

# Polyphenol-eluting stent reduces restenosis and promotes vascular healing in a rat model of arterial angioplasty and stenting

Jim Kleinedler

LSU Health Sciences Center Shreveport

Department of Pharmacology, Toxicology, and  
Neuroscience

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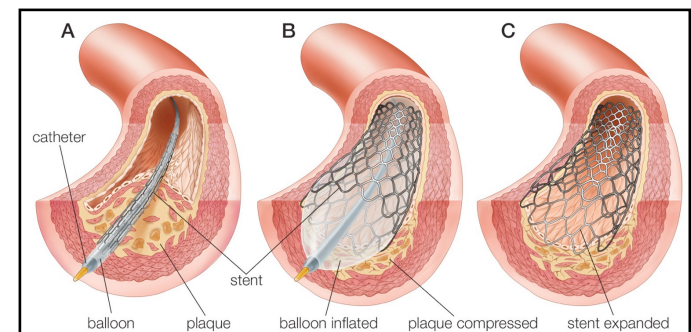
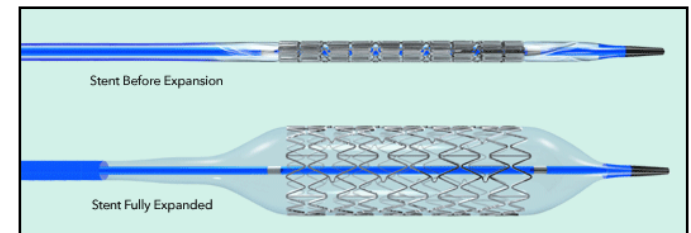
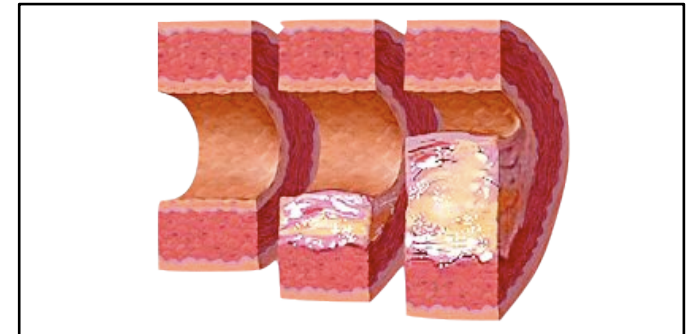


# Disclosure

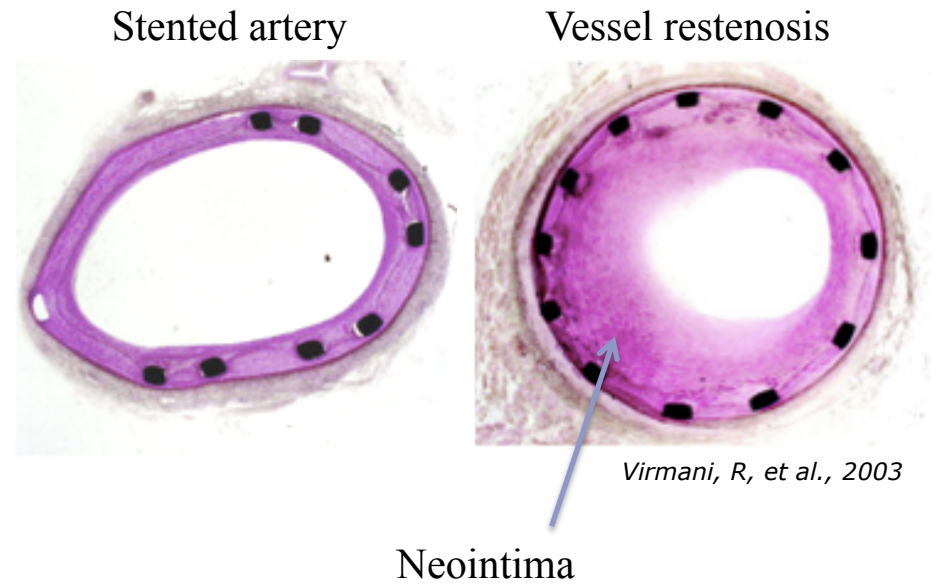
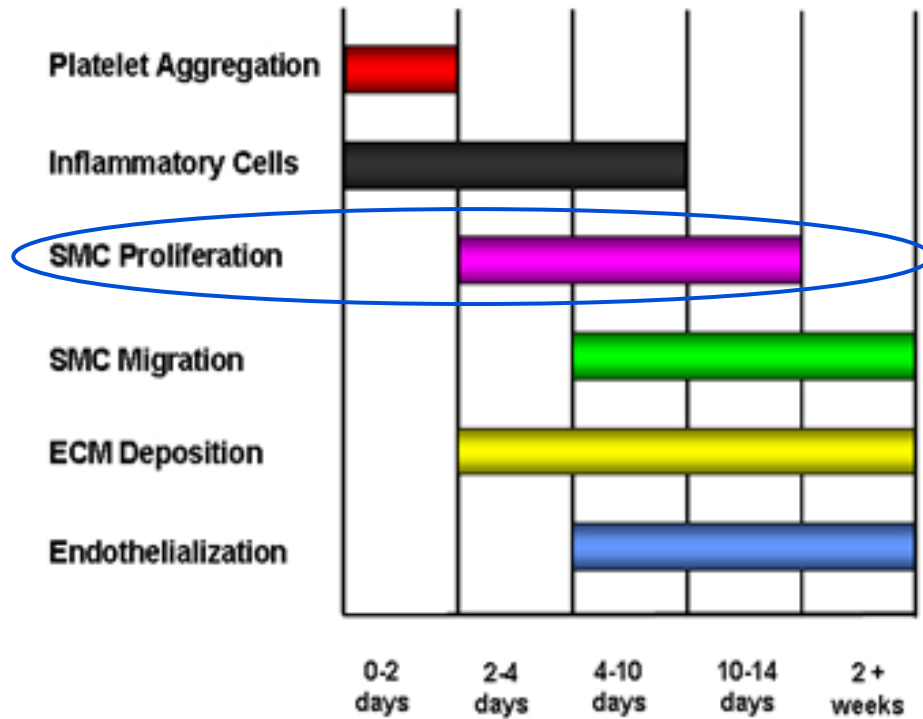
- Intellectual property
- ReQquisite Biomedical

# Stenting and Angioplasty

- Atherosclerosis
- Coronary heart disease is the single leading cause of death in America  
(Roger et al. Circulation 2011;123:e1-e192)
- Angioplasty is used to restore vessel patency
- 90% of angioplasty procedures involves the use of stents (Angioplasty.org, 2008)



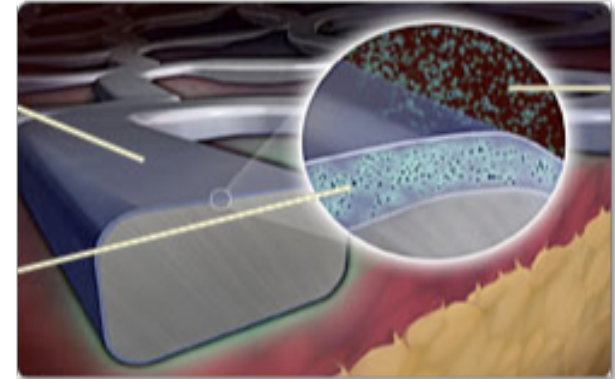
# Stenting and Restenosis





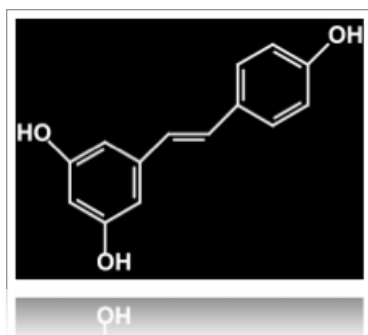
# Drug-Eluting Stents (DES)

- Cypher (Rapamycin)
- Taxus (Paclitaxel)
- Adverse outcomes associated with current generation DES
- Thrombosis due to delayed and incomplete healing after angioplasty (Virmani, 2004; Shuchman, 2006)
- Our approach...



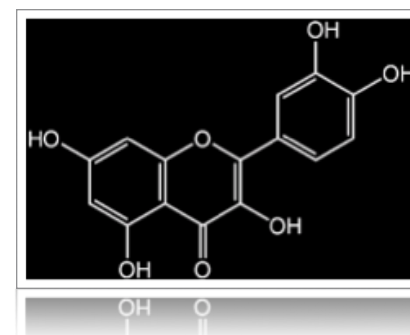
# Incorporate vascular-protective polyphenols into a coating for DES

## Resveratrol



- Oral dosing has been shown to **reduce restenosis** (Zou, J., et al., 2000)
- Oral RESV **accelerates re-endothelialization** in rats (J, Gu, et al., 2006)
- **Reduce inflammatory cell activation** (Csiszar A et al., 2006)

