Interventional Cardiology

Intracoronary Optical Coherence Tomography and Histology at 1 Month and 2, 3, and 4 Years After Implantation of Everolimus-Eluting Bioresorbable Vascular Scaffolds in a Porcine Coronary Artery Model

An Attempt to Decipher the Human Optical Coherence Tomography Images in the ABSORB Trial

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Background—With the use of optical coherence tomography (OCT), alterations of the reflectance characteristics of everolimus-eluting bioresorbable vascular scaffold (BVS) struts have been reported in humans. In the absence of histology, the interpretation of the appearances of the struts by OCT remains speculative. We therefore report OCT findings with corresponding histology in the porcine coronary artery model immediately after and at 28 days and 2, 3, and 4 years after BVS implantation.

Methods and Results—Thirty-five polymeric BVS (3.0×12.0 mm) were singly implanted in the main coronary arteries of 17 pigs that underwent OCT and were then euthanized immediately (n=2), at 28 days (n=2), at 2 years (n=3), at 3 years (n=5), or at 4 years (n=5) after implantation. All BVS-implanted arteries in these animals were evaluated by histology except for 5 arteries examined at 2 years with gel permeation chromatography to assess the biodegradation of the polymeric device. Fourteen arteries with BVS from an additional 6 pigs were examined by gel permeation chromatography at 1 (n=1), 1.5 (n=2), and 3 (n=2) years. Corresponding OCT and histology images were selected with the distal and proximal radiopaque markers used as landmarks. At 28 days, by OCT, 82% of struts showed sharply defined, bright reflection borders, best described as a box-shaped appearance. Histologically, all struts appeared intact with no evidence of resorption. At 2 years, by OCT, 60±20 struts were discernible per BVS with 80.4% of the strut sites as a box-shaped appearance. Despite their defined appearance by OCT, by histology, these structures appeared to be composed of proteoglycan, with polymeric material being at such low level as to be no longer quantifiable by chromatography. At 3 years, by OCT, recognizable struts decreased to 28±9 struts per BVS: 43.7% showed dissolved black box; 34.8%, dissolved bright box; 16.1%, open box; and 5.4%, preserved box appearance. Histology shows that connective tissue cells within a proteoglycan-rich matrix replaced the areas previously occupied by the polymeric struts and coalesced into the arterial wall. At 4 years, by OCT, 10±6 struts were recognizable as either dissolved black or dissolved bright box. In histology, these struts are minimally discernible as foci of low-cellular-density connective tissue. Relative to the prediction of histological type by OCT appearance, the preserved box appearance of OCT corresponds well with 2-year histology (86.4%), whereas the dissolved bright and black box appearances correspond to 3-year histology (88.0% and 90.7%, respectively). Struts indiscernible by OCT correspond to the integrated strut footprints seen at 4 years (100%).

Conclusions—Struts that are still discernible by OCT at 2 years are compatible with largely bioresorbed struts, as demonstrated by histological and gel permeation chromatography analysis. At 3 and 4 years, both OCT and histology confirm complete integration of the struts into the arterial wall. (Circulation. 2010;122:2288-2300.)

Key Words: imaging ■ pathology ■ stents ■ coronary disease

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Bioresorbable vascular scaffolds are a novel approach to the interventional treatment of coronary artery disease, providing short-term vascular scaffolding combined with drug-delivery capability while avoiding the long-term limitations of metallic drug-eluting stents. Such limitations of metallic drug-eluting stents include retardation of the growth of healthy endothelium over stent struts or endothelial dysfunction resulting in abnormal endothelial responses to acetylcholine. Permanent metallic stenting may also preclude surgical revascularization, jail side branches, prevent late luminal enlargement, and impair noninvasive imaging of coronary arteries with multislice computed tomography and magnetic resonance. Recently, an everolimus-eluting bioresorbable vascular scaffold (BVS; Abbott Vascular, Santa Clara, Calif) was tested in the first-in-humans ABSORB study with a series of 30 patients. In this trial, BVS demonstrated excellent long-term clinical results up to 2 years with a low major adverse cardiac event rate of 3.4%. The device consists of a backbone of poly-L-lactide (PLLA) coated with poly-D, L-lactide (PDLLA), which contains and controls the release of the antiproliferative drug everolimus (Novartis, Basel, Switzerland).

