Mechanisms of DES healing - insights from proteomics and optical coherence tomography

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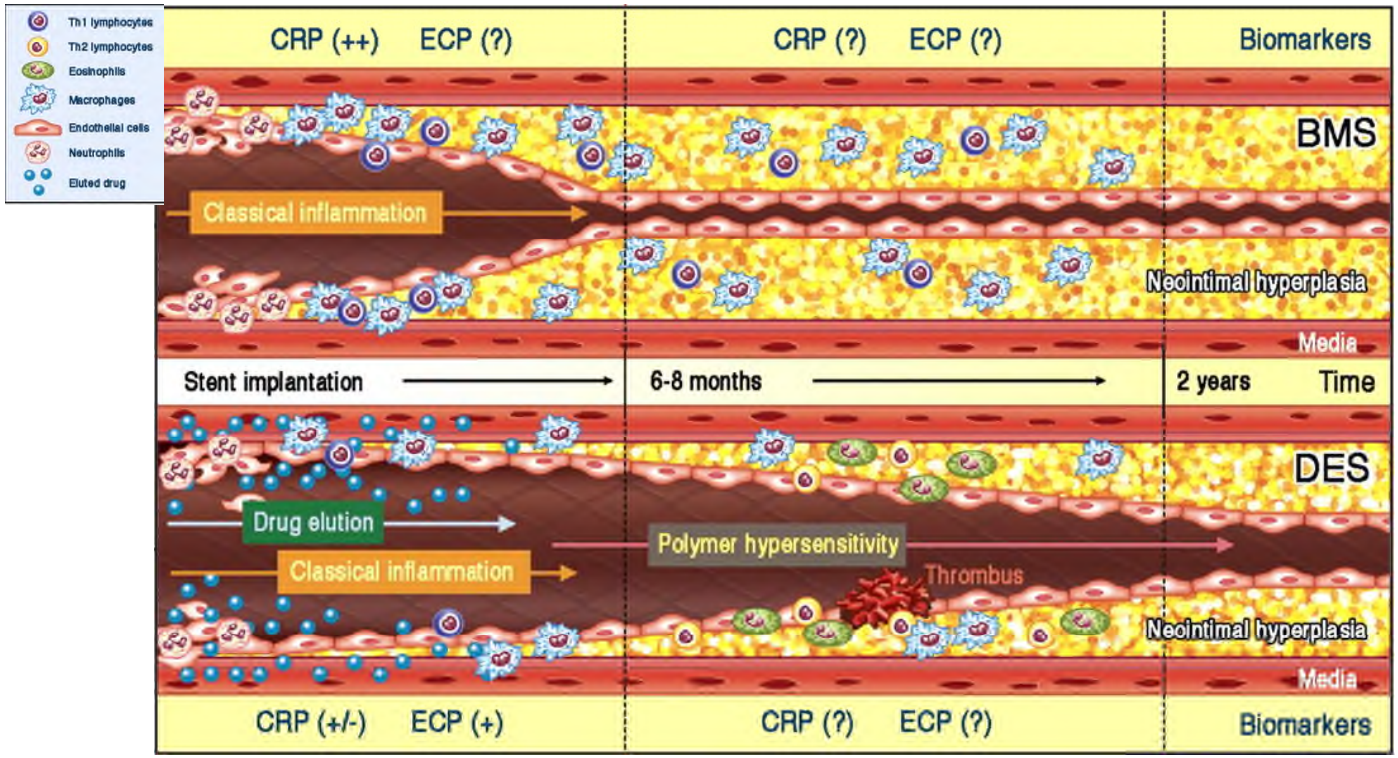
Background

Adverse reaction to Drug Eluting Stents



- endoluminal mural thrombi/old thrombi
- prevalence of eosinophils, giant cells, and fibrin
- development of yellow plaque and plaque rupture
- different SMC phenotype
- different optical properties of neointima
- elevated plasma CRP and ECP

