
Supplemental Methods
Experimental Model
Fifty non-atherosclerotic healthy Yorkshire Crossbred Farm Swine (≤3-months) and 86 Yucatan Mini Swine (6- to 42-months) underwent device implantation, where both the Absorb™ bioresorbable vascular scaffolds (Absorb) and XIENCE V® (XV) were inflated at a steady rate of inflation to a maximum pressure within the range of 5 to 20 atmospheres to the appropriate diameter. The typical times for complete expansion were generally between 5 to 10 seconds for both Absorb and XV. The pressure at maximum expansion was then maintained for up to 30 seconds. Some arteries implanted with 3.0 x 18 mm (1- to 6-months) devices had a second comparable inflation performed in the proximal region in order to ensure proper device apposition proximally. One Yucatan mini swine for 42-months was euthanized at 40-months due to health issues; however, the vascular responses observed in this animal was comparable to other animals examined at 42-months, and therefore the animal was included into the 42-months group.

Quantitative Coronary Angiography (QCA)
Arteries were evaluated at implant and at follow-up for device migration and for quantitative assessment; angiograms (Siemens AXIOM- Artis, Munich, Germany) were acquired at implant and at the designated time of follow-up and were analyzed using a PC-based QCA workstation
The pre-implant, expanded delivery balloon, post-implant and follow-up lumen diameters were measured using the internal digital calipers of the fluoroscope with the guiding catheter serving as a reference for calibration. The balloon to artery ratio \( \text{B:A: balloon diameter / pre-implant mean luminal diameter [MLD]} \) was calculated for each artery. At follow-up, the MLD was measured and late lumen loss was calculated. Percent diameter stenosis \( \% \text{DS} \) was also calculated as \( \frac{(\text{post-implant MLD} - \text{follow up MLD})}{\text{post-implant MLD}} \times 100 \). The results of pre- and post-implant MLD are being reported in a separate manuscript.\(^1\)

**Scanning Electron Microscopy (SEM)**

SEM was used to evaluate the extent and maturation of surface re-endothelialization of implanted arteries. The percentage of endothelium was based on a visual estimate. In brief, the implanted vessels were bisected longitudinally and photographed. Specimens were post-fixed in 1\% osmium tetroxide, dehydrated in graded series of ethanol solutions, critical point dried, and sputter-coated with gold as previously described.\(^2\) All specimens were visualized using a Hitachi 3600N scanning electron microscope.

**Pharmacokinetic (PK) Evaluation**

PK evaluation was conducted at 3 hours and at 1-, 3-, 7-, 14-, 28-, 60- and 90-days post implant, with three animals being evaluated per time point (Table 1) utilizing methods that have been previously published.\(^3\) Blood samples were collected in tubes containing \( \text{K}_2\text{EDTA} \) as anticoagulant and stored frozen until analysis.
Everolimus concentration in arterial tissue and in blood was determined using Liquid Chromatography / Mass Spectrometry (LC/MS). An arterial tissue segment was homogenized in 2 mL extraction solution (5mM ammonium acetate in 75% acetonitrile in water) containing rapamycin as internal standard (IS). After sonication and centrifugation, an aliquot of the supernatant was injected onto a reverse-phase HPLC column for drug quantification by LC/MS analysis. The limit of quantification for everolimus in the extraction solution was 0.5 ng/mL. Everolimus concentration in arterial tissue was determined by dividing the total amount of drug in extraction solution by the tissue weight. To determine drug concentration in blood, 300 µL whole blood was mixed with 30 µL dilution solution (50:50 MeOH/H$_2$O) and 600 µL of precipitation solution (80% MeOH in water with 0.225M ZnSO$_4$) containing rapamycin as internal standard. After vortex and centrifugation, the supernatant from the mixture was injected onto a reverse-phase HPLC column for drug quantification by LC/MS analysis. The limit of quantification for the drug in whole blood was 0.1 ng/mL.

**Gel Permeation Chromatography (GPC)**

A previously reported GPC method, with a slightly modified sample extraction/purification process, was employed to investigate the degradation of polymer over time by evaluating the weight-average molecular weight (Mw), number-average molecular weight (Mn), polydispersity index (PDI) and the content of polymer in the Absorb.$^4$ In the present method, the extraction and purification of the polymer was repeated up to five times until the polymer was fully extracted from the tissue (i.e., the polymer signal in the last extract below the quantitation limit of 0.3 mg/mL). The samples were analyzed prior to device implantation (T0) and at 28 days, 3-, 6-, 12-, 18-, 24-, 30-, 36- and 42-months after implantation. The Mw, Mn, and PDI of polymer were
calculated from the calibration curves obtained for polystyrene standards (Mw range, 500–700,000 Da), whereas the amount of polymer was calculated from the calibration curves constructed by five calibration standards (0.3–4.0 mg/mL) of polylactide (PDLLA) certified reference material with a Mw of 90,000 Da.

References