OCT and Histology of the Bioresorbable Scaffold

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selected arteries at 2, 3, and 4 years also were stained with Alcian Blue, Masson trichrome, and Von Kossa histochemical stains for the detection of acid mucopolysaccharides (proteoglycans), connective tissue, and calcification, respectively, and/or were immunohistochemically stained for detection of smooth muscle actin (SMA) to identify smooth muscle cells. In pigs that underwent both OCT and histology, the struts were counted, and these struts were classified according to histological findings, including the presence of calcification and cartilaginous metaplasia. Additionally, qualitative characterization of the vascular response around the BVS was performed (e.g., inflammation).

To correlate OCT classification and histology, 1 observer (Y.O.) aware of the histological image selected the matched OCT cross sections at 2, 3, and 4 years using the distance from markers and anatomic landmarks such as side branches. To obtain an even number of struts in histology and OCT, the observer indicated the potentially coregistered strut sites. These matched OCT images with suggested strut sites were sent to 2 independent OCT analysts (H.M.G.-G., N.G.). Although an observer for matching purposes was unblinded to both OCT and histology, the struts were classified according to histological findings, including the presence of calcification and cartilaginous metaplasia. Additionally, qualitative characterization of the vascular response around the BVS was performed (e.g., inflammation).

To characterize the degradation of polymer, the weight-average molecular weight (Mw), number-average molecular weight (Mn), polydispersity index (PDI), and content of polymer in the BVS were analyzed by GPC before stent deployment (T0) and at 1, 1.5, 2, and 3 years after implantation. The polymer was extracted with chloroform, and the tissue residue was then removed by filtration with deactivated glass wool packing. Measurements were performed with 2 PLgel Mixed-D columns (Polymer Laboratories, Stretton, UK) equipped with a refractive index detector. The Mw, Mn, and PDI of polymer were calculated from the calibration curves obtained for polystyrene standards (Mw range, 3.6 to 380 kDa), whereas the amount of polymer was calculated from the calibration curves of PLLA standards.

Statistical Analysis
Categorical data are expressed as percentages, and continuous variables are presented as mean with SD. Generalized estimating equations modeling was performed with SAS software version 9.1 (SAS Institute, Cary, NC) to take into account the clustered nature of scaffolds analyzed from same pigs, which might result in unknown correlations among measurements within these scaffold clusters. A log link function was used for the categorical and continuous variables; a logit link function was used for the binary variables. An exchangeable structure working correlation matrix was used for the analyses. All statistical tests were 2 tailed, and a value of \( P < 0.05 \) was considered statistically significant. Agreement between observers was measured by calculating the Cohen \( \kappa \) value, which takes into account the proportion of agreements occurring by chance.

Results

OCT Findings
Examples of strut appearances assessed with OCT are shown in Figure 2. Immediately after implantation, all struts had a
preserved box appearance (Figure 3) and were well apposed to the vessel wall. In 1 of the 2 pigs euthanized immediately after implantation, the BVS was deployed after heparinization but without antiplatelet therapy. In this animal, by OCT, a strut of 1 BVS was covered with highly reflective structures without shadowing, suggesting the presence of a white thrombus (Figure 3A and 3E).

The OCT findings in porcine animals at 28 days and at 2, 3, and 4 years are summarized with the human observation up to 2 years in Table 1, and representative OCT images are provided in Figures 3 through 7. At 28 days, 82% of struts presented a preserved box appearance, and 18% had an open box appearance (Figure 3). At 2 years, on average, 60 struts were discernible per BVS device. The endoluminal lining of the implanted segment was smooth and circular (Figure 4). Four fifths of the struts showed a preserved box appearance, 17.2% showed a dissolved black box, and only a few struts (2.4%) demonstrated open box appearance.

At 3 years, the number of recognizable struts decreased to 28±9 struts per BVS: 43.7% of discernible struts showed a dissolved black box appearance, 34.8% showed a dissolved bright box appearance, 16.1% showed an open box appearance, and 5.4% remained preserved box appearances (Figures 5 and 6). At 4 years, only 10±6 struts per BVS were recognized by OCT: 51.2% of struts were classified as dissolved bright box and 48.8% as dissolved black box appearance (Figure 7). At 2, 3, and 4 years, all visible struts were fully apposed and covered by tissue.

**Histological Findings**

The qualitative analysis of histology is summarized in Table 2. Immediately after implantation, the indentation of the media by the struts was visible (Figure 3C and 3G). At 28 days, the struts were completely sequestered from the lumen by a thin, fibromuscular neointima and had well-defined and squared edges. The polymeric material was not stained with Alcian Blue (Figure 3K, 3N, and 3O).