J Am Coll Cardiol 2010;56:1783–93

Background

- The extracellular matrix plays a critical role in providing structural support to the vessel wall and influences cell behavior and signaling
- Platelet activation and inflammation as early response to stent deployment result in increased SMC migration, proliferation and ECM production
- Little is known about changes in ECM composition upon vascular stent injury, even though more than 50% of the neointimal hyperplasia consists of ECM proteins
- The strut coverage in OCT is used as a surrogate marker of chronic stent healing, however less data is available regarding the acute healing response in the first week

Aims

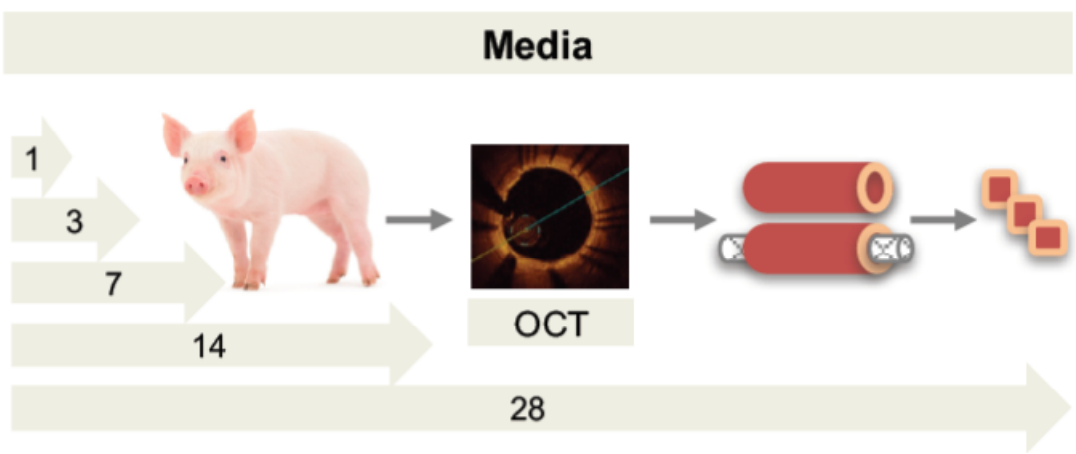
- Proteomics is a powerful technology to profile not just individual ECM proteins by antibody staining but characterize the different stages of ECM remodeling by liquid chromatography tandem mass spectrometry (LC-MS/MS).
- Comparative analysis of protein expression at sequential stages of stent healing

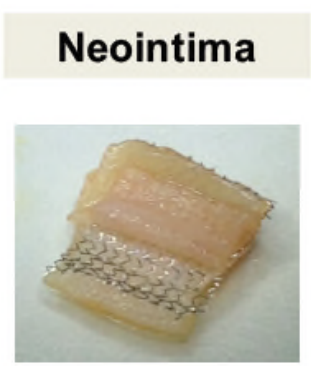
<u>The aim of the study:</u>

- Characterize ECM remodelling in porcine coronary arteries stented with BMS and DES by proteomics
- Evaluate the early healing response by OCT