## Quercetin

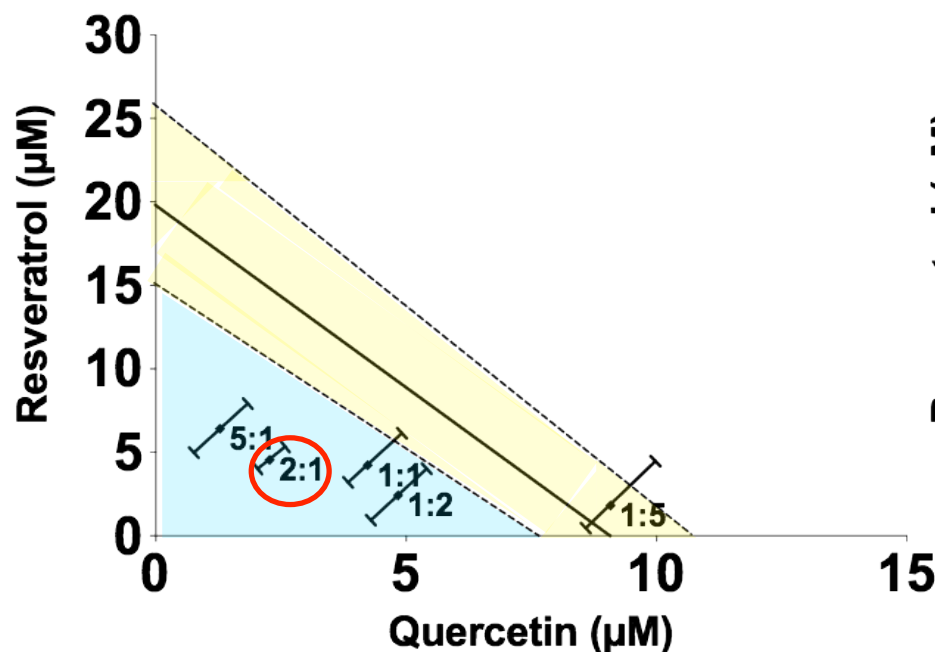


- Potent **inhibitor of platelet activation** (Hubbard GP, et al., 2004)
- Inhibits VSMC proliferation (Moon, S.K., et al., 2003)
- Strong **antioxidant** (Ishizawa, K., et al., 2009)

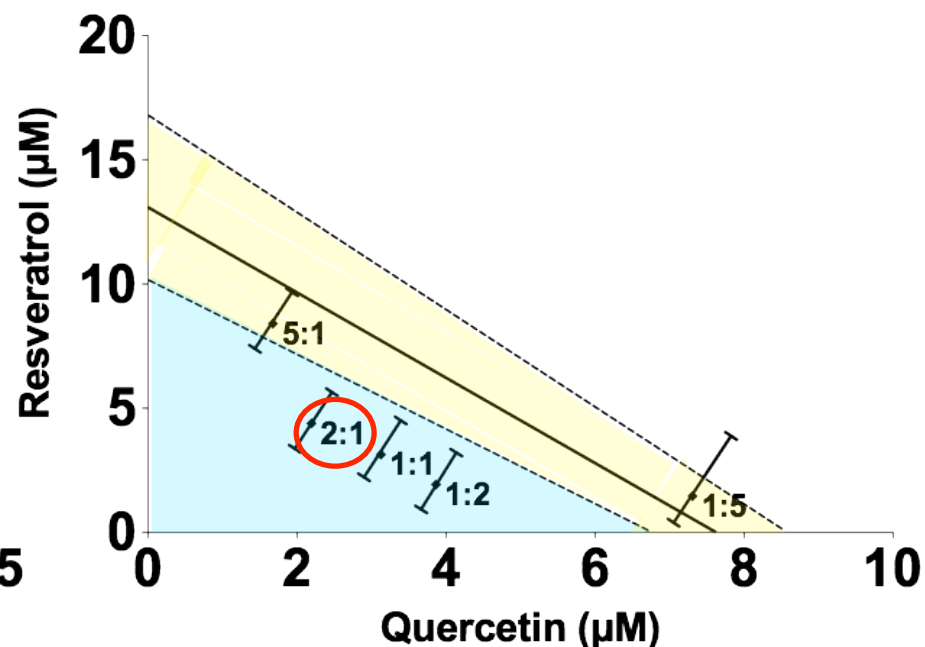
# What ratio of RESV and QUER gives the best response?

*RESV and QUER synergize in a 2:1 ratio*

Inhibition of VSMC Proliferation



Inhibition of Macrophage Activation

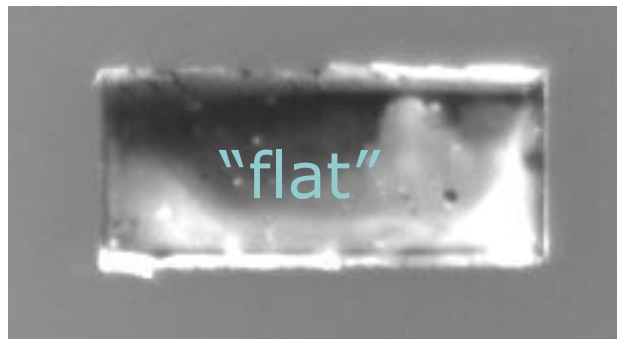


All isobologram ratios defined as RESV:QUER

Error bars represent 95% CI

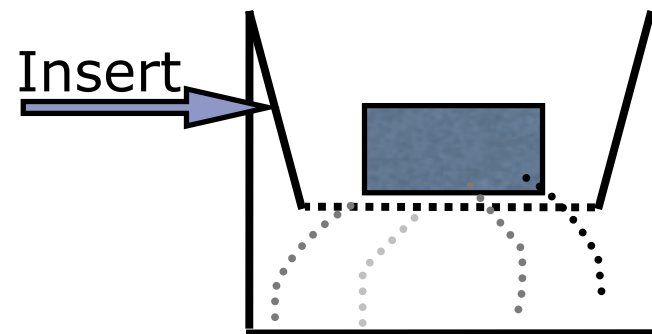
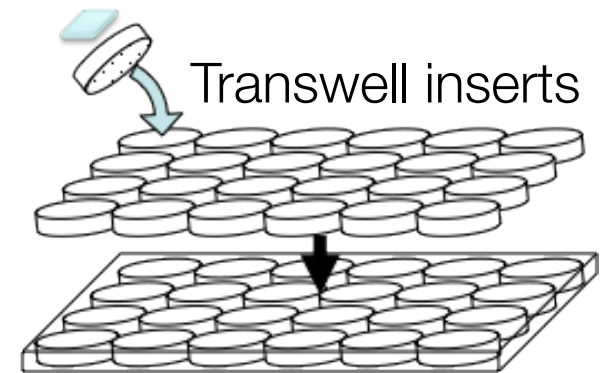
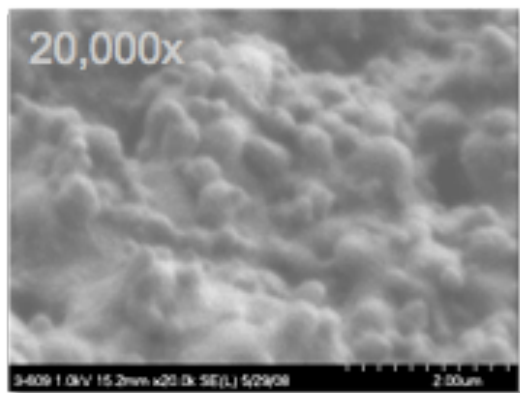
# Incorporating the drugs into a polymer coating