At 2 years, 6 histological images containing 76 struts were evaluated. All struts were morphologically identical as open acellular regions with well-defined borders. In such strut footprints, these “preserved box” structures likewise had discrete borders and were composed of faintly hyaline material that stained positively with Alcian Blue, indicating that they were composed of acid mucopolysaccharides (proteoglycans; Figure 4E). Because of the high water content of the matrix replacing the polymer, there was likely processing-induced swelling that made these footprints appear falsely larger by histology compared with OCT. Minimal calcification was present around all struts (100%, 76 of 76; Figure 4H). Preexisting struts were completely sequestered within a
fibromuscular neointima, with no to minimal inflammatory cells (macrophages, multinucleated giant cells) being immediately associated with the struts (Figure 4D).

At 3 years, 23 histological images with 195 struts were analyzed. One hundred eleven strut footprints were recognized as a defined accretion of hyaline (proteinaceous) material separated by extracellular matrix (proteoglycans) and cells (Figure 5). The hyaline material was identified as nonfibrillar glycoprotein by transmission electron microscopy (Figure 5H). The majority of cells integrating into these sites did not stain positively for SMA (Figure 5G). In this histological classification, 74.8% of struts (83 of 111) showed calcification (Figure 5F). The remaining 84 areas previously occupied with struts were recognized as regions without hyaline material but with connective tissue with low to moderately cellular density, which indicated complete, benign involution of the struts into the arterial wall (Figure 6). In this morphology, cells replacing preexisting struts were irregularly arranged, and minimal calcification that was partially to completely circumferential around preexisting struts was observed around 36.9% of struts (31 of 84). For both morphologies, just as at 2 years, macro-
phages and multinucleated giant cells were noted only occasionally.

At 4 years, 36 histological specimens with 239 strut footprints were analyzed. Although 5 strut footprints remained as a defined accretion of hyaline material similar to what was observed at 3 years, the rest of the strut footprints were minimally discernible in histology as regions of integrated connective tissue (Figure 7). Again, as for 3 years,

Table 1. Results of OCT Evaluation in the Present Porcine Model and in the First-in-Humans ABSORB Trial

<table>
<thead>
<tr>
<th>OCT Findings</th>
<th>ABSORB Study Immediately After Implantation</th>
<th>ABSORB Study at 2 y†</th>
<th>Porcine Model at 28 d</th>
<th>Porcine Model at 2 y‡</th>
<th>Porcine Model at 3 y‡</th>
<th>Porcine Model at 4 y‡</th>
<th>P§</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVSS evaluated, n</td>
<td>7</td>
<td>7</td>
<td>4†</td>
<td>7</td>
<td>8</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>
| Mean discernible struts per BVS, n    | 58±19                                       | 38±30                | 41±27                | 60±20                | 28±9                 | 10±6                 | 0.002
| Strut appearance, %                  |                                            |                      |                      |                      |                      |                      | 0.001
| Preserved box                         | 100                                        | 3                    | 82                   | 80.4                 | 5.4                  | 0                    |
| Open box                              | 0                                          | 21.7                 | 18                   | 2.4                  | 16.1                 | 0                    |
| Dissolved bright box                   | 0                                          | 62                   | 0                    | 0                    | 34.8                 | 51.2                 |
| Dissolved black box                    | 0                                          | 13.3                 | 0                    | 17.2                 | 43.7                 | 48.8                 |
| Strut/vessel wall interaction, %      |                                            |                      |                      |                      |                      |                      | 0.298
| Strat apposition                      | 90.1                                       | 99.2                 | 100                  | 100                  | 100                  | 100                  |
| Strat alignment                       | 4.1                                        | 0                    | 0                    | 0                    | 0                    | 0                    |
| Incomplete strut apposition            | 5.8                                        | 0.8                  | 0                    | 0                    | 0                    | 0                    |
| Strut coverage, %                     |                                            |                      |                      |                      |                      |                      | 0.104
| Complete                              | 0                                          | 100                  | 99                   | 100                  | 100                  | 100                  |
| Incomplete                            | 0                                          | 0                    | 0                    | 0                    | 0                    | 0                    |
| Not visible                           | 100                                        | 0                    | 1                    | 0                    | 0                    | 0                    |
| Quantitative assessment, mm²          |                                            |                      |                      |                      |                      |                      | 0.001
| Lumen area                            | 6.53±0.91                                  | 5.80±1.13            | 2.87±1.28            | 5.13±0.84            | 4.87±1.14            | 6.85±1.25            |
| Stent area                            | 6.53±0.91                                  | NA                   | 5.73±0.56            | 5.92±0.83            | NA                   | NA                   |

The percentage was calculated or recalculated as follows: No. of struts in each category/total No. of discernible struts at that time point.

