Thinking inside the graft: applications of optical coherence tomography in coronary artery bypass grafting

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1 Introduction

Coronary artery bypass grafting (CABG) is the most common major surgical procedure in the United States with over 300,000 cases performed each year. To restore blood flow to the affected myocardium, a vessel from another part of the body is procured to create a bypass around a critically stenosed coronary artery. The internal thoracic artery (ITA) remains the conduit of first choice due to its superior long-term patency, which is primarily a result of near perfect integrity of its inner blood-contacting lining, the “intima.”1 However, almost all patients referred for CABG require additional grafts to provide a complete revascularization. This necessitates the harvest of other vessels, most commonly the saphenous vein (SV) and/or radial artery (RA). These conduits have higher rates of intimal irregularities2 and early graft occlusion compared3,4 to the ITA.

Strategies aimed at screening the intimal quality of potential conduits as a means of improving bypass graft patency would be clinically beneficial since graft failure is associated with increased morbidity and mortality and often requires reoperation.5,6 Intraoperative assessment of a conduit is currently limited to gross inspection by the surgeon for externally apparent abnormalities such as lacerations, branch avulsions, or varicosities with no effort to assess the intima. However, injury to this inner vascular layer is more likely to directly influence the risk of early failure by hampering the antiinflammatory and antithrombotic role of the endothelium.7 Since the vascular endothelium is also the body’s major source of endogenous vasodilators such as prostacyclin and nitric oxide, atherosclerotic plaque or traumatic injury may increase the risk of postoperative spasm, particularly for the RA graft.8 In a prospective analysis of patients who ultimately developed graft failure, our group found that the degree of endothelial cell disruption detected in surplus segments of the SV graft immediately after harvest was directly associated with the risk of failure.2 “Bench-to-bedside” translation of our understanding that endothelial integrity plays a key role in conduit selection practices has been hindered by the lack of a convenient means to objectively assess endothelial quality in the operating room setting. Immunohistochemistry (IHC) remains the “gold stan-
standard” for analyzing vascular endothelium, but cannot be obtained in real time and is insensitive to heterogeneous abnormalities of the endothelium throughout the conduit. Intravascular ultrasound (IVUS) and high-resolution computed tomography (CT) scanning can provide a more global method for screening the entire in situ RA graft in real time, but have insufficient resolution for detecting most intimal abnormalities.

Catheter-based optical coherence tomography (OCT) is an emerging imaging technology capable of an axial resolution in the range of 2 to 15 μm, at least a 10-fold improvement over IVUS. The feasibility of OCT for visualization of coronary plaques in patients was first demonstrated in 2002. OCT has been applied for plaque and thrombus characterization, determining the risk of plaque rupture by macrophage detection, and therapeutic guidance of coronary interventions for stent visualization. The superior resolution of OCT versus IVUS enables more precise evaluation of stent deployment.

Despite a wide array of advantages, the adoption of OCT has been slow in the cardiology field. The problem with intracoronary OCT imaging in live patients is that blood must be flushed out of the coronary artery for the image to be obtained. This requires that a proximal segment of the vessel be occluded with a high-pressure balloon for approximately 30 s. Such localized pressure can injure the endothelium and increase the risk of subsequent thrombosis. Second, periods of coronary occlusion may not be safe in unstable patients. Finally, because of the finite distance between the balloon and imaging positions, plaques close to branch points cannot be imaged.

The challenge has been to identify applications that will optimally utilize the strength of catheter-based OCT without these limitations. In this paper, we propose and present preliminary human data on a novel cardiac application for OCT: intraoperative screening of harvested conduits in CABG procedures. Graft imaging during CABG is performed in a segment of vessel that is exsanguinated and bathed in crystalloid preservation solution. Consequently, there is no need for the endothelium to be subjected to the potentially damaging pressure of an inflated intraluminal balloon. In this paper, we report the feasibility of OCT for real-time analysis of luminal abnormalities within bypass conduits that result from atherosclerosis, trauma (e.g., intimal tear, medial dissection), or retention of thrombi.

2 Methods
2.1 Subject Enrollment and Study Design
Following IRB (Institutional Review Board) approval (protocol H25350), all clinical subjects in whom the RA and/or SV were considered as a conduit between March and December 2006 provided informed consent before enrollment into a prospective observational study assessing the feasibility of OCT for evaluating bypass conduits. A total of 27 RA and 33 SV conduits were evaluated from 35 patients.