*Developing a surrogate stent surface for in vitro experiments*



0.75 cm

0.50 cm



*poly (styrene-isobutylene-styrene) triblock polymer*



# How much drug to load into a coating for DES?

## HD Combination

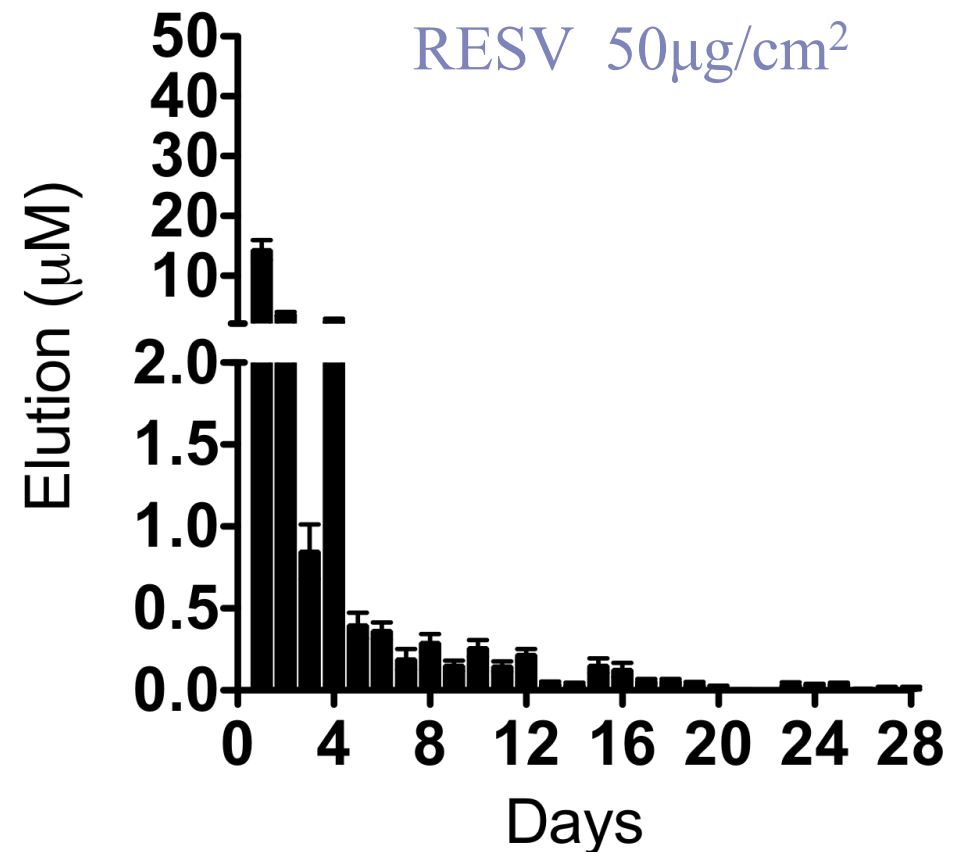
RESV  $100\mu\text{g}/\text{cm}^2$

QUER  $50\mu\text{g}/\text{cm}^2$

## LD Combination

RESV  $50\mu\text{g}/\text{cm}^2$

QUER  $25\mu\text{g}/\text{cm}^2$



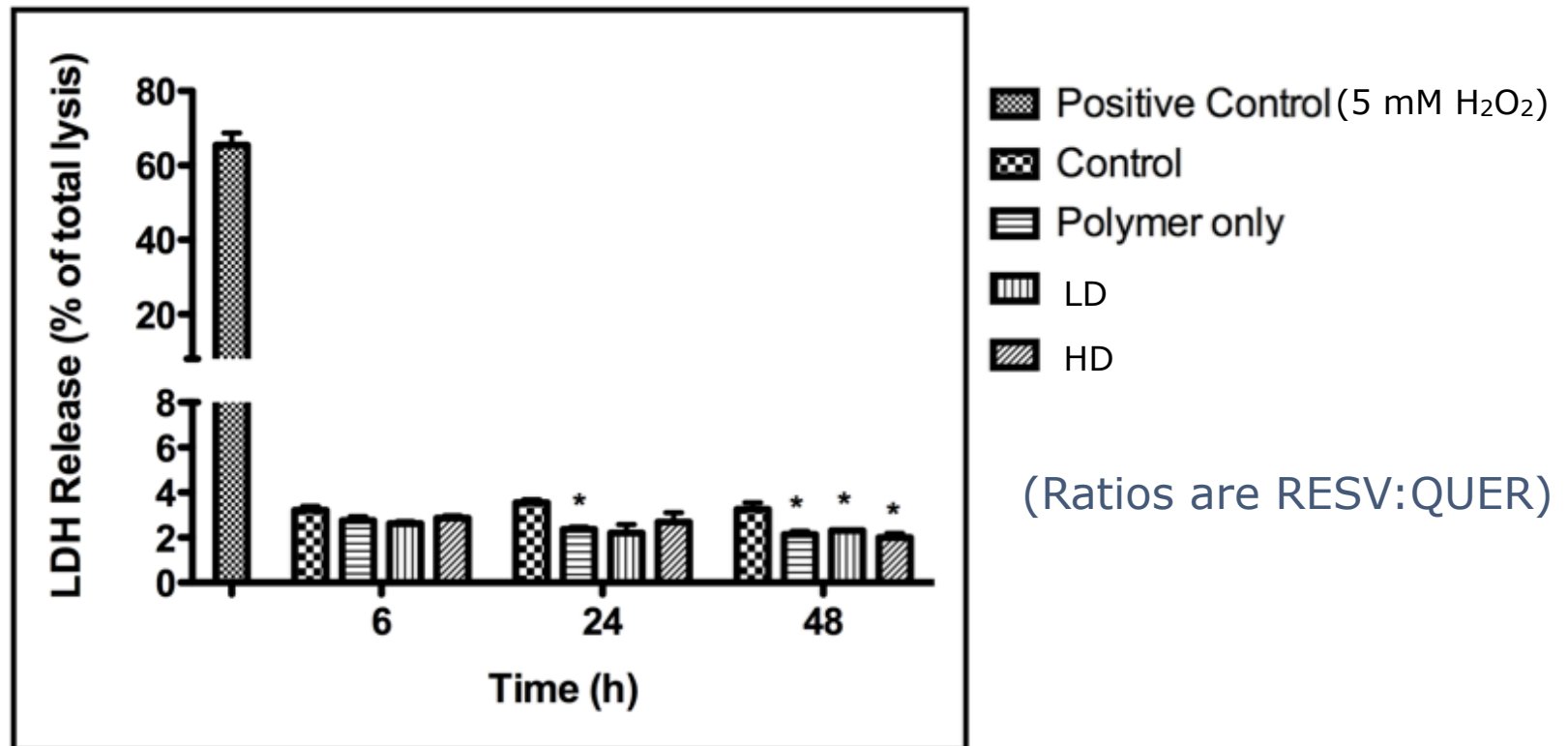
# RESV and QUER have a wide therapeutic window

Unpublished Data: Available Under CDA

VSMC toxicity assessed by LDH assay. Efficacy determined by inhibition of proliferation as assessed by BrdU incorporation assay. n = 3. Data presented as means  $\pm$  SE.

# No toxicity associated with drug-eluting coatings

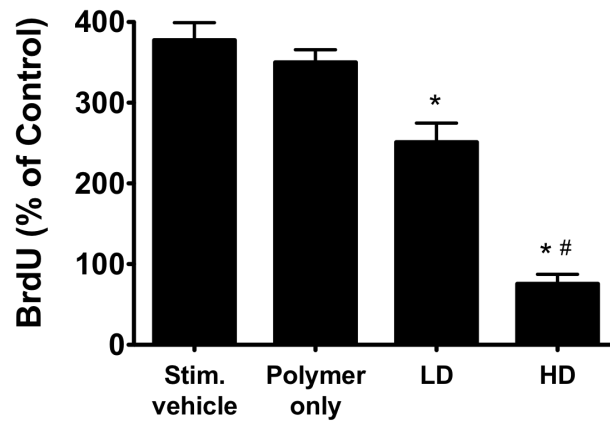
*Cytotoxicity assessed in VSMC*



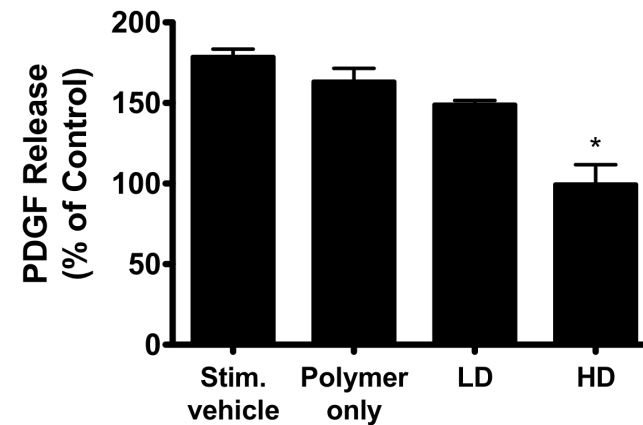
Error bars represent SEM. \* Indicates a significant difference from the control group.  $p < 0.05$ .  $n = 3$

# RESV and QUER show efficacy after elution from polymer coatings

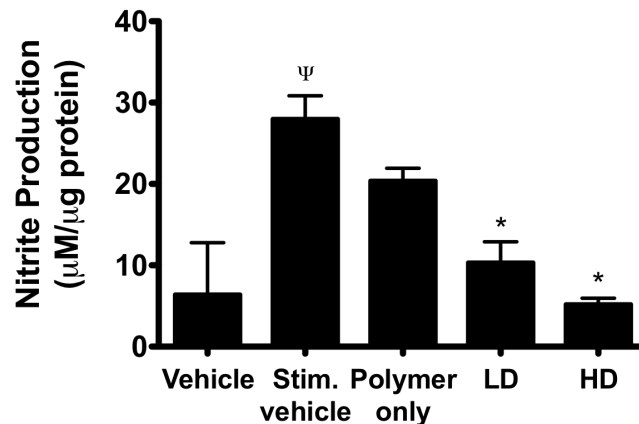
## VSMC Proliferation



## Platelet Activation



## Macrophage Activation

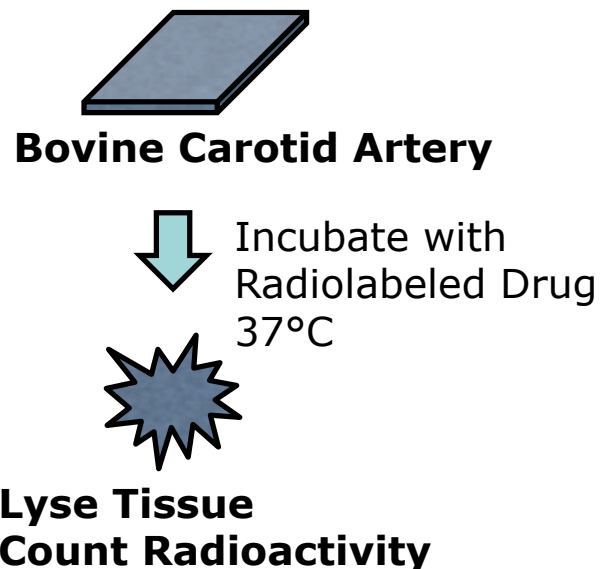


*Significant reduction in VSMC proliferation, macrophage activation, and platelet activation*

Error bars represent SEM. \* Indicates difference from the stimulated control.  $p < 0.05$ .  $n = 3$

# Implications of arterial transport on a RESV and QUER formulation for local delivery

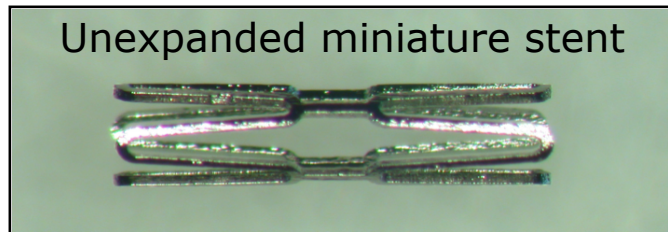
| Octanol:Water Partition Coefficient |      |      |
|-------------------------------------|------|------|
| Paclitaxel                          | Resv | Quer |
| 3.63                                | 3.14 | 1.48 |