*Data in the matched 7 patients who underwent serial OCT assessment at baseline, 6 months, and 2 years.5,8
†In 2 of 4 pullbacks, struts were not visible because of the eccentric position of the catheter.
‡Although designated as struts, GPC analysis confirms that these structures were actually tissue that had replaced the preexisting struts.
§Comparison was made between 4 groups of the porcine model.

Figure 4. OCT images and corresponding histology at 2 years after BVS implantation. Struts conforming to the “preserved box” subcategory as visible by OCT (A and B) and corresponding histological photomicrographs (C through H). Locations of bioresorbed struts are readily visible in histological sections stained with standard hematoxylin and eosin, but material staining positively for Alcian Blue fills in the regions previously occupied by the struts (E, proteoglycan). In the location of bioresorbed struts, neither SMA (brown) nor collagen (red) is observed in SMA immunohistochemical staining (F) and trichrome staining (G). A small rim of calcification was observed in von Kossa staining, corresponding to the location of the PDLLA coating (H, black arrows).
only a paucity of cells within the strut footprints stained positively with SMA (Figure 7G). One hundred sixty-two struts presented as a circumscribed area of dense connective tissue with low cellularity in which cells are arranged in circumferential pattern. Of these, calcification was present in 16.0%, whereas cartilaginous metaplasia was found in 4.4%. Calcification when observed was minimal, typically being only scant linear foci associated with the connective tissue replacing struts. The remaining 72 strut footprints were recognized as poorly circumscribed regions of dense connective tissue with moderate to low cellularity in which cells were not regularly arranged. In this morphology, 16.7% of struts showed calcification (Figure 7H), and 4.2% demonstrated cartilaginous metaplasia. Overall, from 3 to 4 years, the frequency of strut footprints with calcification decreased significantly from 58.5% (114 of 195) to 17.2% (41 of 239; \( P < 0.001 \)).

Relative to inflammatory responses, the percentages of struts with granuloma were 13.8 ± 25.1%, 3.96 ± 6.93%, and 0.37 ± 1.11% at 1, 6, and 24 months, respectively; the percentages of struts with giant cells were 34.8 ± 20.5%, 14.7 ± 18.9%, and 1.6 ± 2.9%, respectively. At 3 and 4 years, no granuloma/ giant cells were observed in the area previously occupied with polymeric struts.

Comparison Between OCT and Histology
The comparison between OCT and histological classification at 2, 3, and 4 years in matched struts is summarized in Table 3. Of 510 struts analyzed by histology, 49 struts sites were excluded. Sixteen struts sites were not visualized with OCT because they were located out of range, and 33 struts were of poor imaging quality. The \( \kappa \) value of interobserver agreement was 0.58. In histology, the struts were classified into 5 categories, as shown in Figure 2.

In the strut footprints with histological category 1 (open acellular regions with well-defined borders), which is uniformly observed by histology at 2 years after procedure, 91.9% of strut footprints were recognized as the preserved box appearance by OCT (Figure 4). In 4.8%, the strut sites were recognized as dissolved black box owing to blurring of OCT image by artifacts. In the strut footprints with histological category 2 (a region with hyaline material separated by extracellular matrix and cells, which was observed frequently at 3 years and rarely at 4 years), 81.5% of strut footprints were classified as dissolved black and preserved box, respectively. In particular struts with preserved box appearance, histology showed that the strut footprints coalesced with the surrounding neointimal tissue, with demarcating lines of calcification delineating preexisting strut locations (Figure 5). In the strut footprints with histological category 3 (a region without hyaline material but with low to moderately cellular connective tissue observed at 3 years), 80.7% and 15.7% of strut footprints were classified as dissolved black and bright boxes, respectively. In struts with histological categories observed at 4 years, regions of strut sites were poorly discernible in OCT (category 4, 90.9% indiscernible; category 5, 92.3% indiscernible).
When predicting histological type from OCT classification, the preserved box corresponds to an open acellular region with hyaline material at 2 years (category 1) in 86.4% and less frequently to a region with accretion of hyaline at 3 years (category 2) in 12.1%. The open box appearance was observed uncommonly (8 struts, 1.7%) in this animal experimental model. The dissolved bright box appearance corresponds to 3-year histology: category 2 in 36% and category 3 in 52%. The dissolved black box also corresponds to 3-year histology but more frequently with category 2 (51.5%) than category 3 (39.2%). Indiscernibility with OCT accurately predicts 4-year histology (31.4% in category 4 and 68.6% in category 5).