2.2 Surgical Technique
CABG was performed via a median sternotomy and the left ITA, SV, and/or RA were harvested in all patients. RAs were procured using either endoscopic (56%) or pedicle (44%) techniques as described previously, whereas all SVs were harvested endoscopically (VasoView6; Guidant Systems, Inc., Minneapolis, Minnesota). Endoscopic harvest was initiated by inflating a tourniquet while exsanguinating the leg or arm with an Esmark bandage. A longitudinal 3-cm skin incision was made over the distal portion of the vessel and a trochar port inserted with the balloon inflated to establish a seal, which is necessary to create CO2 pressure (8 to 10 mm Hg) within a subcutaneous tunnel. Anterior and posterior exposure around the vessel was created by blunt dissection with endoscopic visualization. Division of branches was performed with minimal tension using bipolar electrocautery. Proximal RA or SV ligation was performed through a separate stab incision. The vessel was then removed from the tunnel and flushed with a plasmalyte solution containing glyceryl trinitrate and verapamil.

2.3 OCT Analysis of Bypass Grafts
Conduits were imaged using OCT in situ prior to harvest and ex vivo after harvest (LightLab Imaging, Inc., Westford, Massachusetts) (Fig. 1). The in situ OCT examination was performed by inserting a 1.2 F (0.4-mm) imaging probe (ImageWire®, LightLab Imaging, Westford, Massachusetts) into the exsanguinated vessel. Additional clearance of blood from the vessel was facilitated by infusing heparinized saline during imaging. Vessels were imaged at a rate of 0.5 mm/s and data were processed using proprietary software according to the principles of OCT imaging described elsewhere. Ex vivo examination was performed in a similar manner, except for evaluating bypass conduits. A total of 27 RA and 33 SV conduits were evaluated from 35 patients.

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![Fig. 1 Schematic of in situ and ex vivo scanning protocol. In situ OCT examination (A) was performed prior to any surgical manipulation by exsanguinating the forearm using a tourniquet, inserting the OCT probe into the distal RA, and advancing it proximally. Gentle Hank's balanced salt solution (HBSS) infusion via a Y-connector connected to the cannula was used to inflate the vessel to facilitate optimal imaging. This examination identified preexisting intimal pathology, such as atherosclerotic plaque. The excised RA underwent a second ex vivo OCT examination (B) to assess any damage incurred during harvest.]
that one end of the vessel was occluded with a spring-loaded vascular clip to allow for gentle distension of the vessel during imaging (Fig. 1). Plaques visualized by cross-sectional OCT imaging were categorized as fibrous, fibrocalcific, or fibroatheromatous, based on the American Heart Associations scientific statement for advanced coronary lesions, and intimal disease was quantified by intimal-medial ratio, as previously described. Harvesting injury was categorized as mild when intimal disruption was restricted to the ostium of branch points and severe when the tear affected the luminal surface. Intraluminal thrombus was identified as a lobulated mass with high signal intensity and characteristic radial shadowing, as previously described.

In a subset of SVs (n=3), the OCT imaging wire was introduced via a small venotomy in the body of the graft and advanced into the distal anastomosis. This allowed for imaging of the patency of this graft-to-coronary connection, as previously described using coronary ultrasound.

2.4 Histological Analyses

Biopsy specimens for histological processing were procured from discarded conduit segments. To exactly register the OCT images with the corresponding histological sections, the vessel site at which the biopsy specimen was obtained was marked externally at the location of the catheter, visualized by the rotating infrared light at the catheter tip. These “image-guided” biopsy specimens were then stored in solution before being embedded and frozen in cutting compound (Tissue-Tek O.C.T., Redding, California). Frozen sections were analyzed via van Giesen staining (elastin) to visualize the internal (IEL) and external elastic lamina (EEL), and the intimal-medial ratio was measured. Selected frozen sections were also analyzed for the presence of macrophages with anti-CD68 mAb (Invitrogen, Carlsbad, California), as previously described.

2.5 Tissue Factor Activity

Selected vessel segments were incubated at 37°C in a custom designed chamber containing Tris buffer (pH 7.4), 50 mM CaCl₂, 2 U/ml Factor VII, and 2 U/ml Factor X (American Diagnostica, Stamford, Connecticut). After 60 min, reaction was stopped by adding 25 mM EDTA. This incubation solution was combined with Tris buffer (pH 8.6) and 5 mM chromogenic FXa substrate (Spectrozyme FXa, American Diagnostica, Stamford, Connecticut) in a 96-well plate, and incubated for 60 min at 37°C. The absorption of the reaction buffer was assessed at 405 nm and then compared with a standard curve to determine tissue factor activity.