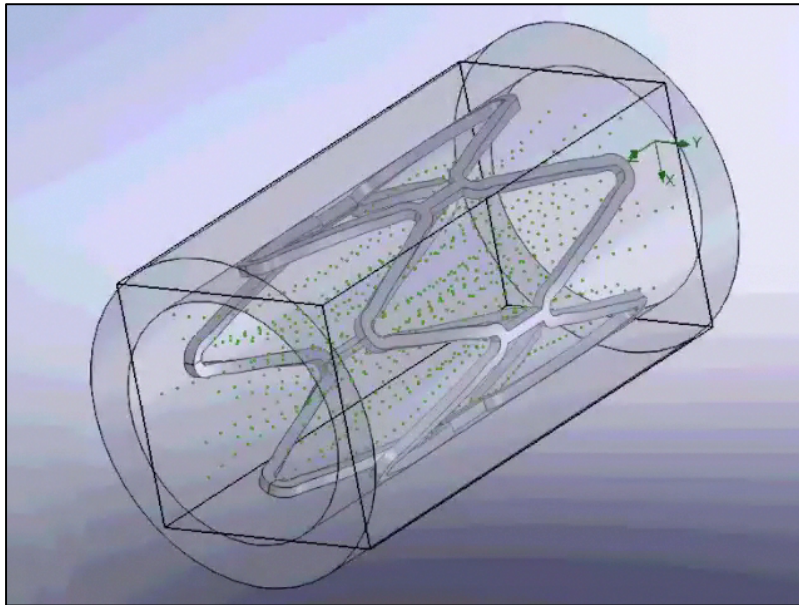
Unpublished Data:  
Available Under CDA



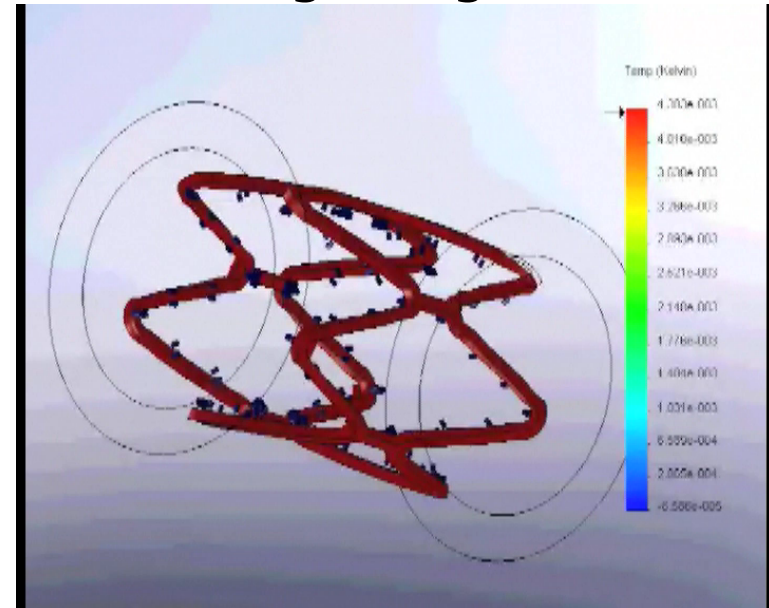
# Computational modeling to aid future coating development



Flow Simulation

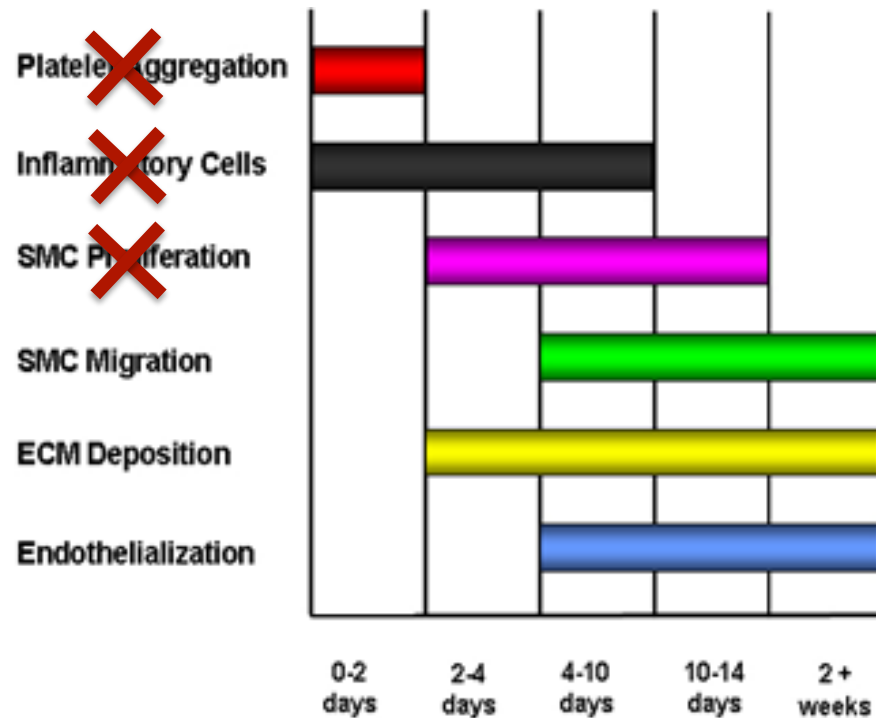


Modeling Drug Elution



Goal: Predict stent release profiles, tissue accumulation, and arterial residence time

## *in vitro* findings



- 2:1 ratio of RESV:QUER synergistically inhibits VSMC proliferation and macrophage activation
- Polymer coatings loaded with 2:1 ratios of RESV:QUER dose-dependently inhibited VSMC proliferation, macrophage and platelet activation
- Polymer coatings provided controlled release RESV:QUER out to 28 d

# Comparison: Preclinical and clinical trials

## Efficacy to reduce neointimal hyperplasia

| Drug                        | Rat | Porcine | Human |
|-----------------------------|-----|---------|-------|
| Statins                     | +   | +/-     | -     |
| ACE Inhibitors              | +   | -       | -     |
| Paclitaxel *                | +   | +       | +     |
| Rapamycin*                  | +   | +       | +     |
| Ca <sup>2+</sup> C Blockers | +   | +/-     | -     |
| <i>c-myc</i> Antisense*     | +   | +       | -     |
| Dexamethasone *             | +   | +       | +/-   |
| Heparin*                    | +   | +/-     | -     |

\* Indicate stent-based therapy. Others are provided systemically.

# A dietary approach to promote restenosis in a rat model of stenting angioplasty

## Objective

- Develop a modified rat model that shows greater face validity to human lesions than the current model

## Advantage

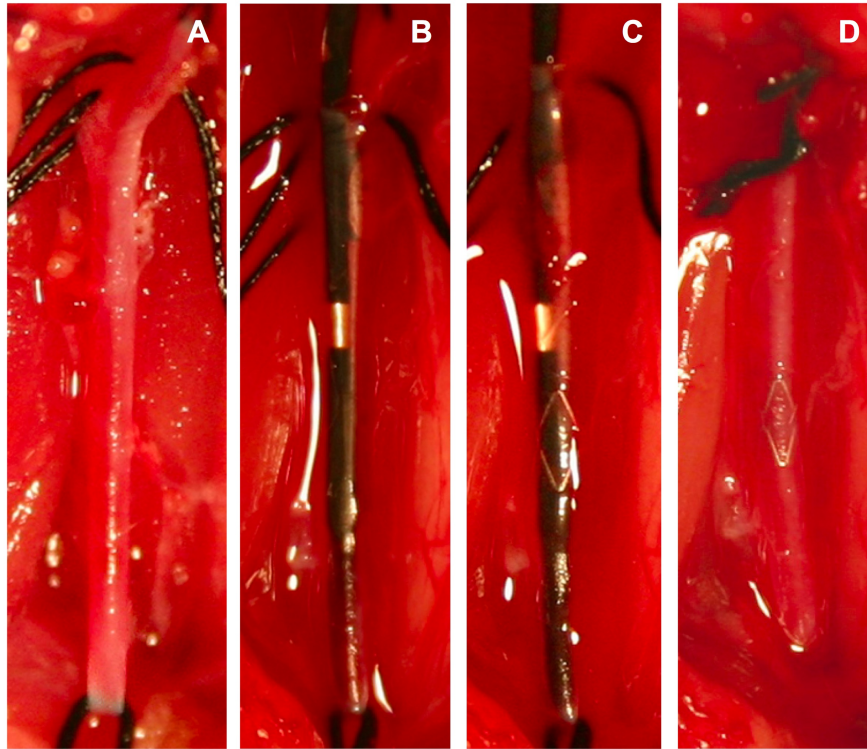
- Expedite the investigation of new therapies targeting restenosis

## Challenge

- Will increased face validity translate into increased predictive validity?