**GPC Analysis**

GPC analysis demonstrated an expected decrease in the Mw, Mn, PDI, and content of polymer in the BVSs over time. The Mw and Mn of PLLA dropped by $\approx 100\%$, from 200 and 100 kDa at T₀ to unquantifiable at 2 years after implantation, and the polymer mass loss increased by $\approx 20\%$, $\approx 60\%$, and $\approx 90\%$ at 1, 1.5, and 2 years, respectively, becoming undetectable at 3 years (Figure 8). In addition, the polymer peak on the chromatograms obtained from BVS samples at 1, 1.5, and 2 years after stent implantation shifted to the right (lower Mw range) relative to the polymer peak on the chromatogram from samples at T₀, consistent with polymer degradation. In 24-month chromatograms, only a very small peak with a magnitude (S:N, $\approx 10:1$) close to the limit of quantification (0.3 mg/mL of PLLA, ie, 5.7% of the gravimetric weight [5.3 mg] of the stent at T₀) was observed. It is likely that the observed peak is attributed mainly to the trace tissue species (extracted together with the polymer), which may be superimposed on the polymer peak on the significant shift of the latter to the right as the polymer has degraded to very low molecular weight. Taking into consideration the limit of quantification of the GPC method used, the measurement uncertainty at the limit of quantification levels ($\pm 20\%$), and the possible constant systematic error caused by the interference of the tissue species, which can give rise to a false signal, the BVS can be considered to be fully resorbed at 24 months after implantation. Complete degradation was confirmed at 3 years, with polymer being undetectable (limit of detection, 0.1 mg/mL of PLLA, ie, 1.9% of the gravimetric weight of the stent at T₀ [5.3 mg]). Therefore, the structures visible by OCT and histology at 2, 3, and 4 years represent locations of bioresorbed BVS struts.

**Discussion**

In the present study, we describe the preclinical experience using BVSs in a porcine coronary artery model. Twenty-eight days after BVS implantation, on OCT, 82% of the struts had the preserved box appearance, which corresponded to intact polymeric struts. Two years after BVS implantation, OCT images demonstrated that 80% of the struts had the preserved box appearance, which corresponded to intact polymeric struts. Two years after BVS implantation, OCT images demonstrated that 80% of the struts still had a preserved box appearance. Nonetheless, the corresponding histological images and polymer degradation assessment with GPC showed that the polymeric struts could, for all intents and purposes, be considered to be fully resorbed and replaced by proteoglycan. Three years after BVS implantation, OCT images demonstrated that only 5% of the struts retained a preserved box appearance, whereas 44% had evolved to a...
dissolved black box appearance. In addition, at the 3-year follow-up, we observed on OCT dissolved black boxes that, on histology, reflected infiltration of the sites of preexisting struts by connective tissue cells. At 3 years, the absolute count of discernible struts was reduced by \( \approx 50\% \), and the no-longer-discernible strut footprints on OCT seem to correspond in histology to complete integration into the surrounding arterial wall. Furthermore, at 4 years, only one sixth of the struts were discernible in OCT with a dissolved black or dissolved bright box appearance, which were minimally discernible in histology. Relative to prediction of histological type by OCT appearance, the preserved box appearance of OCT corresponds well with 2-year histology (86.4%), whereas dissolved bright and black box appearance corresponds well to 3-year histology (88.0% and 90.7%, respectively). Indiscernible struts in OCT can confirm the integrated strut footprints seen at 4 years (100%).

**Histological Response After BVS Implantation**

The present study suggests the following temporal progression after implantation of this PLLA/PDLLA BVS as viewed with histology. First, the polymeric struts are covered by a

**Table 2. Summary of Qualitative Histology at 1 Month and at 2, 3, and 4 Years**

<table>
<thead>
<tr>
<th>Time After Procedure</th>
<th>Struts, n</th>
<th>Frequency of calcification, n (% in category)</th>
<th>Frequency of cartilaginous metaplasia, n (% in category)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 1 mo</td>
<td>49 (100)</td>
<td>42 (86)</td>
<td>0</td>
</tr>
<tr>
<td>At 2 y</td>
<td>76 (100)</td>
<td>76 (100)</td>
<td>0</td>
</tr>
<tr>
<td>At 3 y</td>
<td>111 (56.9)</td>
<td>83 (74.8)</td>
<td>0</td>
</tr>
<tr>
<td>At 4 y</td>
<td>239</td>
<td>12 (16.7)</td>
<td>3 (4.2)</td>
</tr>
<tr>
<td>Frequency of calcification, n (% in category)</td>
<td>42 (86)</td>
<td>76 (100)</td>
<td>83 (74.8)</td>
</tr>
<tr>
<td>Frequency of cartilaginous metaplasia, n (% in category)</td>
<td>0</td>
<td>0</td>
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</table>
fibromuscular neointima. Second, struts are replaced by proteoglycan matrix, which corresponds to resorption demonstrated by GPC analysis. Third, there is inspissation of the proteoglycan matrix, and connective tissue cells infiltrate the region of the preexisting struts. Fourth, the areas of preexisting struts become fully integrated into the arterial wall and are difficult to discern. Strictly speaking, the process of “biodegradation” finishes at the second phase. Subsequent phases represent an “integration” process of the struts within the arterial wall; ultimately, the locations of preexisting struts become indiscernible on histology.

