activation of the sealing mechanisms (58). The platelets, fibrin, and red blood cells accumulate at the site of injury and form thrombus, which is usually responsible for immediate occlusion of the graft. The process of neointima formation also starts from the thrombotic phase, followed by the cellular recruitment (migration) and proliferation phases (59). After the thrombotic phase, ECs are recruited together with monocytes and lymphocytes infiltration, causing an inflammatory response with reendothelialization (60). In the proliferative phase, actin-positive cells from the lumen side forms a “cap” on the lumen surface and gradually replace the deeper thrombotic material. The ECM secretion and additional recruitment also likely add to neointimal volume during this phase (60). As the three phases in neointima formation are usually exaggerated, the formation process ends up as neointimal hyperplasia. In addition, the vSMCs play a pivotal role in causing restenosis, and are involved in all the above phases (61). The platelets, ECs, and inflammatory cells produce large amount of mitogens such as PDGF, thrombin and endothelial and FB growth factors. These mitogens stimulate the migration and proliferation of vSMCs from the media into the intima, followed by the formation of fibrocellular tissue with an abundant proteoglycan matrix (61). The failure of secreting inhibitors for vSMC proliferation such as NO and heparin sulfate, which are normally released by intact endothelium, aggravates the vSMC proliferation (57). The thrombus formed also provides an absorbable matrix for vSMC infiltration and proliferation (60). Moreover, the mismatch of mechanical compliance between the graft and host tissue induces vSMCs at the anastomotic sites to hyperproliferates and secretes excessive ECM (17). As a result, the neointima is further thickened by excessive cells and ECM deposition. Recently, it has been found that a number of cell types such as the stem or progenitor cells in circulation and the FBs/myofibroblasts and progenitor cells located within the adventitial layer of the damaged vessel, can also exhibit the potential to differentiate into a vSMC phenotype and show the potential to partially contribute to the neointimal hyperplasia (62). Since neointimal hyperplastic lesions result from the healing of injury, the distribution pattern may be diffuse throughout the vessel, focal at the anastomotic sites or within the body of the vessel (63).
Although the theory that restenosis is an excessive healing response to injury is widely accepted, it is not entirely undisputed. Besides the tissue responses to the injury, which is considered biological, the restenosis also depends on mechanical processes that result in a geometric change in the circumference of the damaged vessel, especially for the coronary arteries after angioplasty. The mechanical processes involve both an acute and a chronic phase (64). In the acute phase, elastic recoil due to the elastic properties of the arterial wall contributes to the vessel closure (65). In the chronic phase (3-6 months), negative arterial remodeling resulting from the mechanical stretch damage to the vessel wall leads to the shrinkage of the arterial wall diameter. The remodeling is largely an adventitia-based process that is restricted to dilation injury and involves the adventitial myofibroblasts and ECM (65-67). The adventitial FBs undergo phenotypic conversion to myofibroblasts, which in turn produce large amount of ECM materials that contributes most to the stiffness of the arterial wall. They also secrete pro-inflammatory factors that result in an alteration of the tensile force of the artery. In addition, the endothelial dysfunction due to the injury diminishes the endothelium-dependent relaxation, which may also lead to vasoconstriction and eventually, the remodeling (67). It is interesting to note that a negative correlation between neointimal hyperplasia and the reduction of the total arterial circumference has been demonstrated (68), and increased neointimal hyperplasia along with restricted remodeling by stents (67). A theory proposed to explain these findings is that neointimal hyperplasia and geometric remodeling compete to re-narrow the artery (67).

Taking into account all those proposed theories, the restenosis probably reflects a complex equilibrium of vessel recoil, neointimal hyperplasia and geometric remodeling of the vessel (69). The understanding of these factors involved in the pathophysiology of restenosis would be much helpful to determine the effective therapeutic control of restenosis.

**Therapeutic Control of Restenosis**

In clinical treatment, the most commonly strategy to treat restenosis is repeat angioplasty, which however, may cause recurrent restenosis for a significant percentage of patients (70). Alternatively, CABG is considered for those patients whose clinical characteristics suggest a high likelihood of recurrent restenosis or those who are at high risk for angioplasty. Rather than surgical treatment, more attempted solutions have been made on pharmacologic interventions and improved mechanical devices (54). Diverse pharmacological agents have been evaluated as restenosis inhibitors (54, 57, 63, 67). Heparin has been routinely used post-surgery to reduce the acute complications like thrombosis and decrease vSMC proliferation due to its anticoagulant and antithrombotic properties. However, extended heparin therapy results in more bleeding complications (57, 71). Vitamins E and C have been found to directly control the ECM synthesis and subsequently promote favorable vascular remodeling after injury (72). Antimetabolite agents and growth inhibitors such as methotrexate and somatostatin analogues have been studied to prevent vSMC proliferation. However, the effects of these agents on vSMC proliferation and neointimal hyperplasia development are still controversial (73-75). As each pharmacologic intervention usually targets one specific process implicated in restenosis, it may not sufficiently modify the complex underlying pathology for clinical benefits.