# Modified rat model of carotid artery stenting angioplasty



## Stenting Paradigm

1. Isolate carotid
2. Denude endothelium
3. Place stent
4. Restore blood flow

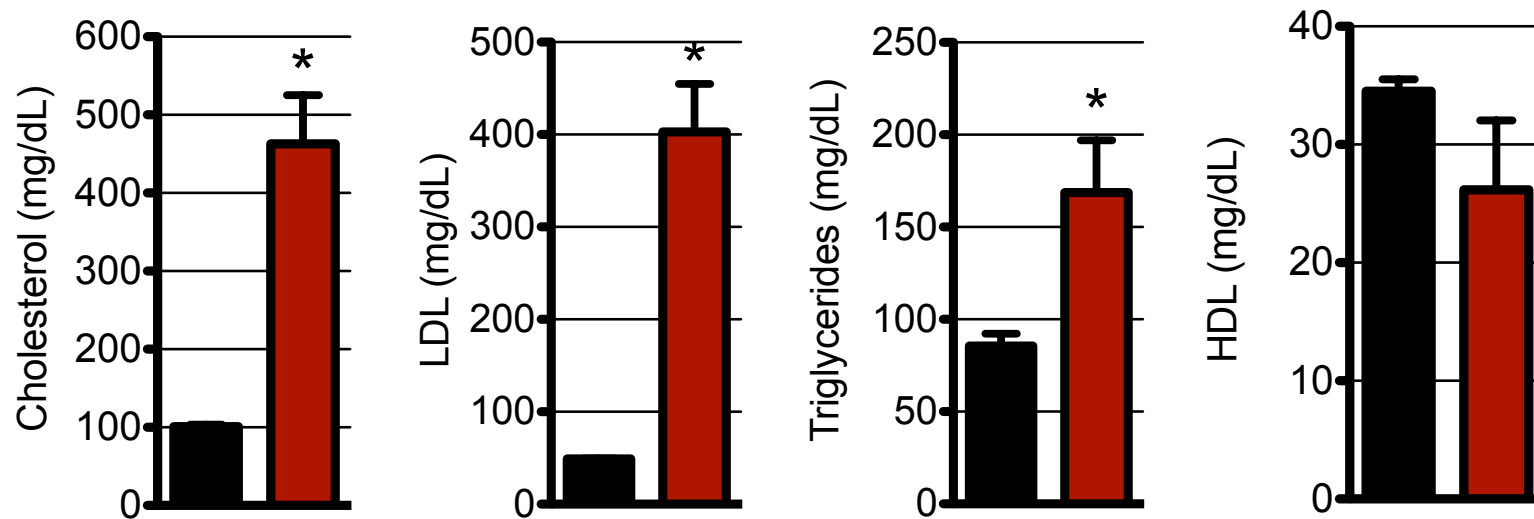


## Study Paradigm

1. Feed atherogenic diet
2. Sacrifice after 28d
3. Harvest carotid artery



# Addition of an atherogenic diet induces hyperlipidemia 4 weeks post-stent



Hypercholesterolemia  
Hypertriglyceridemia



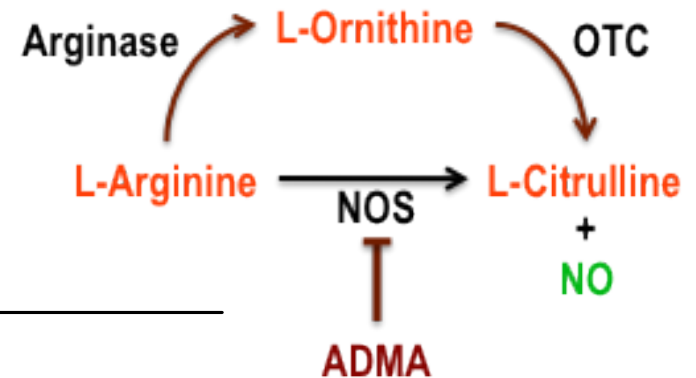
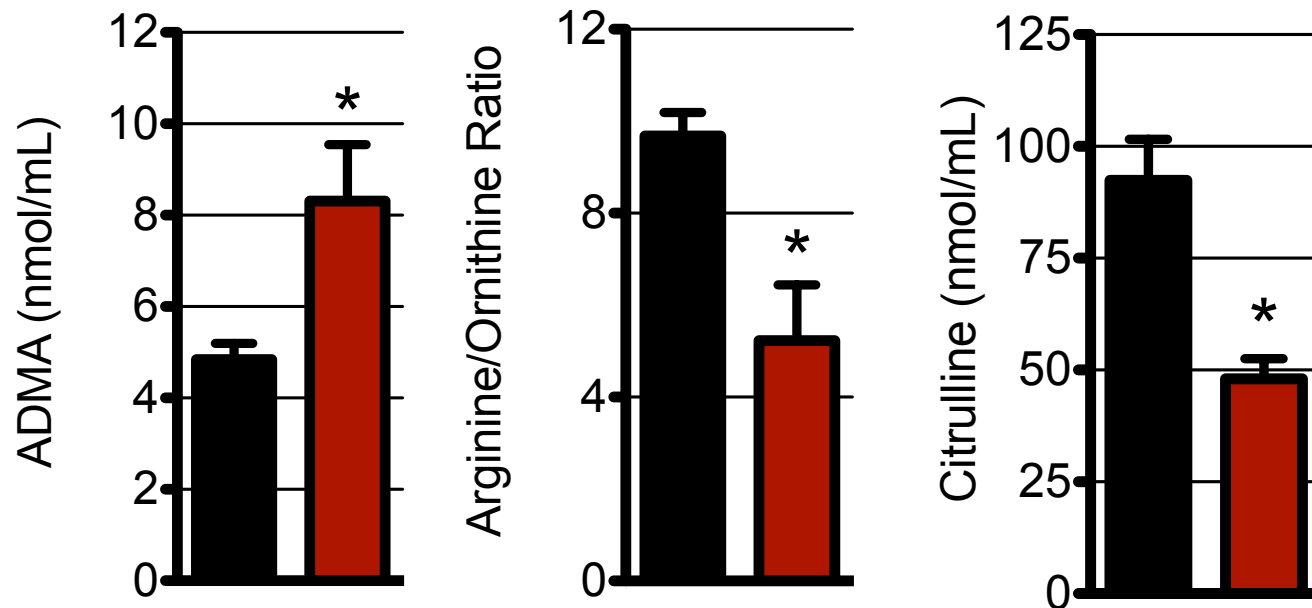
Risk factors known to exacerbate  
lesion formation

- Normal Chow
- Atherogenic Diet

Effect of treatment evaluated using a Student's t-test. \* $p < 0.05$ .  $n = 6$ .

# Atherogenic diet induces endothelial dysfunction 4 weeks post-stent

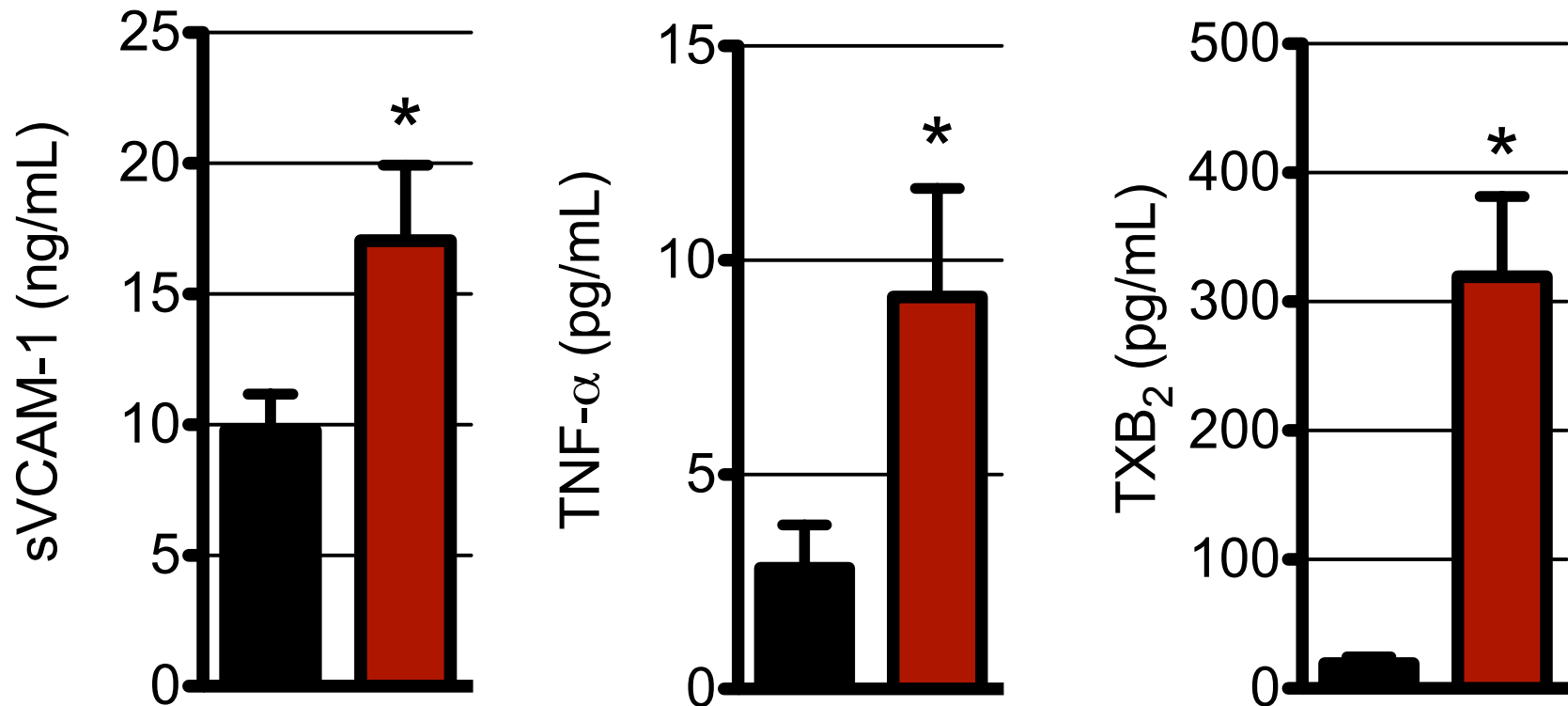
■ Normal Chow  
■ Atherogenic Diet



Quantification by HPLC at 28d post-stenting

Effect of treatment evaluated using a Student's t-test. \*p<0.05. n=6.

