Neointimal patterns obtained by optical coherence tomography correlate with specific histological components and neointimal proliferation in a swine model of restenosis

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Aims
Although optical coherence tomography (OCT) is capable to detect microscopic peri-strut changes that seem to be related to neointimal inhibition and healing, its ability to characterize these components is still limited. In this study, we aimed to compare different OCT morphological characteristics with different in-stent neointimal tissue types analysed by histology.

Methods
A total of 69 stents (39 drug eluting and 30 bare metal stents) were implanted in coronary arteries of 27 swine. By OCT, neointimal type was classified as homogeneous, heterogeneous, or layered according to its pattern of backscatter and optical intensity. The resulting optical patterns were correlated with several histological findings [external elastic lamina (EEL) disruption, fibrin deposition, circumferential rim of peri-strut inflammatory cell infiltration, and fibrous connective deposition] in every single cross-section (CS) analysed.

Results
A total of 197 matched OCT and histological CS were analysed. The heterogeneous (0.44 ± 0.21 mm) and layered (0.65 ± 0.16 mm) patterns had a significantly higher degree of neointimal thickness compared with the homogeneous pattern (0.25 ± 0.16 mm, \(P < 0.001\)). Fibrous connective tissue deposition was more frequently present in the homogeneous pattern (71.6%, \(P < 0.001\)), whereas significant fibrin deposits were more commonly seen in the heterogeneous pattern (56.9%, \(P = 0.007\)). Peri-strut inflammation was less frequently found in the homogeneous pattern (19.8%, \(P < 0.001\)) in comparison with the layered (73.9%) or heterogeneous patterns (43.1%). The presence of EEL rupture was also more commonly seen in layered (73.9%) and heterogeneous (46.6%) patterns than in the homogeneous pattern (22.4%, \(P < 0.001\)).

Conclusion
The optical characteristics of neointimal formation seen in OCT properly correlated with the presence of several histological findings involved in stent healing. The biological implications of these findings in clinical outcomes require further investigation.

Keywords
Optical coherence tomography • Optical intensity • Histology • Neointima • Stent

Introduction
Drug-eluting stents (DESs) are considered the standard of care for the interventional therapy of coronary artery disease.1,2 Following the introduction of these devices, the incidence of in-stent restenosis (ISR) and repeated revascularization remarkably decreased.1,2 However, late and very late stent thrombotic events still occur and appear to be related to abnormal healing response occurring...
following stent implantation. Due to its high resolution, optical coherence tomography (OCT) has emerged as the ideal tool for the evaluation of stent healing. Recent animal and human data validated this tool in the evaluation of peri-strut neointimal formation and coverage. In addition, recent studies suggest that different characteristics of neointimal tissue seen by OCT may correlate with clinical events such as late stent thrombosis. However, although OCT has a value in the evaluation of strut coverage, its ability to characterize neointimal tissue types is still limited. In this study, we aimed to compare different OCT morphological characteristics with in-stent neointimal tissue types analysed by histology in a porcine model of ISR.

**Methods**

**Study design**

The study protocol was approved by the local institutional animal care and use committee (IACUC). All animals received humane care in compliance with the Animal Welfare Act and the ‘Guide for the Care and Use of Laboratory Animals’ formulated by the Institute of Laboratory Animal Research (National Research Press, 1996). Twenty-seven swine weighing between 25 and 40 kg were studied for 28 or 90 days to achieve different degrees of healing and neointimal formation. At the time of deployment, 69 stents [39 DES: 19 Taxus Liberté (Boston Scientific, Natick, MA, USA), 13 Xience V (Abbott Vascular, Santa Clara, CA, USA), 7 Promus (Boston Scientific), and 30 bare metal stents [BMS; 20 Liberté (Boston Scientific) and 10 ML Vision (Abbott Vascular)]] were randomly assigned to either coronary artery in each animal. Stents of 12 mm length and diameters of 3.0, 3.5, or 4.0 mm were implanted depending on the vessel size.