Methods

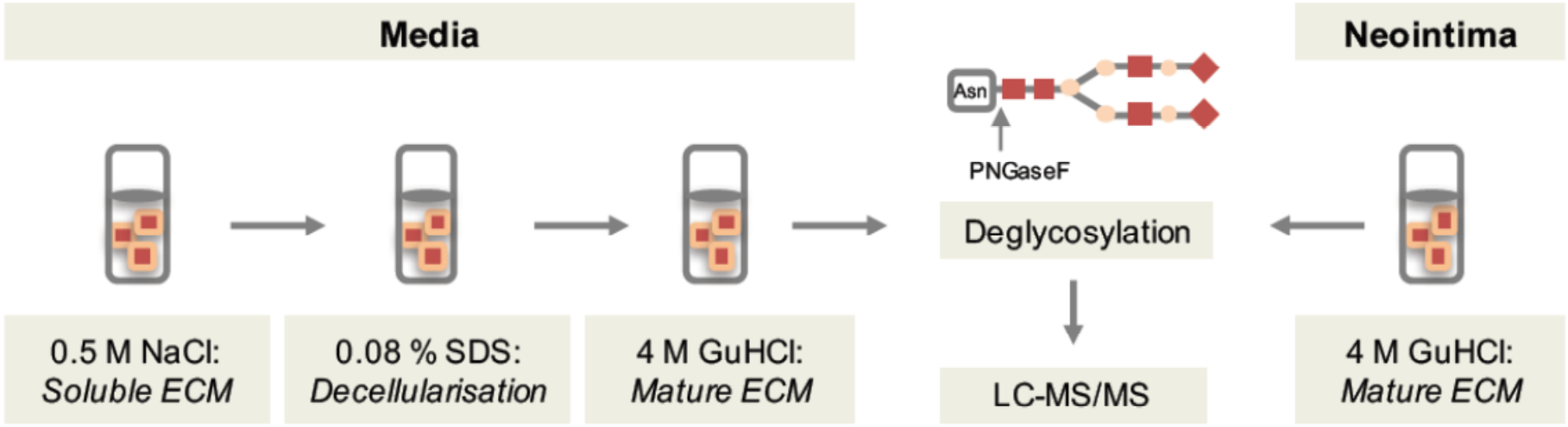
- 12 domestic pigs underwent PCI with BMS (ML Vision, Abbott) or DES (Xience, Abbott) or POBA
- OCT was performed at 1, 3, 7, 14 and 28 days after stent deployment
- The neointima and media samples were analyzed separately





