Endothelial Cell Recovery, Acute Thrombogenicity, and Cell Adhesion Assessments of Fluorinated Copolymer and Phosphorylcholine Polymer

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Statement of Purpose:
The safety of drug-eluting stents (DES) has been a heated topic because of the potential for late and very late stent thrombosis (LST) in a small subset of patients.[1-4] While the increased incidence of LST with DES is multifactorial, delayed vascular healing, and specifically delayed re-endothelialization, is one of the mechanisms contributing to occurrence of LST.[3-5] Current preclinical models, including the rabbit iliac arterial model, have shown value for evaluating the vascular safety of a DES system.[6-7] In order to better understand the factors that contribute to improve vascular healing after stenting, the components that make up a DES can be isolated and assessed independently. The use of polymers in current DES has been a topic of discussion as it relates to arterial healing and LST. Therefore, we sought to assess and to compare the effects of fluorinated copolymer (FP) and phosphorylcholine polymer (PC), two polymers used in Abbott's DES program, on vascular re-endothelialization, thrombogenicity, and cell adhesion using preclinical models.

Methods:
Iliac arteries of New Zealand White rabbits were treated by balloon denudation and implanted bilaterally with ML VISION™ stents (Abbott Vascular, Santa Clara, CA) coated with either FP or PC (without drug) at approximately the same thickness (~4.55 µm). Endothelial coverage at 14 days post-implantation was measured by morphometric analysis of images acquired through en face scanning electron microscopy (SEM) as well as dual fluorescent immunolabeling via confocal microscopy for platelet endothelial cell adhesion molecule-1 (PECAM-1) and thrombomodulin (TM) (n=6). Acute thrombogenicity was also assessed in an *ex vivo* Chandler loop model from stents deployed into tygon tubing in the presence of fresh heparinized porcine blood for 30 minutes at a pulsatile flow rate of 200 mL/min, where adhered thrombus weight was measured (n=10). In addition, *in vitro* cell adhesion assays were performed on glass coupon surfaces spin-coated with FP or PC using human umbilical vein endothelial cells (HUVEC), human coronary artery endothelial cells (HCAEC), or THP-1 monocytes (n=3). Lastly, the expression levels of a panel of inflammatory cytokines, including macrophage inflammatory protein-1 alpha and beta (MIP-1 alpha, MIP-1 beta, RANTES, interleukin-6 (IL-6), and monocyte chemoattractant protein 1 (MCP1), from adhered THP-1 monocytes after 24 hours was assessed from cell culture media using the Bioplex bead-based cytokine ELISA assay (Bio-Rad Laboratories, Hercules, CA) to compare FP and PC effects on adhered monocyte behavior (n=3).

Results:
Overall endothelialization was equivalent for FP and PC coated stents, as largely complete endothelial coverage occurred for both groups (>80% by SEM) by 14 days (**Figure 1**). Expression of PECAM-1 was also equivalent for FP as compared to PC, though both had less than 40% coverage, providing evidence of a transitional healing surface. Acute thrombus adherence was similar for FP, PC, and ML VISION™ control stents (clot weight ratio normalized to ML VISION™, FP = 0.99 $\pm$ 0.15, PC = 1.04 $\pm$ 0.17). The number of endothelial cells adhered to FP and control glass surfaces were equivalent and significantly greater than that adhered onto PC surfaces ($p<0.05$) in an *in vitro* cell adhesion model. In regards to THP-1 monocyte adhesion, no differences were observed among all surfaces. Secreted expression levels of MIP-1 alpha, MIP-1 beta, RANTES, IL-6, and MCP-1 from adhered THP-1 monocytes were also similar across all surfaces.

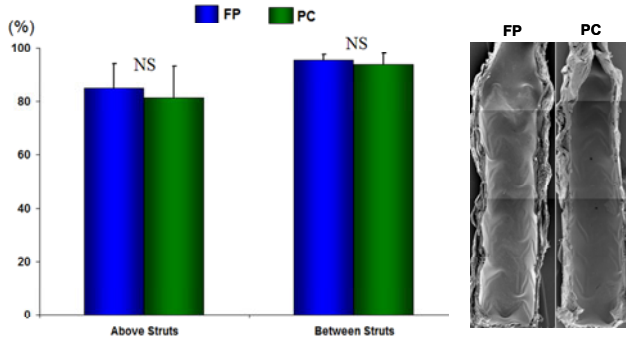

Figure 1 % Endothelial coverage above and between struts as assessed by SEM at 14 days in rabbit iliac arteries showed no significant (NS) differences between FP and PC coated stents.

Conclusions:
The data collected from *in vivo*, *ex vivo*, and *in vitro* preclinical models supports that both FP and PC are biocompatible and hemocompatible, which may be attributed to their own inherent properties. Other features of a DES, such as coating integrity, strut thickness, drug dose and release profile, and ease of good wall apposition, may be possible contributors to late stent thrombosis.

References:
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