Of note, a thin rim of calcification around a majority of struts at the tissue interface was observed on histology at 1 month and at 2 and 3 years. This precipitation of calcium phosphate might be the result of benign, localized drop in pH caused by acidic polymer degradants at the strut-tissue interface.11 However, this is not unique to BVS or PLA but has been observed with bioprosthetic and synthetic materials.12,13 In the present study, the total amount of calcification is minimal and is demonstrated to be largely resolved at 4 years after implantation.

### Assessment of the Biodegradation Process by OCT
In the first-in-humans trial using the BVS, the OCT classification of the 4 strut appearances was developed to characterize the process of biodegradation.5,8 Immediately after implantation, the polymeric struts appeared on OCT as clear-cut boxes lying on the vessel wall rather than being embedded (Figure 3). The disappearance of this box appearance was considered to be the initial stages of the biodegradation process because it was no longer seen at 6 months.5,8 The first optical change of this box appearance in the ABSORB trial was the “opening” of the extremities of the box in its short axis, which was considered a first sign of biochemical or histological alteration (Figure 1), observed in 34% of struts at 6 months and still in 14% at 2 years. The dissolved black box

### Table 3. Comparison of OCT Classification and Histological Classification in the Matched Cross Sections at 2, 3, and 4 Years

<table>
<thead>
<tr>
<th>Histology Category*</th>
<th>OCT Classification</th>
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</thead>
<tbody>
<tr>
<td>1: An open acellular region with well-defined borders</td>
<td></td>
<td>Preserved Box</td>
<td>Open Box</td>
<td>Dissolved Bright</td>
<td>Dissolved Black</td>
<td>Indiscernible</td>
<td>Total</td>
<td></td>
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<tr>
<td></td>
<td>n</td>
<td>57</td>
<td>1</td>
<td>1</td>
<td>3</td>
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<td>62</td>
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<tr>
<td></td>
<td>Within histological category, %</td>
<td>91.9</td>
<td>1.6</td>
<td>1.6</td>
<td>4.8</td>
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<td>100.0</td>
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<tr>
<td></td>
<td>Within OCT category, %</td>
<td>86.4</td>
<td>12.5</td>
<td>4.0</td>
<td>1.8</td>
<td>0.0</td>
<td>13.4</td>
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</tr>
<tr>
<td>2: A region with hyaline material (proteinaceous) separated by extracellular matrix (proteoglycans) and cells</td>
<td>n</td>
<td>8</td>
<td>3</td>
<td>9</td>
<td>88</td>
<td>0</td>
<td>108</td>
<td></td>
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<tr>
<td></td>
<td>Within histological category, %</td>
<td>7.4</td>
<td>2.8</td>
<td>8.3</td>
<td>81.5</td>
<td>0.0</td>
<td>100.0</td>
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<tr>
<td></td>
<td>Within OCT category, %</td>
<td>12.1</td>
<td>37.5</td>
<td>36.0</td>
<td>51.5</td>
<td>0.0</td>
<td>23.4</td>
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<tr>
<td>3: A region without hyaline material but with low to moderately cellular connective tissue</td>
<td>n</td>
<td>0</td>
<td>3</td>
<td>13</td>
<td>67</td>
<td>0</td>
<td>83</td>
<td></td>
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<tr>
<td></td>
<td>Within histological category, %</td>
<td>0.0</td>
<td>3.6</td>
<td>15.7</td>
<td>80.7</td>
<td>0.0</td>
<td>100.0</td>
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<tr>
<td></td>
<td>Within OCT category, %</td>
<td>0.0</td>
<td>37.5</td>
<td>52.0</td>
<td>39.2</td>
<td>0.0</td>
<td>18.0</td>
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<tr>
<td>4: A poorly circumscribed region of dense connective tissue with moderate to low cellularity in which cells were not regularly arranged</td>
<td>n</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>60</td>
<td>66</td>
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<tr>
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<td>Within histological category, %</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>4.5</td>
<td>90.9</td>
<td>100.0</td>
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<tr>
<td></td>
<td>Within OCT category, %</td>
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<td>12.5</td>
<td>4.0</td>
<td>1.8</td>
<td>31.4</td>
<td>14.3</td>
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<tr>
<td>5: A circumscribed region of dense connective tissue with low cellularity</td>
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<td>0</td>
<td>1</td>
<td>10</td>
<td>131</td>
<td>142</td>
<td></td>
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</tr>
<tr>
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<td>Within histological category, %</td>
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<td>0.0</td>
<td>0.7</td>
<td>7.0</td>
<td>92.3</td>
<td>100.0</td>
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<tr>
<td></td>
<td>Within OCT category, %</td>
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<td>0.0</td>
<td>4.0</td>
<td>5.8</td>
<td>68.6</td>
<td>30.8</td>
<td></td>
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<tr>
<td>Total</td>
<td>n</td>
<td>66</td>
<td>8</td>
<td>25</td>
<td>171</td>
<td>191</td>
<td>461</td>
<td></td>
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<tr>
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<td>Within histological category, %</td>
<td>14.3</td>
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<td>37.1</td>
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<tr>
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<td>Within OCT category, %</td>
<td>100.0</td>
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<td>100.0</td>
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<td>100.0</td>
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*Histological category corresponds to Figure 2.
appearance and dissolved bright box appearance were considered to reflect further stages of bioresorption and vessel wall integration. The inability to discern the strut footprints on OCT was interpreted as a sign of complete bioresorption. At 2 years, 36.3% of the struts were indiscernible by OCT in humans. So far, no attempt has been made to interpret these human OCT observations by correlating OCT and histological observation in an animal model.