# Atherogenic diet increases inflammation and platelet activation 4 weeks post-stent



■ Normal Chow  
■ Atherogenic Diet

Quantification by ELISA at 28d post-stenting

Effect of treatment evaluated using a Student's t-test. \*p<0.05. n=6.

# Addition of atherogenic diet induces...

Hypercholesterolemia

Hypertriglyceridemia

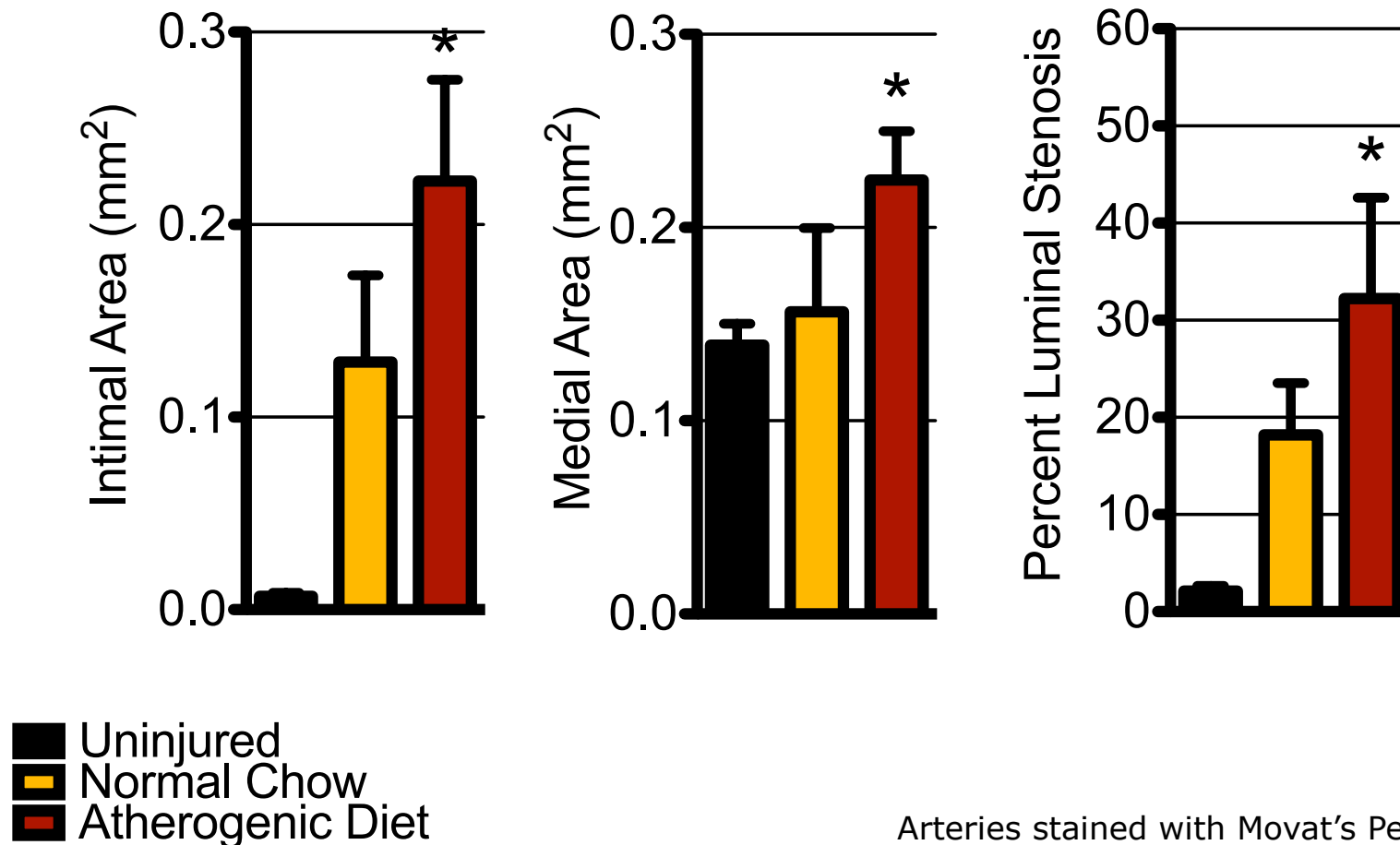
Endothelial Dysfunction

Adhesion Molecule Expression

Inflammation

Platelet Activation

# Atherogenic diet promotes stenosis at 4 weeks post-stent compared with traditional model

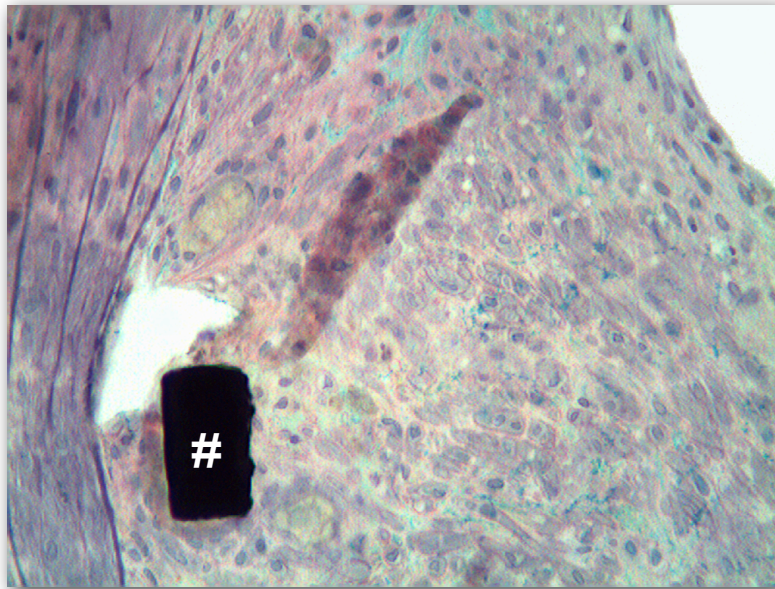


Arteries stained with Movat's Pentachrome.  
Morphometry using ImageJ software.

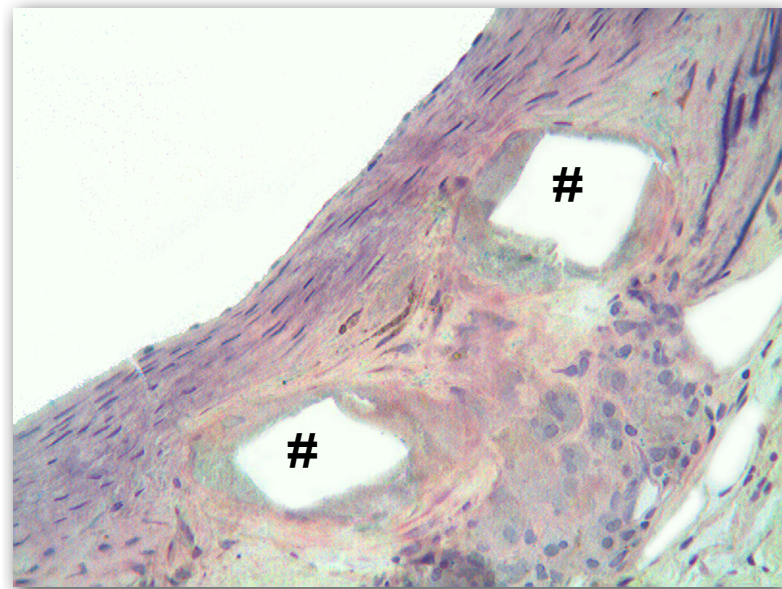
Effect of treatment evaluated using a one-way ANOVA. \* $p < 0.05$ .  $n = 6$ .