For device approach, coronary stenting technology is effective in preventing acute vessel re-occlusion and reduced post-angioplasty restenosis by sealing the dissection flaps and preventing vessel recoil (76). Unfortunately, they fail to eliminate restenosis as neointimal hyperplasia is accelerated by restricted remodeling and contributes to the in-stent restenosis (67, 77). Seeding EC on the prosthetic vascular grafts and the angioplasty sites has been proposed as a solution, yet the efficacy of EC seeding is often low with a rapid loss of the seeded cells (78-79). Drug-eluting stents and prosthetic grafts have been developed with the concept of delivering selective antiproliferative drugs at the site of neointima formation with high local concentrations and low systemic effects (3, 76). Sirolimus- or paclitaxel-eluting stents have been applied in clinical trials and shown reduced rates of restenosis and associated clinical events (80-82). Heparin-coated or paclitaxel-eluting synthetic vascular grafts have also shown the effects on reducing thrombosis and neointima formation in vitro and in vivo (83-84). Nonetheless, the risk of late restenosis and the associated endothelium-dependent vasomotor dysfunction remain the concerns for these devices (85). Besides the candidate drugs, extensive efforts have also been made in finding the optimal material of the device since coronary restenosis may be partially attributed to the material-induced cellular responses. It has been suggested that the ideal device should have a selective
antiproliferative effect on vSMCs while being inert toward ECs or even promoting their growth (76). This requires the identification of drugs/intracellular molecules that and an optimal combination of the candidate drug and device material. Messenger molecules such as NO have been extensively studied as it plays differential roles in vSMC and EC growth. Various types of NO donors have been developed in terms of membrane, hydrogel and nanoparticles to achieve prolonged release (86-88). There is also an increasing interest in applying gene therapy such as locally delivering antisense oligonucleotide or a class of endogenous, small, noncoding RNAs (microRNA) to negatively regulate gene expression and prevent vSMC proliferation and neointimal hyperplasia (63, 89).

On the other hand, the concept of vascular tissue engineering (VTE), which is an interdisciplinary field involving the principles of engineering and life sciences to develop biological substitutes, has been introduced to improve the function and patency of the vascular grafts (90). It is expected that with the use of cells, the biocompliance and remodeling ability of the vascular grafts will be improved. Different approaches and strategies have been studied to improve the performance of the vascular graft or promote vascular regeneration mainly including in vitro tissue engineered blood vessel (TEBV) through cell-scaffold hybrid and cell self-assembled TEBV. In vitro TEBVs have been developed based on synthetic biodegradable scaffolds using polyglycolic acid (PGA) and fibrin-polylactide (91-93). During the in vitro development process, the cultured vascular cells produce large amount of matrix proteins while the polymer scaffold undergoes degradation. Biochemical and mechanical stimuli were introduced during the development to promote the synthesis of ECM proteins and enhance the mechanical strength of the graft (91). Biomimetic synthetic grafts with anisotropic geometries have also been developed to help regulate cell orientation and the myogenic differentiation of stem cells (94-95). For cell self-assembled TEBVs, different combinations of cells such as vSMCs, ECs and FBs, or ECs and FBs have been used for the graft construction (96-97). The cells were first cultured in medium to secrete ECM and form cell sheets, and the cell sheets were then wrapped around a temporary inert tubular support and allowed for maturation for some time to form a cylinder composed of concentric sheet layers (96). Some success of TEBV has been achieved, especially in large diameter applications. However, TEBV still faces the problems such as the mismatch of the degradation rate of the scaffold and the cell proliferation rate, and the long culture time required for the desired characteristics of the construct.

Conclusions and perspective

Coronary artery restenosis is a major post-surgery problem for current cardiovascular disease treatment using synthetic vascular grafts. The restenosis development involves the thrombosis formation and neointimal hyperplasia, and the lack of bioactivity regulation and the mismatch of mechanical properties between the synthetic graft and host tissue further aggravate the problem. Compared to conventional restenosis prevention measures that require extensive anticoagulant/antithrombotic control and have the risk of further hemorrhagic complications, the use of bioactive synthetic grafts and TEBVs are considered as promising approaches to provide regulation towards vascular remodeling and better biocompliance to prevent restenosis. Current research in restenosis prevention has shown a favor in VTE; however, we believe that surface or material modification of synthetic vascular grafts and stents for enhanced remodeling regulation will also be a main research direction due to the ease of synthetic graft availability and the feasibility of bulk production.
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