1. Introduction

Although the introduction of drug-eluting-stents (DES) has dramatically reduced restenosis and the need for repeat revascularization compared with bare-metal stents (BMS), percutaneous coronary intervention (PCI) does not always prevent cardiac events, including acute coronary syndrome (ACS) [1]. Therefore, for all cardiologists, the detection of vulnerable plaques before they rupture is one of ultimate goals to predict and prevent ACS. Vulnerable plaques are characterized as thin fibrous cap (<65 µm), large lipid core, and macrophage infiltration within the cap. Furthermore, plaque neovascularization has been identified recently as a common feature of plaque vulnerability. Increased neovascularization in atherosclerotic plaques plays an important role in plaque progression, plaque instability, and rupture of plaque [2-5]. Until recently, however, in vivo studies assessing neovascularization in atherosclerotic plaques have been difficult because of the lack of sufficient resolution that reliably identifies this feature of vulnerable plaque. The first-generation catheter-based Time-domain optical coherence tomography (TD-OCT) system (M2 and M3 OCT system; LightLab Imaging, Westford, MA, USA), which offers superior resolution of 10-15 µm, has emerged as an intracoronary imaging modality, rendering the detailed micro-structure information of coronary plaques [6-8]. With its excellent resolution, OCT may provide an opportunity to directly detect plaque neovascularization in vivo. This chapter reviews the evidence of plaque neovascularization accumulated so far on OCT and discuss the future perspectives and limitations.

2. Neovascularization in atherosclerosis

Nourishment of normal blood vessels is accomplished by oxygen diffusion from the vessel lumen or from adventitial vasa vasorum [9]. As atherosclerosis progresses, the intima thickens, and oxygen diffusion is impaired. As a result, vasa vasorum proliferates in the inner
layers of the vessel wall, and becomes major source of nutrients. Vasa vasorum neovascularization in early atherosclerosis is associated with inflammatory cell infiltration and lipid deposition, leading to plaque progression [10]. Furthermore, intraplaque hemorrhage from microvessels contributes to expansion of the necrotic core through the accumulation of free cholesterol from erythrocyte membranes. Several human pathologic studies have demonstrated that plaque neovascularization is pronounced among patients with unstable coronary syndromes and that its presence may be a marker of plaque instability and plaque rupture [4, 5, 11]. Therefore, investigations of imaging methods with the ability to visualize neovascularization would appear worthwhile.

3. Potential imaging modalities of neovascularization

Several imaging modalities such as micro-computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound have emerged as potential techniques for imaging neovessels in atherosclerotic plaques. Micro-CT provides high-resolution images of coronary vasa vasorum neovascularization and insight into their structure and function in animal models [12, 13]. Winter et al have reported that molecular MRI with αvβ3-targeted, paramagnetic nanoparticles can detect plaque neovessels in atherosclerotic rabbit model [14]. In addition, more recently, Sirol et al have demonstrated how gadofluorine M-enhanced MRI can accurately identify plaque neovascularization in an animal model of atherosclerosis with good histological correlation [15]. Thus, even though the results from these techniques are promising, further studies are needed for clinical application in humans. Intravascular ultrasound (IVUS) has the potential to detect flow within the plaque and subsequently evaluate functional neovessels. The development of IVUS-based imaging has recently demonstrated the preliminary data imaging neovascularization in coronary plaques in vivo after intravascular injection of microbubbles [16, 17], but further investigations will be required to show the feasibility of this method for routine clinical use.