In contrast to what was initially thought after the first-in-humans BVS trial and according to the present animal study, the preserved box appearance does not exclude the resorption of polymeric struts. The polymer was only no longer accurately quantifiable by GPC at 2 years after BVS implantation, but the structures observed with OCT and histology represented tissue-derived regions corresponding to the location of the former BVS struts. In other words, the preserved box appearance with optical translucency is compatible with complete polymer dissolution.

Assessment of Integration Process by OCT

Although OCT might not be sensitive enough to evaluate the degradation process of the polymer, it may provide more detailed information on the integration process. In the present study, the transition from the preserved box appearance (at 2 years) to the full disappearance of the strut footprints on OCT or the presence of dissolved black box (at 3 years) appears to be related to the replacement of the preexisting struts by proteoglycan and dense connective tissue, respectively. Although the demarcation of the preexisting struts becomes undetectable with OCT imaging, by histology, the struts ultimately coalesce with the arterial wall. This suggests that changes in strut appearances on OCT mirror the integration process of the struts. In a few cases, a demarcating line of calcification seems to be the sole histological remnant of the preexisting strut, and it is still unclear whether this thin line of calcification would be seemingly detectable on OCT (Figure 7).

In humans, we have reported the changes in ultrasound backscattering using gray-scale assessment and backscattering of radiofrequency signal.\textsuperscript{5,7–8} We have also demonstrated the disappearance of the echogenic signature of the polymeric struts with time.\textsuperscript{14} The ultrasonic change in signal was characterized by the disappearance of brightness (gray-scale) and pseudodense calcium (virtual histology), whereas with light assessment, the black appearance (resulting from the homogeneity of the polymeric material) of the struts shortly after stent implantation is progressively replaced by light backscattering undistinguishable from the backscattering of tissue background.

Figure 8. Top, Representative chromatograms of PLA polymer samples before BVS implantation (A) and 1 (B), 1.5 (C), 2 (D), and 3 (E) years after BVS implantation and of extraction solvent (F). Peaks: 1 to 4 PLA polymer. Bottom, Molecular weight loss estimated from GPC data. Molecular weight loss percentage is $\sim 70\%$ and $>90\%$ at 12 and 18 months, respectively, and reaches $98\%$ to $100\%$ at 24 months.
Inflammatory Reaction

Recently, Jiang et al.\textsuperscript{15} demonstrated in vitro and in vivo studies that a strong correlation exists between the rate of material degradation and the degree of inflammatory response to implanted material. In Swiss Webster mice with polymeric disks implanted into the peritoneum, the PLLA disk, which has longer degradation time compared with the mixed polymer of PLLA and PEG, has the least associated inflammation. However, in a rat study by Polimeni et al.,\textsuperscript{16} when the PLA device implanted subcutaneously came in contact with bones, the polymer triggered a foreign body reaction, including multinucleated giant cells, macrophages, and lymphocytes. Experts on biodegradable polymer have repeatedly emphasized the importance of the locus of the implant on its rate of degradation and the inverse relationship between rates of biodegradation and the presence of an inflammatory process.\textsuperscript{15} In the present histological study, relative to the degree of completeness of bioresorption of BVS, there was no significant inflammatory response associated with BVS at 2, 3, or 4 years, although a likely short-term inflammatory response during the active resorption phase of the scaffolds was not evaluated as part of this study.