3 Results and Discussion

OCT imaging of bypass conduits showed that the RAs and SVs possess unique imaging characteristics based on the respective compositions of their vascular walls. Three distinct tissue layers are imaged for the normal RA: an inner high-intensity band of varying thickness representing the vascular intima, a thicker low-intensity band representing the smooth muscle of the media, and an outer heterogeneous band of higher signal intensity representing the connective tissue of the adventitia (Fig. 2). These three layers are demarcated by well-developed internal and external elastic laminae in the RA, but are less discernable in OCT images of the SV, which has a poorly developed internal elastic lamina (Fig. 2). Due to the excellent resolution of tissue layers in the RA, intimal hyperplasia in this vessel is easily detected by OCT. RA intimal-medial ratios measured by OCT showed a strong correlation with the analysis of registered histologic sections (R =0.88, p<0.001, Fig. 2) and a small average discrepancy and consistent variation (−0.07±0.22) as determined by Bland-Altman analysis. Our experience corroborates other reports, concluding that OCT is ideally suited for evaluating the intimal surface of blood vessels.

While patients referred for CABG obviously have atherosclerotic lesions in their coronary arteries, plaques can also occur throughout their vasculature, including the RAs. The main finding of our study was that OCT imaging easily and quickly elucidates RA atherosclerosis. A unique advantage
when using this imaging modality during ex vivo applications is the ease of confirming imaging findings by histological correlations. The OCT probe’s IR light transilluminates the vessel wall, enabling an exact registration with biopsies obtained from surplus segments of the graft. The additional ability to discriminate the underlying medial and adventitia layers illustrates that light penetration is effective in this area. In this example, the boundary between the bright intima and darker media is difficult to discern, but the external elastic membrane that demarcates the boundary between the media and adventitia appears as a sharp line. A fibrocalcific plaque (B) is recognized by discrete areas of poor signal intensity with well-demarcated borders that represent areas of intimal calcification. Here, the concentrically thickened intimal layer is seen as a bright inner band, consistent with the fibrotic neointimal tissue shown in the corresponding histological section (E). A fibroatheroma ([C] and [F]) appeared as shadowed areas capped by a bright overlying layer. High attenuation of light within dense atheromatous tissue creates a shadowing artifact that limits penetration into the deeper medial and adventitia layers. Lipid-laden foam cells (macrophages), which create bright localized reflections with spoke-like shadows on OCT imaging, were identified by histological appearance (G) and CD68 IHC (H). Note the relatively low density of foam cells in the middle of the plaque and the corresponding region of preserved signal penetration in the OCT image (C).

**Fig. 3** Atherosclerotic plaques in the RA. Using histological sections that were registered to the areas of OCT images, we established that OCT was able to differentiate plaque morphology within RA conduits on a microscopic scale. A fibrous plaque [(A) and (D)] is demonstrated by a homogeneous region of signal-rich intensity that is eccentrically located on the interior of the vessel. The additional ability to discriminate the underlying medial and adventitia layers illustrates that light penetration is effective in this area. In this example, the boundary between the bright intima and darker media is difficult to discern, but the external elastic membrane that demarcates the boundary between the media and adventitia appears as a sharp line. A fibrocalcific plaque (B) is recognized by discrete areas of poor signal intensity with well-demarcated borders that represent areas of intimal calcification. Here, the concentrically thickened intimal layer is seen as a bright inner band, consistent with the fibrotic neointimal tissue shown in the corresponding histological section (E). A fibroatheroma ([C] and [F]) appeared as shadowed areas capped by a bright overlying layer. High attenuation of light within dense atheromatous tissue creates a shadowing artifact that limits penetration into the deeper medial and adventitia layers. Lipid-laden foam cells (macrophages), which create bright localized reflections with spoke-like shadows on OCT imaging, were identified by histological appearance (G) and CD68 IHC (H). Note the relatively low density of foam cells in the middle of the plaque and the corresponding region of preserved signal penetration in the OCT image (C).
compared to adjacent areas with intact intima (3.71 versus 0.76 U/cm²). Given the key role that tissue factor plays in thrombosis, this finding suggests that OCT may identify specific areas within the conduit that are likely to be more thrombogenic when exposed to the coronary bloodstream after grafting. Previous investigations have suggested that endoscopic harvest does not affect intimal integrity in the RA or SV, but have been limited to analyses of discarded segments. Given the focal nature of intimal trauma, these studies may not have had sufficient sensitivity to determine the consequences of endoscopic harvest. The concerning pathologies in endoscopically harvested conduits, as detected by OCT imaging in this study, are more in line with recent evidence from a large, multicenter graft patency trial, showing that endoscopic harvest was an independent predictor of bypass graft failure. Undoubtedly, OCT can provide a quality assurance tool for addressing these controversies and directing the development of improved conduit harvesting methods and devices.