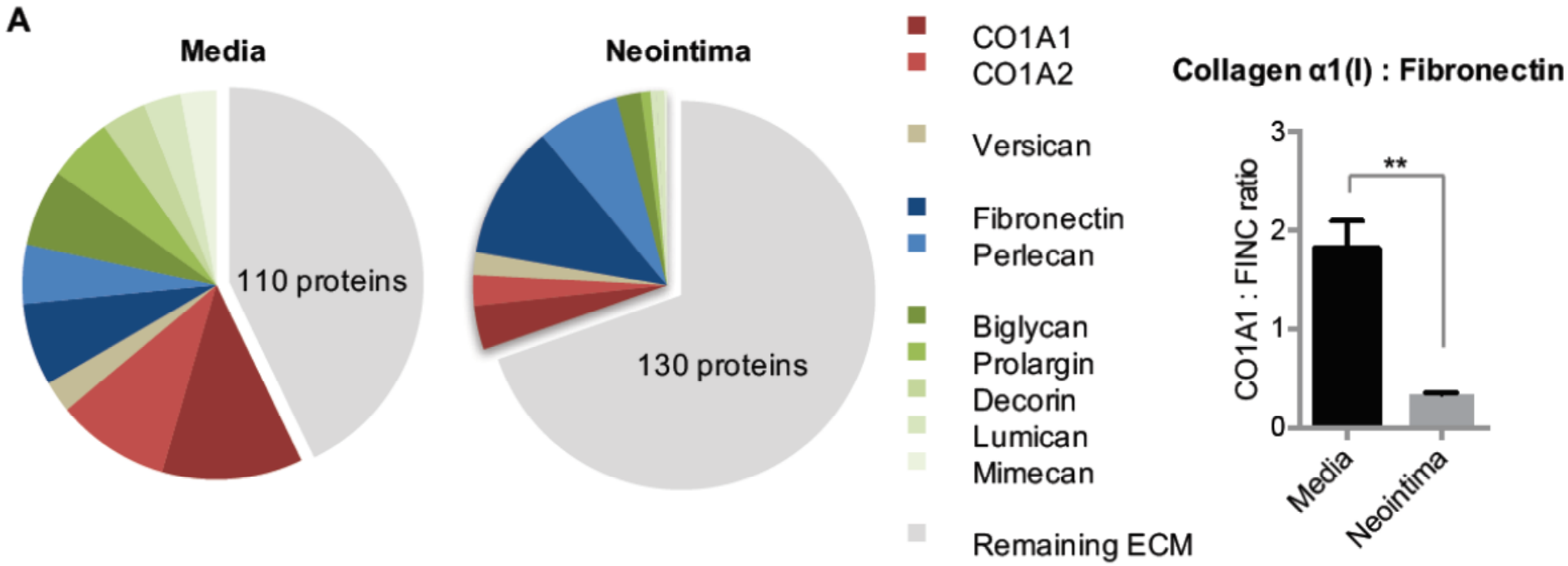
Methods

- A 3-step protein extraction method was applied to the media and a single step for the more fragile neointimal tissue, followed by LC-MS/MS analysis of the deglycosylated, trypsin digested peptides per protocol developed in M. Mayr's lab



Results

Custom-made pig ECM database was generated with list of previously published ECM proteins

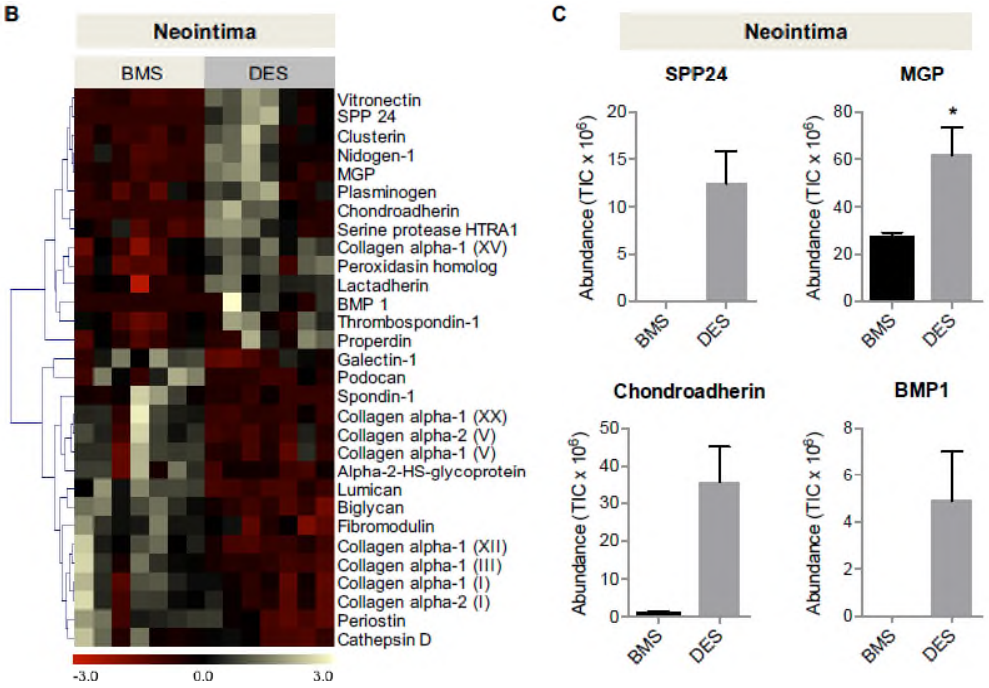


A total of 151 unique ECM proteins were identified overall in the media and neointima