# Lesion morphology is altered in animals fed the atherogenic diet



Atherogenic diet



Normal chow

Black: Nuclei and elastic fibers

Yellow: Collagen

Proteoglycans: Blue

Fibrin: Intense red

Muscle: Red

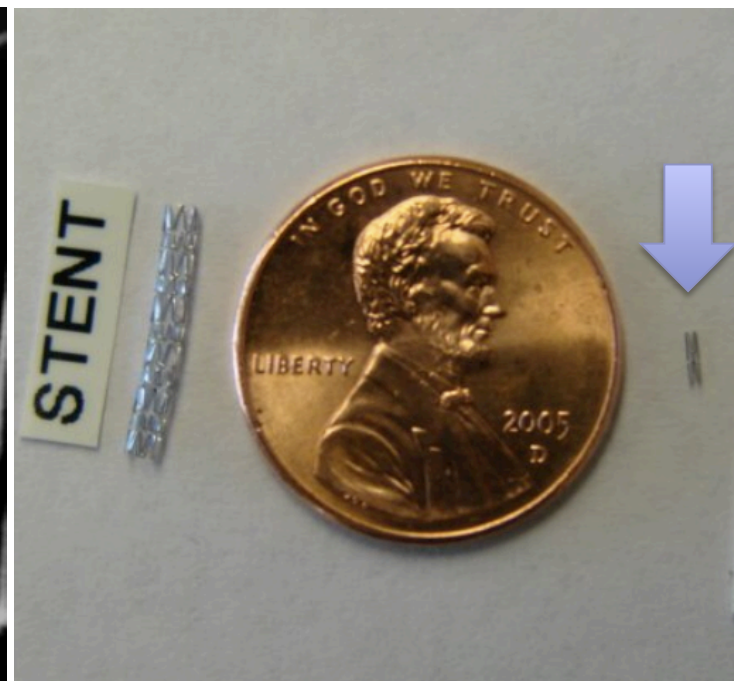
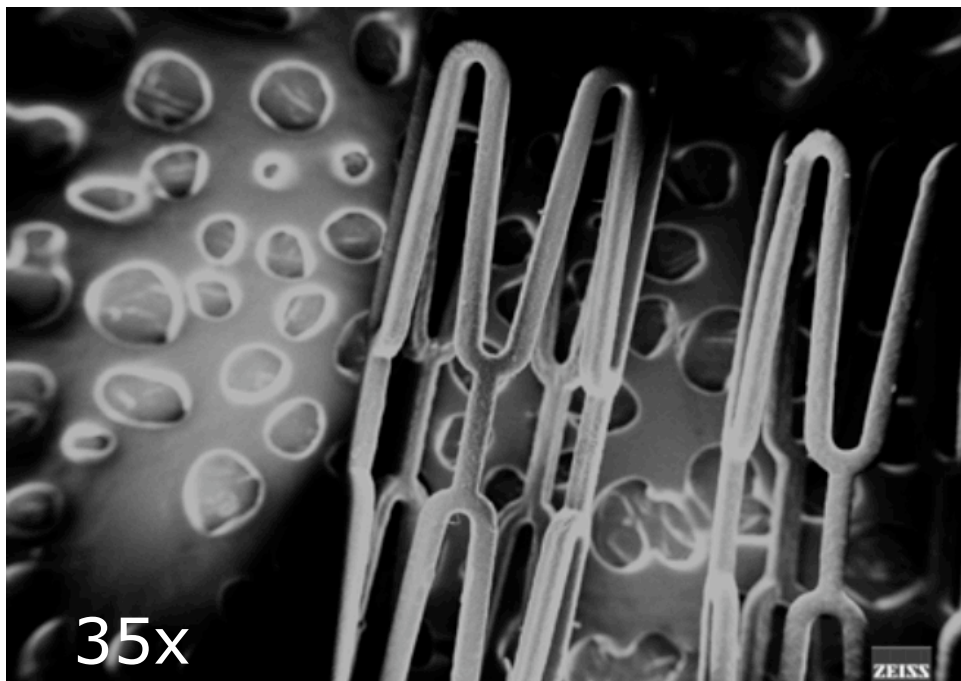
28 days after stenting angioplasty

## Addition of atherogenic diet increases the face validity of this model by:

- Exacerbating luminal stenosis
- Stimulating vascular inflammation
- Increasing sensitivity to thrombogenic stimuli
- Inducing endothelial dysfunction
- Promoting lesions that mimic certain histologic findings in human lesions

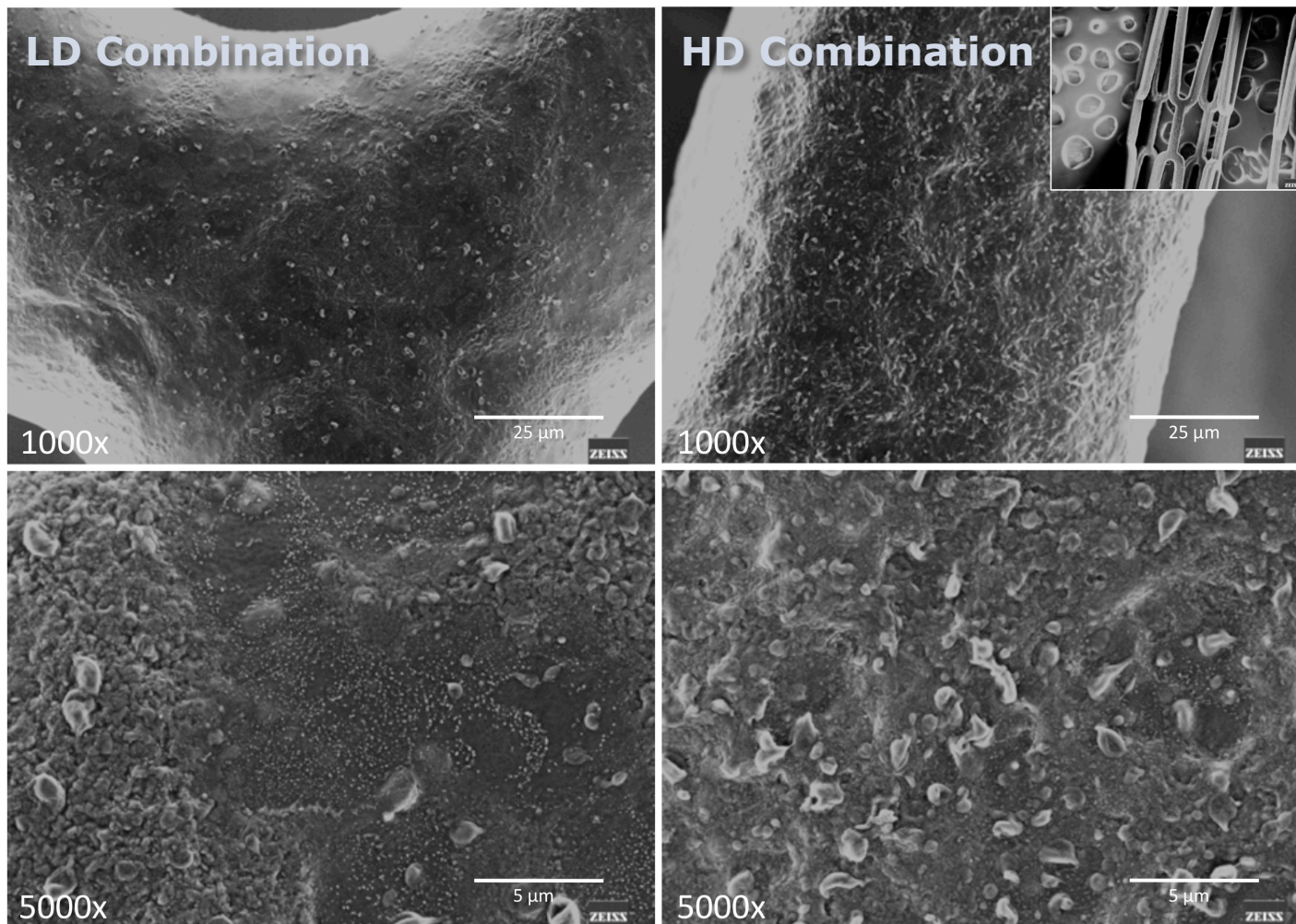
# Hypothesis

Stents providing controlled release of RESV and QUER will inhibit restenosis compared with bare metal stents

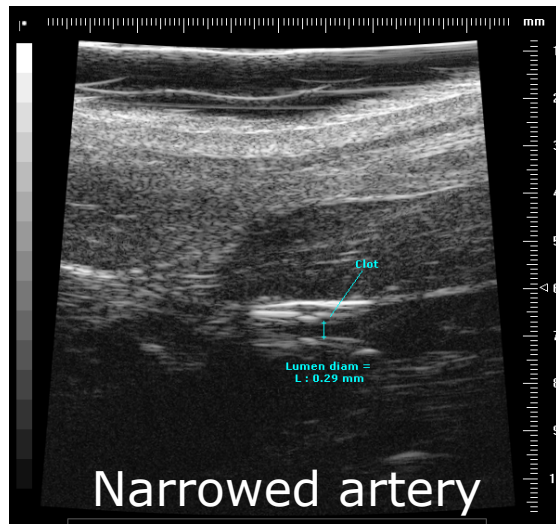
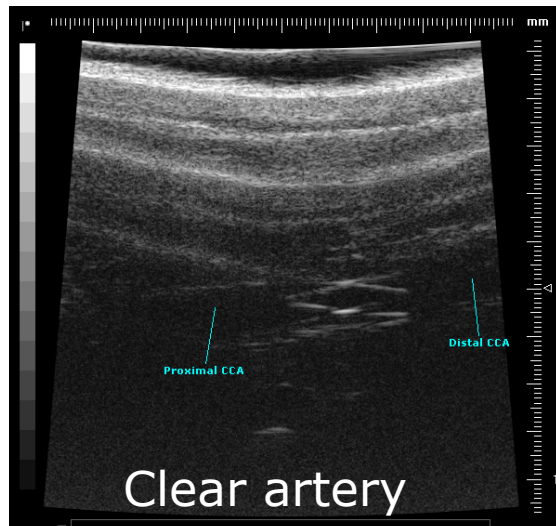




# ElectroNanospray produces a uniform stent coating



# Assessing restenosis via Doppler ultrasound



- Progressive restenosis of the carotid artery observed after stenting with BMS
- Significant reduction in restenosis mediated by HD coating as assessed by ICA/CCA flow changes

Unpublished Data:  
Available Under CDA

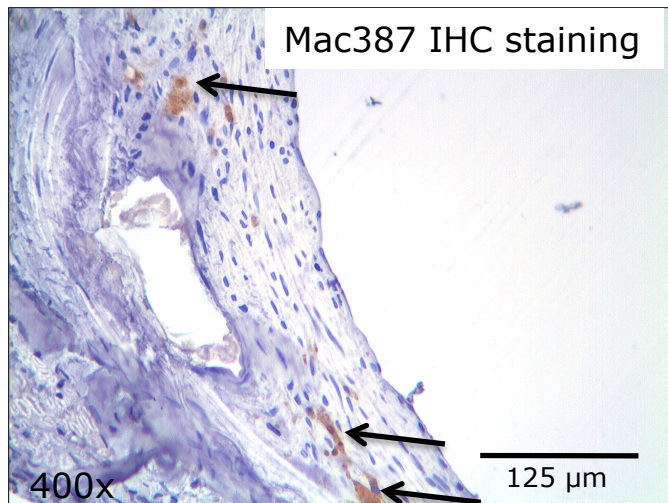
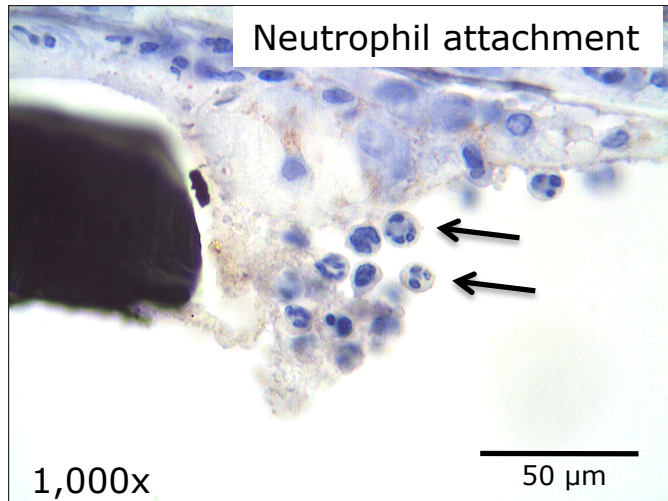