Ex vivo OCT imaging also revealed a high incidence of retained clot within SVs (Fig. 5). All veins in this study were harvested using an endoscopic technique and therefore were exposed to a period of pressurized CO₂ insufflation, which is required to facilitate endoscopic visualization. A side effect of this pressurization is that the vein is compressed leading to stagnation of intraluminal blood that may promote clot formation. These clots might serve as nidus to activate the coagulation cascade and cause acute graft failure. While intraluminal thrombi have been noted anecdotally by surgeons in the past, the use of OCT in this study enabled the first true appreciation of total clot burden contained within these endoscopically harvested bypass grafts. The ability of OCT to accurately quantify the volume of retained clot provides a highly sensitive endpoint for identifying potential risk factors and testing strategies for preventing thrombus formation.

OCT is used widely in ophthalmology, but its application to vascular imaging has not been met with enthusiasm. Up to now, clinical research into catheter-based OCT has been devoted primarily to imaging plaques within the coronary arteries. However, a problem with intracoronary OCT imaging in live patients is that blood must be flushed out of the coronary artery for the image to be obtained. This requires brief occlusion of the coronary artery with an inflated balloon, risking endothelial injury and ischemia. Imaging of bypass conduits provides a more convenient and advantageous application of this powerful technology. Unlike the coronary artery, the limb can be exsanguinated with a tourniquet without subjecting the vessel to the effects of an occlusion.
sive balloon. Since ischemic periods of up to a few hours are well-tolerated in the extremities, a 3 to 5 min period of limb ischemia to perform these examinations was not a concern. In addition, the ability to fully flush all blood out of the vessel produced very high quality OCT images as compared to coronary applications in which the image is often compromised by the presence of residual blood (Fig. 6).

One of the most promising applications of OCT in cardiac surgery is for evidence-based “targeted conduit selection.” The average lengths of harvested conduit in this study were $16.2 \pm 1.6$ cm for RAs and $27.2 \pm 7.3$ cm for SVs, while the average length discarded was $4.9 \pm 3.1$ cm. This means that length in excess of that required for grafting was consistently harvested. OCT imaging enables comprehensive assessment of the bypass conduit in real time, prior to grafting into the coronary circulation. Therefore, the surgeon is able to exclude regions of conduit considered less optimal (i.e., containing a fibroatheroma or thrombus). In the event of severe preexisting intimal disease throughout the conduit, the ability to conduct in situ OCT scanning enables rejection of the entire vessel length prior to harvest, sparing the patient potential morbidity associated with removing a vessel that is not subsequently utilized. Thus, targeted conduit selection using OCT imaging has the potential to improve graft patency and patient outcomes through utilization of bypass grafts with the highest quality intima possible.

Another potential application of OCT toward the assessment of bypass conduits is the imaging of the distal anastomosis (i.e., the location where the bypass graft is sutured to the native coronary vessel). A poor-quality anastomosis can reduce blood flow in the graft and increase the risk of occlusion. A similar technique has been described using 13-MHz epicardial ultrasound, but the resolution offered by OCT is vastly superior and enables detection of much more subtle defects. OCT is unique in that it enables high-resolution visualization of every aspect of the suturing (Fig. 7) and can be used to determine if low flow is in fact due to a poor surgical technique that necessitates revision. In addition, this application would further enhance the value of OCT as a quality-control device, particularly in the training of novice coronary surgeons.

4 Conclusion

Cardiac surgery, and CABG in particular, is a field that truly requires innovation if it is to succeed into the future. Recent discoveries about the importance of intimal quality for bypass graft patency have a tremendous potential to improve outcomes, but not without a suitable means of real-time assessment. OCT has the potential to fill this niche. Despite the vastly superior resolution of OCT as compared to other currently available imaging techniques, it has not yet been widely accepted for intravascular imaging. These data provide support for the initiation of appropriately powered clinical trials to confirm the relationship between intimal quality identified by OCT and bypass graft patency. In this example of “bench-to-bedside” translational research, we identify a novel application that exploits OCT’s high spatial resolution and real-time imaging capabilities while circumventing the problems associated with clearing blood from the coronary vessel. It is also an application with high impact in terms of the potential number of patients affected and the ability to alter clinical outcomes.

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