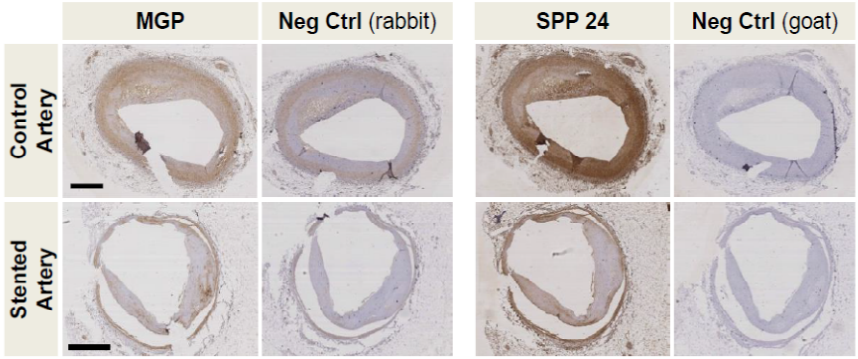
Results

ECM Remodeling in the Neointima

Pig model

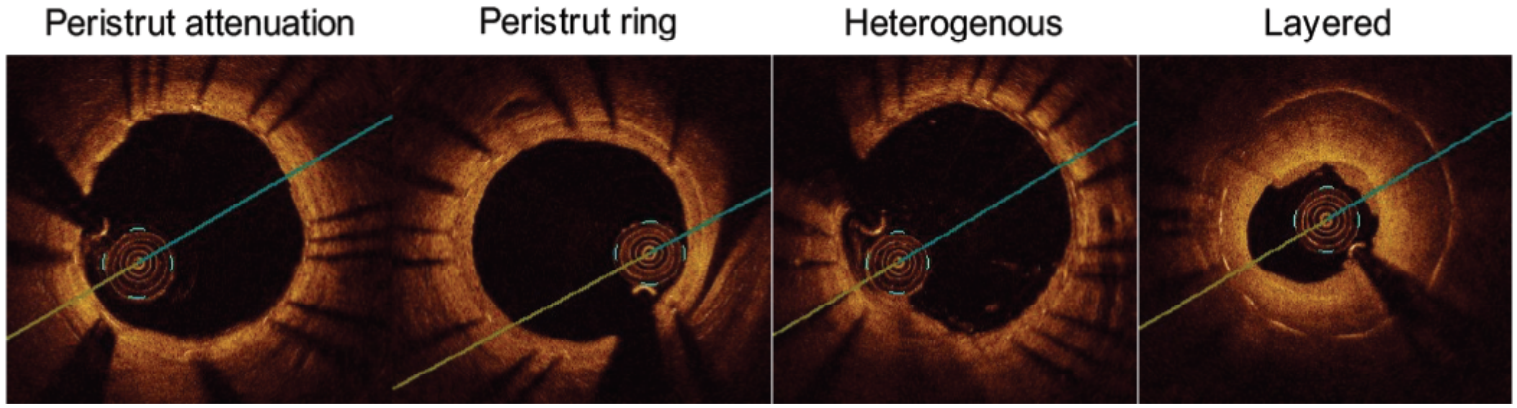


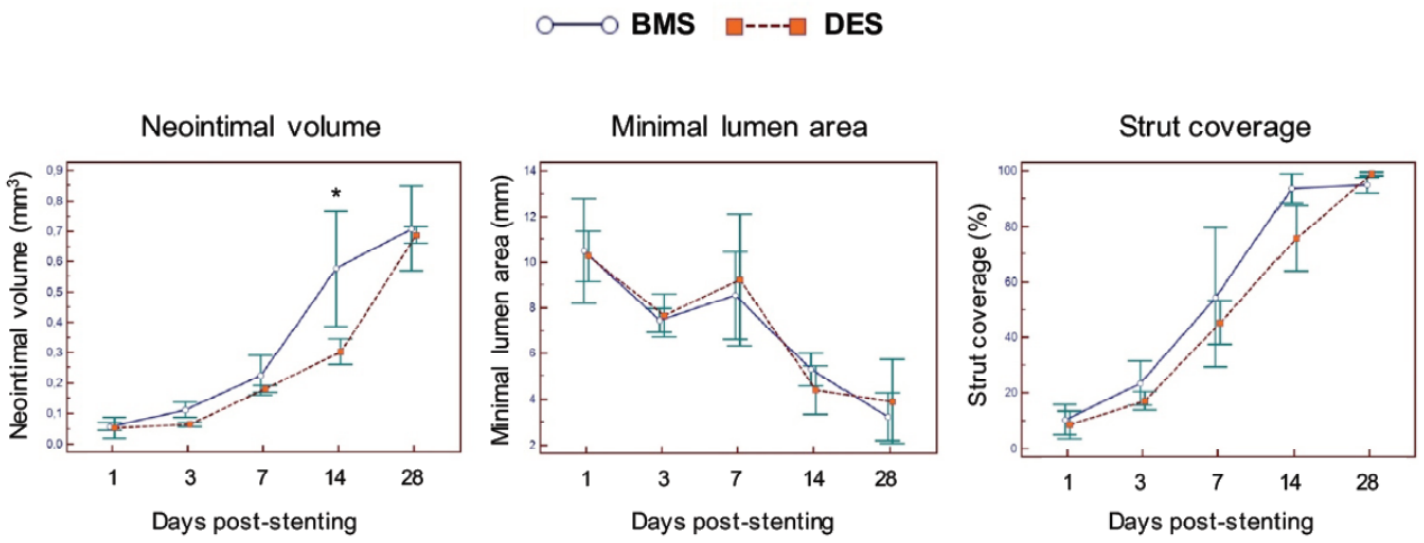
Human stented and control coronary arteries



