PART I
Pathology and Indications: Clinical Trials
CHAPTER 1

The Pathobiology of CTO

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Introduction

Chronic total occlusions (CTOs), defined as occlusions more than 1 month old, are common in patients undergoing diagnostic coronary artery catheterization, with up to 20% of angiograms reported to have one or more CTO [1]. Despite its common occurrence, there is surprisingly little information about the pathophysiology of CTOs, and why some CTOs can be crossed, while in others, crossing is unsuccessful.

Current paradigm of CTO evolution

Composition of the CTO evolves over time and has remarkable spatial variability. Arterial occlusions may develop insidiously with minimal symptoms, or may present as an acute coronary syndrome. The initial acute event leading to the development of a CTO is a ruptured atherosclerotic plaque with bidirectional thrombus formation [2]. In patients with minimal or no symptoms, the timing of the occlusive event can not be clearly identified. In patients with ST-segment elevation myocardial infarction (MI) not treated with reperfusion therapy, an occluded infarct-related artery has been found in 87% of the patients within 4 hours, in 65% within 12–24 hours, and in 45% at 1 month [3,4]. As many as 30% of the patients treated with thrombolytic therapy alone have a chronically occluded artery 3–6 months after MI [5]. In patients treated with percutaneous coronary intervention (PCI) during evolving acute myocardial infarction (AMI), approximately 6–11% will have chronic occlusion of an infarct-related artery at 6 months due to either initial treatment failure or late re-occlusion [6].

The current understanding of CTO development is derived from autopsy studies, imaging in human subjects, and animal CTO models. Characterization of CTO development in human studies is problematic, since CTOs are often diagnosed after a prolonged maturation period, and data regarding initial stages in their evolution is lacking. Several animal models have been developed to systematically define the developmental stages of a CTO; however, these models have certain characteristics that could potentially limit their relevance to humans, such as the lack of underlying atherosclerosis or significant calcification. In this chapter we shall review the current understanding of CTO pathobiology.

Early stages of CTO development: Thrombus and inflammation

Our knowledge of thrombus organization is almost exclusively limited to veins. This process resembles the pattern of wound healing [7]. Initially, the freshly formed thrombus contains platelets and erythrocytes within a fibrin mesh, which is followed by invasion of acute inflammatory cells [8]. Neutrophils predominate at first but are later replaced with mononuclear cells [9,10]. Endothelial cells also invade the fibrin lattice and form tube-like structures and microvessels within the organizing thrombi [7,11]. Inflammatory cell infiltrates
in CTOs consist of macrophages, foam cells, and lymphocytes. Inflammation may exist in the intima, media, and adventitia of CTOs, although it is most predominant in the intima, regardless of lesion age.

**Extracellular matrix**

Collagen is the major structural component of the extracellular matrix (ECM) [12] with predominance of types I and III (and minor amounts of IV, V, and VI) in the fibrous stroma of atherosclerotic plaques [13]. In an autopsy study, cholesterol and foam cell-laden plaque were more frequent in younger lesions, whereas fibrocalcific plaque increased with CTO age [14]. Proteoglycans are important components of the CTO within the first year. It is generally stated that the concentration of collagen-rich fibrous tissue is particularly dense at the proximal and distal ends of the lesion, contributing to a columnlike lesion of calcified, resistant fibrous tissue surrounding a softer core of organized thrombus and lipids. However, there is sparse human CTO histological data to support this concept (see Figure 1.1).

**Neovascularization and angiogenesis**

Presence of microvessels within the CTO may facilitate angioplasty success [15]. There are three types of microvessel formation in arteries with advanced atherosclerotic lesions. The first pattern occurs in the vasa vasorum, which is the fine network of microvessels in the adventitia and outer media. These vessels proliferate in atherosclerosis and in response to vascular injury such as angioplasty and stenting [16–18]. Hypoxia in the outer levels of the vessel wall appears to act as an important stimulus [18]. Occasionally in CTOs, these adventitial blood vessels are well developed and can be recognized as “bridging collaterals.” Such microchannels, which can recanalize the distal lumen, may result from thrombus-derived angiogenic stimuli [19] and are suggested on an angiogram of an old CTO without a well-defined stump. Second, neovascularization can develop within occlusive atherosclerotic intimal plaques, predominantly in response to chronic inflammation [20]. The localization of plaque vessels in so-called “hot spots” in the shoulders of atheromas may predispose these plaques to rupture and acute coronary events [21,22]. The third type is the pattern of intraluminal microvessel formation (known as “recanalization”) that occurs as part of the organization phase in CTO in which thrombus is replaced by fibrous tissue. These microvessels generally range in size from 100 to 200 μm, but can be as large as 500 μm (Figure 1.2) [14]. In contrast to the vasa vasorum which runs in radial directions, these intimal microvessels run within and parallel to the thrombosed parent vessel [8]. This is suggested by a tapered CTO on an angiogram. Such channels may serve as a route for a guidewire to reach the distal vessel and hence may have therapeutic value.