**Procedural description**

Animals were pre-medicated with 325 mg of aspirin and 75 mg of clopidogrel at least 12 h before the procedure and pre-anesthetized with a mixture of glycopyrrolate, telazol, and xylazine based on animal weight. After reaching an adequate anaesthetic status, the animals were intubated and inhaled isoflurane (1–2%) delivered through a precision vaporizer and a circle absorption breathing system under periodic arterial blood gas monitoring. A vascular access sheath (7 Fr) was placed in the carotid artery by cut-down with a sterile technique. Before catheterization, heparin (5000–10 000 U) was injected to maintain an activated clotting time of 250–300 s. Baseline angiography was acquired and the coronary vessels were sized for proper stent placement at a targeted 1.1–1.2 : 1 stent to the artery diameter ratio. Before stent implantation vessels were injured with a targeted 10% balloon overstretch to promote more robust neointimal formation for analysis. Stents were deployed at >110–120% overstretch using diameters derived from manufacturer’s compliance charts, at the predetermined sites over a guide wire using fluoroscopic guidance. Following stent implantation, haemostasis was obtained by arterial ligation using a 2-0 silk suture and the incision site closed in 2–3 layers with appropriate suture material. All animals received aspirin (81 mg) and clopidogrel (75 mg) daily and remained on a normal chow diet.

**Angiographic analysis protocol**

Quantitative coronary angiography (QCA) was performed on baseline, following stent implantation and at the terminal procedure. QAngio XA 7.1 Medis System (Medis Medical Imaging Systems, Leiden, The Netherlands) was used to analyse all angiograms by an operator blinded to both stent type and termination time point. Each stent was divided into three segments along the longitudinal axis to match corresponding histological segments. Each individual segment underwent QCA analysis for the assessment of minimal lumen diameter (MLD) immediately post-implantation and at follow-up. Angiographic late lumen loss was determined at each individual segment by subtracting the MLD at follow-up from the MLD assessed immediately post-stenting. The percent diameter of stenosis was calculated according to the following formula: percent diameter stenosis = [1 – (lumen diameter/mean reference vessel diameter)] × 100.

**OCT imaging protocol and analysis**

OCT images were obtained using the C7-XR OCT imaging system (Light-Lab Imaging, Inc., St. Jude Medical, St. Paul, MN, USA), and imaging was performed using a continuous non-occlusive contrast-saline mixture as a flush. Motorized OCT pullbacks were performed at a rate of 20 mm/s, and all images were acquired at 100 frames per second. Every subsequent millimetre was specified by the OCT frame rate, allowing for precise histological-OCT frame matching. This analysis involved choosing three cross-sectional images per pullback: proximal stent, mid-stent, and distal stent. The integrated OCT image analysis software developed by Light Lab Imaging, Inc. was used for measurements. The lumen area (LA) involved the manual identification of the delimiting contours of the stent. Stent area (SA) was defined as the circumferential area limited by the contours of the struts. Neointimal area (NA) was calculated by subtracting the LA from the SA. To analyse a neointimal thickness (NIT), the distance between the centre of each strut and the luminal border was measured in the direction of the centre of gravity. The percentage area stenosis (AS) was calculated according to the following formula: percent AS = [1 – (LA/SA)] × 100. A stent strut was considered covered if NIT was ≥ 20 μm. Malapposition was defined as detachment from the vessel wall considering stent strut thickness, polymer, and blooming artefact at each type of the stent.

**OCT classification of neointimal type**

Neointimal tissue was evaluated qualitatively in cross-section (CS) over 20 μm of mean NIT using a recently published OCT classification, which is based on tissue structure and backscatter. Homogeneous pattern is defined as neointimal tissue with uniform optical properties without focal variation in the backscattering pattern. The heterogeneous pattern refers to those with focally changing optical properties and various backscattering patterns, and the layered pattern has concentric layers with different optical properties.

