REDUCED PROTEIN DEPOSITION:

A MECHANISM FOR IN VIVO PERSISTENCE OF HEPARIN BIOACTIVITY ON CBAS-ePTFE VASCULAR GRAFTS

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In Vivo Heparin Activity

- CBAS-heparinized grafts showed no statistical difference in activity over time ($p>>0.05$).
- Control-ePTFE grafts had minimal residual activity.

*Fig. 6. Mean heparin activities (pmol/cm²) measured as AT binding on replicate samples from CBAS-ePTFE and control ePTFE grafts explanted from canines at 1, 2, 4, 8, and 12 weeks. Error bars = ±1 sd.

Quantitate and compare *in vivo* protein deposition on the luminal blood interfaces of CBAS-ePTFE and CTRL-ePTFE vascular grafts in a canine model.
**AORTOILIAC MODEL**

**Canines**
- Greyhound (7 ♀ 3 ♂)
- 2 per time point

**Implant Times**
- 1, 2, 4, 8, 12 weeks

**Grafts**
- 6 mm x 12 cm
- 2 per dog

**Pharmacology**
- 81 mg ASA/day
- **No Heparin**
Sample Disc Diameter = 15.5 mm
- **Deproteination:** 2% SDS; 5% SDS
- **Treatment Method:** Plate Shaker
- **Treatment Time:** 8, 12, 48, 48 h

**SAMPLE DISCS**

- CBAS ePTFE
- CTRL-ePTFE

Discs Adhered To Plate Abluminal Side Down With TissueMend® II Surgical Adhesive
Bio-Rad Experion Micro-Fluidic Electrophoresis System
PROTEIN ANALYSIS

CBAS-ePTFE

Sample Peaks = 12
Protein = 1.51 mg/cm²
2% SDS - 8 h

2-wk

CTRL-ePTFE

Sample Peaks = 24
Protein = 8.64 mg/cm²
2% SDS - 8 h

2-wk

Representative Peak Profiles

Proximal Sample

Proximal Sample
PROTEIN ANALYSIS

CBAS-ePTFE

Sample 1

Sample Peaks = 15
Protein = 1.42 mg/cm²
2% SDS - 8 h

CTRL-ePTFE

Sample 5

Sample Peaks = 25
Protein = 3.43 mg/cm²
2% SDS - 8 h

Proximal Sample

Representative Peak Profiles
PROTEIN ABUNDANCE

Sig. Diff. (p<0.05)

μ ± 1 SD; n = 8

Implant Duration (wks)

# Proteins (μΣ(graft)^{-1})

CBAS-ePTFE
CTRL-ePTFE

µ ± 1 SD; n = 8
PROTEIN DENSITY

Protein Density (mg/cm$^2$)$^{-1}$

Implant Duration (wks)

1 2 4 8 12

Protein Density

CBAS-ePTFE
CTRL-ePTFE

µ ± 1 SD; n = 8

Sig. Diff. (p<0.05)
CONCLUSIONS

- CBAS-ePTFE affects *in vivo* protein deposition in the canine model.

- Protein Abundance (# proteins(graft)^-1) was significantly less on CBAS-ePTFE at all time points (p<0.05).

- Protein Density (mg protein(cm^2)^-1) was significantly less on CBAS-ePTFE at all time points p<0.05).
ONGOING WORK

Specific Protein Identification

• Conventional Electrophoretic Separation
  - Standard 1-D SDS-PAGE
  - 2-D (greater separation)

• MALDI-TOF Mass Spectrometry
  - Amino acid sequencing
  - Database comparison

• Western Blot Confirmation
  - Protein-specific antibodies
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