4. Plaque neovascularization by OCT

OCT has been proposed as a high-resolution imaging modality that can identify microstructures in atherosclerotic plaques [8, 18, 19]. The superb high-resolution of OCT may offer an opportunity of studying the spatial distribution of plaque neovascularization in vivo (Figure 1) [20]. In fact, it has been shown that OCT is able to visualize neovascularization of atherosclerotic plaques [21-24]. In addition, Vorpahl et al demonstrated that small black holes in atheromatous plaques observed by OCT were in good agreement with the pathohistological evidence of intra-plaque neoangiogenesis formation in an autopsy case [25]. We recently assessed the relationship between intra-plaque microchannel structures identified by OCT, probably representing neovascularization, and plaque vulnerability in patients with coronary artery disease [21]. In this study, microchannel was defined as a no-signal tubuloluminal structure that was present on at least 3 consecutive OCT cross-sections in pull-back images. As a result, microchannels were seen in 38% of culprit plaques, and plaques with microchannels displayed the
characteristics of vulnerability such as positive remodeling and thin fibrous caps compared with plaques without these structures. Of note, the presence of increased microchannel counts was correlated with a greater frequency of thin-capped fibroatheroma (TCFA) (Figure 2). More recently, in larger study population (356 plaques in 117 patients), Tian et al investigated the clinical significance of intra-plaque neovascularization in culprit lesions and no-culprit lesions of unstable angina pectoris (UAP) and in lesions of stable angina pectoris (SAP) using OCT [22]. Intra-plaque neovascularization was found in 35% of UAP culprit lesions, in 34% of UAP non-culprit lesions, and in 28% of SAP lesions, with no significant difference. Among UAP culprit lesions, plaques with neovessel had thinner fibrous cap thickness (56±20 µm vs. 75±30 µm, p<0.001) and significantly higher incidence of TCFA (81% vs. 47%, p=0.002) compared with those without neovessel. In addition, plaque burden was significantly bigger in UAP culprit lesions with neovascularization (79.8±7.9% vs. 72.8±10.7%, p=0.024). In terms of the non-culprit lesions of UAP patients and lesions of SAP patients, however, no significant difference in plaque characteristics was observed, regardless of the presence or absence of neovascularization. Interestingly, Kato et al reported that although the overall prevalence of microchannel in non-culprit lesions was not significantly different between ACS and non-ACS patients (64.7% versus 55.2%, respectively, P=0.647), the closest distance from the lumen to microchannel was shorter in ACS subjects than in non-ACS (104.6±67.0 µm versus 198.3±133.0 µm, p=0.027) [23]. The authors speculated that because neovascular networks expand from the adventitia into the intima as disease progresses [26], plaques with neovascularization located closer to the lumen might represent an advanced stage of atherosclerosis. Furthermore, Uemura et al revealed that microchannel structure in non-culprit plaques (defined as percent diameter stenosis of < 50%) identified by OCT is a predictor of subsequent plaque progression in patients with coronary artery disease [24].

Figure 1. OCT (M2 system) images of plaque neovessels. Microvessels in the outer plaque (A) near the adventitia and (B) within the thickened intima can appear as signal-poor voids (white arrows) that are sharply delineated.
5. Neovascularization inside the implanted stent by OCT

Although neovascularization within the neointima after stent implantation has been already reported in histopathologic studies [27, 28], Regar et al first reported in 2005 that OCT has an ability to visualize microvessels within the neointima inside the stents in a living human [29]. Later the presence of neovascularization in the stent restenosis was noted by Gonzalo et al [30]. However, the role of microvessels in restenotic tissue behavior has been unknown. More recently, Kim et al evaluated the characteristics of in-stent restenosis (ISR) lesions with microvessels detected by OCT [31]. Microvessels were detected in 21 (27%) of 78 ISR lesions. At the minimum lumen area site, the neointimal area (5.4 ± 1.7 mm² vs. 4.2 ± 2.1 mm², p=0.024) and percent neointimal area (79±12% vs. 67±16%, p=0.001) were significantly greater in ISR lesions with microvessels. These results suggest that microvessels within the neointima might be associated with restenosis by the excessive neointimal growth following stent implantation.

Furthermore, it has been shown that neointima in both BMS and DES can transform into atherosclerotic tissue with time although it occurs earlier in DES than BMS and that neoa-therosclerosis progression inside the implanted stents may be associated with very late coronary events such as very late stent thrombosis after BMS and DES implantation [28, 32-40]. Using OCT, Takano et al examined the differences in neointima between early phase (< 6 months) and late phase (≥ 5 years) [35]. When compared with normal neointima proliferated
homogeneously in the early phase, neointima inside the BMS ≥ 5 years after implantation was characterized by marked signal attenuation and a diffuse border, suggesting lipid-laden intima. Its frequency was 67% and lipid-laden intima was not observed in the early phase. TCFA-like intima was also found in 29% of the patients in the late phase. Intimal disruption and thrombus were observed more frequently in the late phase as compared with the early phase (38% vs. 0% and 52% vs. 5%, respectively; p < 0.05). Notably, although there was no significant difference in terms of the incidence of persistent neovascularization (Figure 3A) between the 2 phases (81% vs. 60%, p=0.14), intraintima neovascularization (Figure 3B) was seen more frequently in the late phase than in the early phase (62% vs. 0%, p < 0.01) and in segments with lipid-laden intima than those in without lipid-laden intima (79% vs. 29%, p=0.026). Moreover, Habara et al evaluated the difference of tissue characteristics between early (within the first year) and very late (> 5 years, without restenosis within the first years) restenotic lesions after BMS implantation by using OCT [39]. There was a significant difference in the morphological characteristics of restenotic tissue between very late ISR (characterized by heterogeneous intima) and early ISR (characterized by homogeneous intima). Intraintima microvessels were observed only in the very late ISR group (16.3% vs. 0%, p=0.01). Thus, expansion of neovascularization from persistent to intraintimal area with time may contribute to atherosclerosis progression of neointima, as well as intra-plaque neovascularization of nonstent segments in native coronary arteries.