# Local delivery of RESV and QUER inhibits neointimal formation

Unpublished Data: Available Under CDA

Error bars represent SEM. \* Indicates a significant difference from uninjured arteries. # Indicates a significant difference from BMS group. n=6.  $p < 0.05$

# Polyphenol-eluting stent inhibits local inflammation

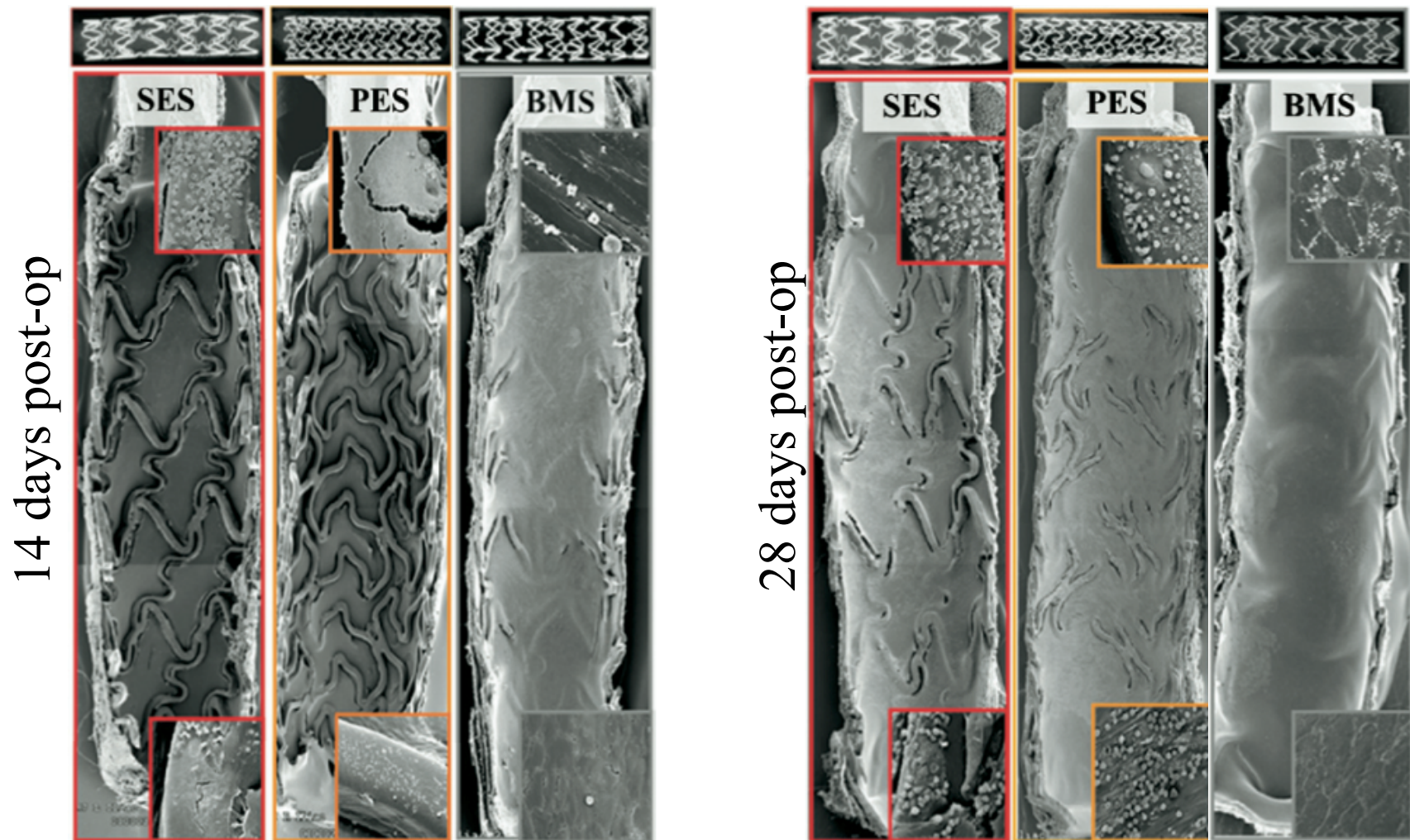


Unpublished Data:  
Available Under CDA

Data averaged from 9 equally-spaced cross-sections of the stent per animal. Error bars represent SEM. \* Indicates a significant difference from BMS as assessed by one-way ANOVA with Tukey's post hoc.

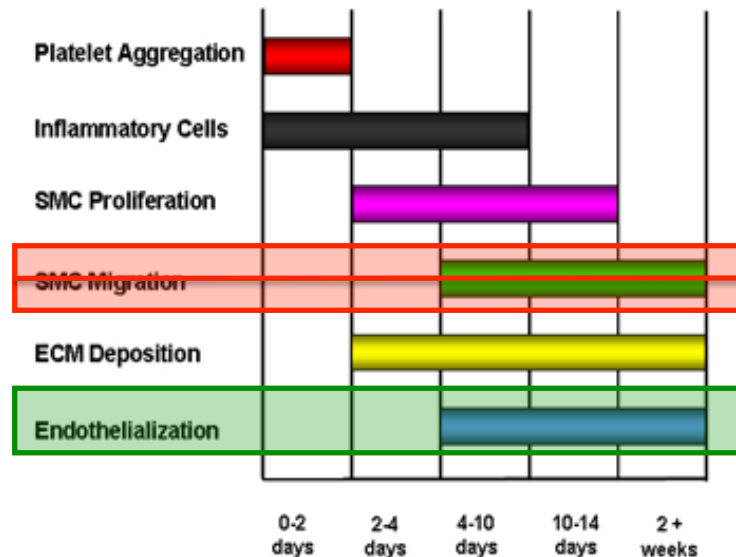


# The importance of developing a novel prohealing approach...



Joner M, Finn AV, Farb A, et al. Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. J Am Coll Cardiol 2006;48:193-202

# RESV and QUER show greater selectivity to inhibit VSMC wound closure



Unpublished Data:  
Available Under CDA

- VSMC simulated with 20 ng/mL PDGF-BB
- HUVEC stimulated with 25 ng/mL VEGF-165

Migration into wounded area assessed at 24 hours. n = 3. One-way ANOVA followed by Dunnett's post-hoc comparing all groups to stimulated vehicle. \*Indicates a significant difference from stimulated vehicle.  $p < 0.05$



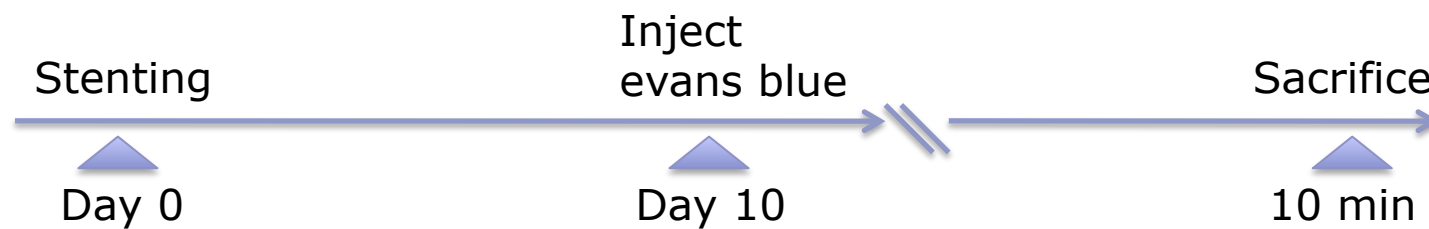
# Low-dose RESV promotes aortic endothelial cell wound closure under laminar shear stress

Unpublished Data: Available Under CDA

Migration into wounded area assessed at 18 hours. n = 3. Two-way ANOVA followed by Bonferroni's post-hoc. \*Indicates a significant difference from stimulated vehicle.  $p < 0.05$

# Hypothesis II

Stents providing controlled release of RESV and QUER will promote re-endothelialization compared with bare metal stents



# Polyphenol-eluting stent accelerates reendothelialization compared with BMS

Unpublished Data: Available Under CDA

Significant difference detected by two-tailed unpaired t-test. Data presented as mean  $\pm$  SE. n = 5.

# Conclusions

- Modified rat model may be useful in rapid evaluation of new therapies aimed at inhibiting restenosis
- ElectroNanospray generated a stent coating free of defects
- A stent coating containing combinations of RESV and QUER attenuated restenosis *in vivo*
  - Also reduced local inflammation
- RESV accelerates endothelial wound closure under LSS
- Local delivery of RESV and QUER promote reendothelialization after stenting injury



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# Questions