**Tissue harvesting and histology**

Under anaesthesia, all animals were euthanized immediately after follow-up imaging. Hearts were excised and pressure-perfused with 0.9% saline followed by pressure-perfusion fixation in 10% neutral-buffered formalin until hardening of the heart muscle was clearly perceptible as previously described. Prior to histological processing, intact hearts with stented vessels were imaged by capturing high-contrast film-based radiographs (Faxitron X-ray Corp., Model 4385SA, National X-Ray, Lawrenceville, GA, USA) to locate and assess stent location. The stented arterial segments were then carefully dissected. After polymerization, three sections were sawed from each stent, beginning at the distal stent edge. Individual slides were cut on a rotary microtome at 4–6 μm, mounted, and stained with haematoxylin and eosin and Masson’s trichrome. For the quantitative analysis: the cross-sectional areas [external elastic lamina (EEL) representing SA, internal elastic lamina (IEL), and LA] of each section were measured using ImagePro Plus 4.5 (Media Cybernetics, Bethesda, MD, USA). NIT was measured as the distance from the inner surface of each stent strut to the luminal border in the direction of the stent centre of gravity. Area measurements were calculated...
as follows: neointima (IEL area - LA), and % AS [1 - (LA/IEL area) x 100]. For the qualitative evaluation, all sections were examined by light microscopy. Histological parameters were analysed on a cross-sectional level. For a CS to be considered to have fibrin present, its mean fibrin deposition has to have a score of 1 (at least a light spotting of fibrin around stent struts or/and in neointima). While for inflammatory cells, a score of 1 referred to a notable solid rim of inflammatory cells not effacing the neointima, media, and or adventitia. EEL rupture was noted when a break in EEL was observed, neovascularization was identified by presence of: microvessels in the neointima and complete strut coverage was defined as 100% of struts covered with neointima in each CS. Fibrous connective tissue was determined if a densely stained collagen predominated (>50%) rather than a loose connective tissue containing stellate cells. 16 Figure 1 shows some representative images of some of these parameters and their corresponding appearance in OCT.

OCT histology co-registration
The histological images were compared with their corresponding OCT pullback stent segments (proximal, middle, and distal) until the best and closest visual match was found (Figure 1). This match was done taking into consideration anatomical features (luminal shape, NIT, presence of neovascularization, etc.), proximity to side branches, and the length from the stent edge to the location of the histology samples (provided by the histological laboratory). Generally, samples were taken within 5 mm to the proximal and distal stent edges and 5 mm within equidistance between the proximal and distal for OCT purposes.

Statistical analysis
Continuous variables were expressed as a mean ± standard deviation (SD). Student’s t-test was performed to compare continuous variables; a Mann–Whitney U-test was used for skewed distributions. The categorical variables were expressed as both numbers and percentages and were compared using the χ² test or Fisher’s exact test. For OCT analysis at our core laboratory, inter- and intra-observer variabilities in measured distance and area had been reported in a previous study. 6 Additionally, to determine inter- and intra-observer variabilities of the characteristics of neointimal tissue in OCT, two observers were blinded to the histological findings. Sixty OCT CSs were randomly selected and the observers classified the neointimal characteristic based on the definitions. The association between OCT and histological analysis was calculated using Spearman’s correlation coefficient. The agreement between NIT as measured by OCT and histological analysis was examined by Bland–Altman plots. Data were analysed with the SPSS 16.0 software for Windows (SPSS, Chicago, IL, USA). A P-value of <0.05 was considered statistically significant.

Results
Morphometric analysis
A total of 69 stents comprising of 39 DESs and 30 BMSs were implanted. This yielded that 197 OCT histology matched CSs were included in the final analysis. The baseline vessel diameter was 2.91 ± 0.56 mm (Table 1). The mean balloon overstretch ratio was 1.14 ± 0.05, while the mean stent-to-artery ratio was 1.18 ± 0.13. This yielded an average MLD of 3.29 ± 0.72 mm immediately after stent implantation. The angiographic late lumen loss was significantly lower in the homogeneous pattern of 0.75 ± 0.89 mm (P < 0.001, Table 1) compared with that in the heterogeneous (1.12 ± 0.88 mm) and layered patterns (1.66 ± 0.36 mm).

There were no differences in SAs between the different neointimal patterns for both OCT and histology as shown in the morphometric data in Table 1. Inter- and intra-observer variabilities for characteristics of neointimal tissue in OCT was κ = 0.85 and 0.98, respectively. The OCT LA of the homogeneous pattern (7.01 ± 2.29 mm², P < 0.001) was larger than that of both heterogeneous (5.73 ± 2.57 mm²) and layered patterns (3.45 ± 1.11 mm²). These results were confirmed in histology as seen in Table 1. Conversely, the mean NIT of the homogeneous pattern had a significantly lower degree of NIT (0.25 ± 0.16 mm, P < 0.001) compared with the heterogeneous (0.44 ± 0.21 mm) and layered (0.65 ± 0.16 mm).