Figure 3. Neovascularization within neointima inside the implanted stent. (A) OCT (M2 system) image of persistent microvessels (arrows) demonstrating no-signal small vesicular and tubular structures locating around the struts. (B) OCT (M2 system) image of intraintima microvessels (arrows) showing small black holes locating near the vessel lumen within the neointimal tissue.
6. Future perspectives (neovascularization as a therapeutic target for plaque stabilization)

For all cardiologists, stabilizing vulnerable plaques remains a major concern. Previous clinical trials have demonstrated that lipid-lowering therapy by statins stabilizes vulnerable plaques, thereby preventing cardiac events. Experimental studies have also shown that anti-atherosclerotic therapies can reduce plaque neovascularization [41-43] and that the inhibition of plaque neovascularization reduces progression of advanced atherosclerosis [41, 42]. Therefore, monitoring treatment effects of anti-atherosclerotic drugs using reliable surrogate markers may be useful to appropriately manage the patients. The thickness of fibrous cap in coronary plaque is a major determinant of plaque destabilization [44]. We recently reported that statins increased the fibrous cap thickness of plaques as assessed by OCT, indicating plaque stabilization [45, 46]. More recently, Tian et al investigated whether there was a difference in the effects of statin therapy between lesions with and without neovascularization [47]. As a result, despite a comparable reduction in serum cholesterol levels, the fibrous cap thickening was smaller in lesions with neovascularization than those without neovascularization after 6 and 12 months of statin treatment, which suggests that a more aggressive anti-atherosclerotic therapy may be required in patients with plaque with neovascularization. Thus, OCT allows us to monitor the response to anti-atherosclerotic therapies such as statins, and micro-channels in plaques identified by OCT could become a therapeutic target for plaque stabilization as important as the thickness of fibrous cap (Figure 4).

Figure 4. Plaque stabilization and elimination of neovascularization by statin treatment. (A) OCT (M2 system) demonstrates lipid-rich plaque covered by thin fibrous cap of 60 μm (thin-capped fibroatheroma) and microvessels at the shoulder region of the plaque (dotted circle). (B) Six months after statin treatment, the minimum fibrous cap thickness (FCT) increased from 60 μm to 170 μm and microvessels disappeared.
Moreover, a newer-generation Frequency-domain OCT (FD-OCT; C7 system, LightLab Imaging) has recently been developed to overcome many of the technical limitations of TD-OCT system by imaging at much higher frame rates (100 frame/s), a larger scan diameter (10 mm) and a faster image acquisition rate (20 mm/s) without loss of image quality, and unlike TD-OCT, this technology does not require proximal balloon occlusion [48]. The imaging catheter of FD-OCT, which is designed for rapid exchange delivery, has a 2.7-Fr crossing profile and can be delivered over a 0.014-inch guidewire through a 6-Fr or larger guide catheter. Intracoronary injection of contrast media via the guide catheter (3 to 4 ml/s; 2-3 s) can achieve effective clearing of blood for the FD-OCT imaging. In combination with a short, nonocclusive flush and a faster pullback speed, the FD-OCT enables imaging of longer segments of coronary arteries without significant ischemia and motion artifact [49]. Thus, we would be able to more precisely and easily assess not only culprit but also nonculprit lesion morphologies in coronary artery disease by use of FD-OCT.

7. Limitations

First, because the penetration depth of OCT is relatively shallow (<2 mm), and OCT light signals are limited behind the lipid component or red thrombus, previous OCT studies may underestimate the presence of neovascularization. Second, neovessel size has been inconsistently defined by a wide range because it is unknown whether there is a threshold for the size of these vessels within the intima [50]. Finally, a direct comparison of OCT-derived microchannels with histology has not been done to date. Therefore, histological studies that properly validate these structures observed with in vivo OCT imaging are mandatory in the near future.

8. Conclusions

OCT has the potential to directly visualize neovascularization of atherosclerotic plaques in vivo. Microchannel structure in coronary plaques identified by OCT could be a marker of plaque vulnerability to improve patient risk stratification and a therapeutic target for plaque stabilization.

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Author details

Hironori Kitabata and Takashi Akasaka
Wakayama Medical University, Japan

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Visualization of Plaque Neovascularization by OCT