There is little published data on the process of intraluminal microvessel formation in thrombin within arterial occlusions. Inflammation may play a role since high concentrations of macrophages have been detected in regions of recanalization in spontaneous human thrombi and in experimental animal arterial thrombi [10,23]. Frequent co-localization of inflammation and neovascularization within the intimal plaque and adventitia suggests that these findings are closely related, although it is unclear whether inflammation is a cause or an effect of neovascularization in CTOs [14]. Lymphocytes and monocytes/macrophages may play an active role in both angiogenesis and atherosclerotic lesion progression.

![Figure 1.1 H&E stained cross-section of a fibrotic CTO (H&E) containing predominantly collagen-rich extracellular matrix (* indicates intraluminal microvessel).](image-url)
by producing a variety of mitogenic and angiogenic actors [24]. The local ECM (extracellular matrix) environment is probably an additional important modifier, with specific matrix components exerting either pro-angiogenic (hyaluronan, fibronectin, perlecan, versican), or anti-angiogenic (type I collagen, decorin) effects.

**Advanced CTO**

Vessels with fibrotic CTO lesions typically undergo negative remodeling [25]. Intravascular ultrasound has demonstrated a positive correlation between degree of plaque calcification and duration of the occlusion [26]. These changes negatively impact the likelihood of successful angioplasty.

**Current research in CTO pathobiology**

Identification of specific components of the CTO at the various stages of development is critical to understanding CTO pathobiology and improving guidewire crossing success rates. Complementary information is required from the following areas of research.

**Human CTO samples**

Samples of CTOs collected during autopsies, amputations, endarterectomies, and transplants provide an important but very infrequent opportunity to study these highly heterogeneous lesions. Information regarding the three-dimensional (3D) architecture of occluded segments, the mechanical properties of their components, and changes in the integrity of the vessel wall layers over the natural history of CTO development are important areas of further study. Histological analysis provides an opportunity to assess a large range of features related to composition and structure.

**Animal models of CTO**

The optimal animal CTO model should be reproducible, contain fibro-calcific tissue, allow serial device evaluation and be able to be utilized with intravascular ultrasound or other technologies. A challenge in developing models of CTO is the lack of spontaneous atherosclerosis in animals. Different approaches have included external arterial constriction, thermal injury, gas-drying of the artery, injection of autologous blood above a stenosis, copper stents, stents with occluded outflow, alcohol injection, and

![Figure 1.2](#) H&E stained cross-sections of CTOs containing prominent fibro-calcific tissue (a) and intraluminal microvessels (b) (* indicates calcium deposits and arrows identify microvessels).
insertion of polymer plugs. We have developed a rabbit CTO model in which thrombin is injected into an isolated femoral artery segment [27]. This model is being used to investigate the temporal and spatial evolution of CTO, and correlate histological findings with non-invasive imaging modalities.

**Imaging techniques**

Non-invasive imaging provides an opportunity to observe features of CTOs at several stages in their development in patients and experimental models. Magnetic resonance imaging (MRI) has spatial resolution down to 100–200 μm in plane and about 1 mm through the plane and can determine composition of atherosclerotic plaque components such as lipid, thrombus, fibrous tissue, and calcium based on signal intensities in T1-, T2-, and proton-density-weighted images. Administration of contrast agents (Gd-DTPA (gadolinium diethylentriaminepentaacetic acid), Clariscan) permits calculations of relative extracellular volume and blood volume within regions of the CTO. Sequential MRI scans can follow the evolution of CTO at different time points. In human studies, CT angiography can provide insight into the presence of calcifications, vessel tortuosity, lesion length, and bridging collaterals [28]. Micro-computerized tomography (micro-CT) performed on ex vivo specimens (and therefore limited to autopsy material or animal models) has a spatial resolution of 17 μm and can be used to visualize specific microchannels within the CTO. Direct magnetic resonance direct thrombus imaging (MRDTI) can help estimate the presence and age of thrombus in occluded segments [29].

Pilot studies with human tissue and animal models have shown the potential of invasive optical coherence tomography (OCT) and ultrasound (in forward-looking configurations) to identify the layers of the vessel wall. OCT has sufficient resolution to identify microchannels in vivo [30]. These techniques are being adapted for in vivo use to enable nondestructive serial assessment of composition at the proximal entry point of CTOs. The ability to understand and non-invasively characterize the specific histologic and spatial features of CTOs, particularly online during revascularization, may dramatically impact on procedural success rates, particularly in complex CTOs.

See Plate 1 in the color plate section.

**References**