![Figure 1](https://academic.oup.com/ehjcimaging/article-abstract/15/3/292/2399818/1)
patterns. An adequate degree of correlation was found between OCT and histological images in the assessment of LA (R = 0.76), NIT (R = 0.83), NA (R = 0.72), and % AS (R = 0.87). The Bland–Altman analysis is shown in Figure 2, demonstrating the agreement between LA, NIT, NA, and % AS obtained by OCT and histological analysis. There was 99.7% strut coverage without malapposition in this study (99.5% in homogeneous, 100% heterogeneous, and 100% layered pattern).

**OCT characterization of different tissue types**

The tissue characterization analysis is presented in Figure 3. The overall prevalence of fibrous connective tissue, fibrin deposits, peri-strut inflammation, neovascularization, and EEL rupture were 54.3, 40.1, 33.0, 20.8, and 35.5%, respectively. Fibrous connective tissue was predominant in the homogeneous pattern (71.6%) than that in the other two patterns (31.0% in heterogeneous and 26.1% in the layered patterns). Fibrin deposits were more frequently detected in the heterogeneous pattern (56.9%) than that in the other two other patterns (31.9% in homogeneous and 39.1% in the layered patterns), while peri-strut inflammation and EEL rupture were more frequently found in the layered pattern (73.9 and 73.9%, respectively) than that in the other two patterns (19.8 and 22.4% in the homogeneous pattern and 43.1 and 39.7% in the heterogeneous pattern). In terms of neovascularization, heterogeneous (39.7%) and layered patterns (47.8%) showed a higher frequency than the homogeneous pattern (60.0%). In a sub-analysis of stent types, the heterogeneous pattern was more frequently observed in the DES group in total, 28 and 90 days after stent implantation, while the layered pattern was seen more frequently in the BMS group (Table 2). The overall homogeneous pattern was seen with a similar frequency between BMS (66.0%) and DES (53.2%, P = 0.07).

**Discussion**

OCT is emerging as an important imaging tool for the evaluation of stent healing in humans. It provides cross-sectional images of vascular structures at a definition which is 10 times higher than intravascular ultrasound imaging. Due to its high resolution, we hypothesized that OCT could be capable of characterizing neointimal tissue based on the different optical properties displayed by the components of the neointima using an ISR swine model. In our study, the optical characteristics of neointimal formation seen in OCT properly correlated with the presence of several histological findings involved in stent healing. In addition, the homogeneous pattern of neointimal formation.
formation appeared to correlate with less neointimal formation and more favourable vessel healing characteristics when compared with the other optical patterns.

Different studies have used OCT as a tool to characterize different atherosclerotic tissue types. In a study of non-stented human atherosclerotic lesions, Yabushita et al. showed that certain OCT tissue characteristics highly matched with some specific histology findings. A recent study using OCT demonstrated that most of the neointimal tissue at the early phase of BMS healing (≤6 months after stent implantation) frequently displayed a signal rich, homogenous OCT pattern. However, various OCT patterns of neointima were observed in the late period of healing (>5-year follow-up) that were similar to native atherosclerotic lesions. Additionally, a study using electron microscopy demonstrated that the evaluation of optical intensity in neointimal tissue could be useful to differentiate between fibrin and normal neointimal tissue over stent struts. Despite these findings, there is little data for the validation of neointimal characteristics characterized by OCT using animal models.

In this study using the same OCT classification for the patterns of stent restenosis, we observed that the homogeneous pattern had a higher proportion of fibrous connective tissue in histology. This supports the findings of Yabushita et al. where fibrous plaques were found to exhibit homogeneous highly backscattering (signal rich) region similar to atherosclerotic lesions. This is also supported by a previous case report that showed bright high-optical intensity lesion in OCT, indicating smooth muscle cells in histology and hypolucent or patched low-optical intensity lesion representing myxomatous tissue or fibrin thrombus. Previous studies demonstrated that neointima rich in smooth muscle cell with dense collagen fibre has high backscatter and high-optical density, whereas neointima rich in proteoglycan, fibrinoid, and thrombus with a low density of smooth muscle cells has a low and various optical densities. Based on this observation, the homogeneous pattern could be more favourable and has a normal healing pattern after stent implantation. Fibrin deposits and inflammatory cells have been associated with delayed vascular healing and arterial toxicity following DES implantation.