Differences Between Human and Porcine Coronary Artery

At the 2-year follow-up, the distribution of OCT strut appearances observed in the porcine animal model differed from the results of the first-in-humans trial (Table 1). More specifically, the open box appearance and dissolved bright box appearance observed in 21.7% and 62.0% of struts in the ABSORB trial were scarcely observed at 2 years in the present animal study (open box, 2.4%; dissolved bright box, 0%). Furthermore, in the ABSORB trial, the number of discernible struts per device was 38±30 at 2 years, whereas in the present animal model, 60±20 strut voids per device were still discernible at 2 years. This suggests a slower integration process of the strut voids filled with proteoglycans in porcine arteries than in human arteries.

One possible explanation is the absence of underlying atherosclerotic plaque with or without inflammatory component in the implanted lesion of the animal model. The vascular response of aged, human atherosclerotic arteries is distinct from that of juvenile, healthy animal arteries. In some human cases, the deployed polymeric struts are presumably the slower hydrolysis time of the polymeric strut from the ends of each chain or from the presence of free residual monomer in the polymer matrix. The polymeric BVS coronary system used in the first-in-humans trial has greater monomer content in its polymer than the device used in the present animal study. These facts may explain the faster integration process observed in the first-in-humans trial than in the present animal study.

Study Limitations

The limitations of this study are the following. The number of specimens examined was small. The sampling rate of histological slices from each scaffold is insufficient to describe the 3-dimensional distribution of strut bioresorption/integration. In addition, as discussed above, differences between the healthy porcine coronary artery and diseased human coronary artery could limit the ability to generalize the hypothetical concepts put forward in this report.

Conclusions

The present porcine animal study potentially elucidates the histological responses after implantation of the BVS: bioresorption and integration. OCT might be more sensitive for assessing the integration process rather than the polymer alteration. Ultimately, the absence of the BVS device footprint on OCT suggests complete integration of the struts into the arterial wall.

Acknowledgments

We thank Jim Yu for his intellectual input into the statistical analyses.

Disclosures

J.C. Powers and Drs Perkins, Kamberi, and Rapoza are employees of Abbott Vascular. Dr Virmani has been on the advisory boards for Medtronic Vascular and Valve, Abbott Vascular, and BVS; has been a consultant to W.L. Gore and Atrium Medical Corp; and has received honoraria from Medtronic, Abbott Vascular, W.L. Gore, Terumo, and Atrium Medical Corp. The other authors report no conflicts.

References


CLINICAL PERSPECTIVE

Alterations of the reflectance characteristics of the everolimus-eluting bioresorbable vascular scaffold struts have been reported in humans. However, in the absence of histology, the interpretation of the appearances of the struts by optical coherence tomography (OCT) remains speculative. In the present animal study, the bioresorbable vascular scaffold (3.0×12.0 mm) was implanted in the coronary arteries of pigs that underwent OCT and then were examined by histology immediately or at 28 days or 2, 3, or 4 years after implantation. To assess the biodegradation, gel permeation chromatography was performed. At 2 years, OCT showed that 80.4% of the strut sites had a box-shaped appearance. On histology, these structures were composed of proteoglycan. By gel permeation chromatography, the polymeric material of bioresorbable vascular scaffold was no longer quantifiable, suggesting complete bioresorption. At 3 years, by OCT, most struts showed dissolved appearances (dissolved black, 43.7%; dissolved bright, 34.8%). Histology showed connective tissue cells within a proteoglycan-rich matrix, signifying the beginning of the integration process. At 4 years, only a few struts were recognizable by OCT, and on histology, struts indiscernible by OCT are also minimally detectable, which suggests complete integration. In summary, despite their defined appearance by OCT, struts at 2 years were largely bioabsorbed, and at 4 years, struts indiscernible by OCT corresponded well to the completely integrated struts. OCT might be more sensitive to assess the integration process rather than the polymer alteration. This animal study will potentially serve as a guide for interpretation of OCT after bioresorbable vascular scaffold implantation in the clinical setting.
Intracoronary Optical Coherence Tomography and Histology at 1 Month and 2, 3, and 4 Years After Implantation of Everolimus-Eluting Bioresorbable Vascular Scaffolds in a Porcine Coronary Artery Model: An Attempt to Decipher the Human Optical Coherence Tomography Images in the ABSORB Trial


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