Figure 2 Bland–Altman plot (two lines: limit of agreements) are shown, demonstrating the agreement between LA (A), NA (B), NIT (C), and AS (D) in histology and OCT analysis.
Interestingly, the heterogeneous pattern correlated more with the presence of fibrin deposits than the other patterns. Additionally, in our study, the layered pattern was associated with a higher degree of ISR than both the homogeneous and heterogeneous patterns. This might be a phenomenon secondary to the progressive attenuation of optical signal as light progresses through an increased neointima and limitation of the current OCT system to evaluate the thick neointimal tissue.\textsuperscript{13} Another interesting finding in the layered pattern was the higher incidence of peri-strut inflammation, neovascularization, and EEL rupture, which might be the cause of a higher degree of vessel injury leading to a higher degree of neointimal proliferation.\textsuperscript{24} According to the type of stents, the heterogeneous pattern was observed more frequently in DES compared with BMS, and this pattern presented the highest prevalence of fibrin in our study. Previous studies demonstrated that DESs are associated with delayed healing including the persistence of fibrin deposits and incomplete endothelialization compared with BMS.\textsuperscript{25,26} It was also interesting to see the layered pattern being more prevalent in BMS than in DES. This could be explained that DESs suppressed an enough neointimal formation by EEL rupture and adventitial inflammation, which might cause to have a more heterogeneous than layered pattern compared with BMS. In addition, the difference in the layered pattern between 28 and 90 days most likely might be related with previous clinical observation and the hypothetical decrease of inflammation or fibrinoid tissue around struts.\textsuperscript{24}

**Table 2** Qualitative parameters assessed in vivo by OCT and ex vivo by histology in a total of 197 matched CS according to the type of stents

<table>
<thead>
<tr>
<th>Total</th>
<th>BMS (N = 88)</th>
<th>DES (N = 109)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneous pattern</td>
<td>58 (66.0%)</td>
<td>58 (53.3%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Heterogeneous pattern</td>
<td>15 (17.0%)</td>
<td>43 (39.4%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Layered pattern</td>
<td>15 (17.0%)</td>
<td>8 (7.3%)</td>
<td>0.04</td>
</tr>
<tr>
<td>28 days</td>
<td>N = 63</td>
<td>N = 83</td>
<td>0.76</td>
</tr>
<tr>
<td>Homogeneous pattern</td>
<td>41 (65.1%)</td>
<td>52 (62.7%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Heterogeneous pattern</td>
<td>9 (14.3%)</td>
<td>25 (30.1%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Layered pattern</td>
<td>13 (20.6%)</td>
<td>6 (7.2%)</td>
<td>0.01</td>
</tr>
<tr>
<td>90 days</td>
<td>N = 25</td>
<td>N = 26</td>
<td>0.01</td>
</tr>
<tr>
<td>Homogeneous pattern</td>
<td>17 (68.0%)</td>
<td>6 (23.1%)</td>
<td></td>
</tr>
<tr>
<td>Heterogeneous pattern</td>
<td>6 (24.0%)</td>
<td>18 (69.2%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Layered pattern</td>
<td>2 (8.0%)</td>
<td>2 (7.7%)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Values are presented as n (%).
The main limitation of the study was the experimental nature of the procedure, and the fact that stents were implanted in the absence of atherosclerotic disease. Although widely used for the validation of stents, this model does not provide the complex atherosclerotic milieu seen in humans. Compared with OCT findings after DES implantation in human, lipid rich neointima, thin cap fibroatheroma like neointima, neointimal rupture, or intracoronary thrombus are not easily detected in the animal model.

Therefore, the results of this study need to be interpreted with caution and within the context of an ISR model. Also, we acknowledge that immunohistochemical and special staining would have been more accurate in determining the fibrous connective tissue contents and specific loose connective tissue (proteoglycan and hyalurano). Another limitation relates to the challenges of in vivo imaging, co-registration, and histological tissue processing potentially causing some data variability. However, proper and meticulous co-registration procedures were followed in order to minimize potential confounding variables. In addition, the study included a large sample size including stents displaying a wide variety of degrees of neointimal proliferation allowing an accurate statistical analysis. Finally, signal attenuation due to inherent limited penetration depth should be considered to evaluate the neointimal characteristics, although previous investigations demonstrated the association of neointimal findings in OCT and histology.8,22

In summary, the current study aimed to compare different OCT morphological characteristics with in-stent neointimal tissue types analysed by histology using an ISR model. In this study, the optical characteristics of neointimal formation seen in OCT properly were correlated with the presence of several histological findings involved in stent healing.20 However, the biological implications of these findings need to be further investigated in humans.

Conflict of interest: none declared.

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References