Results

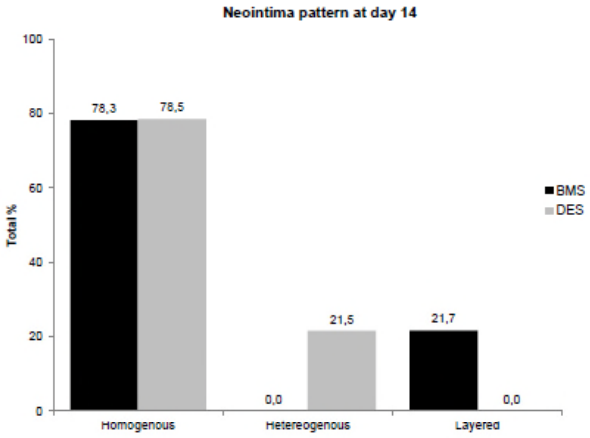
Quantitative analysis of the neointima by OCT

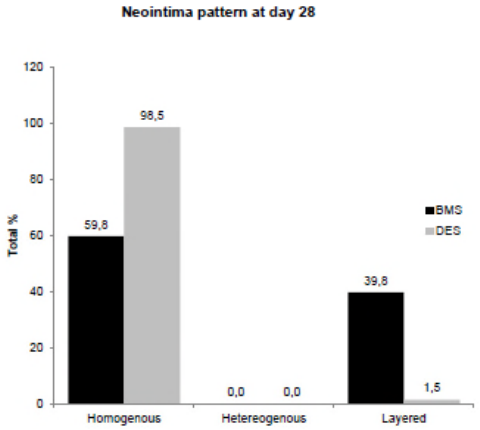


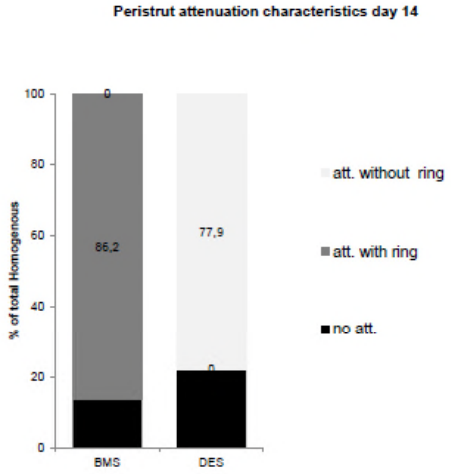


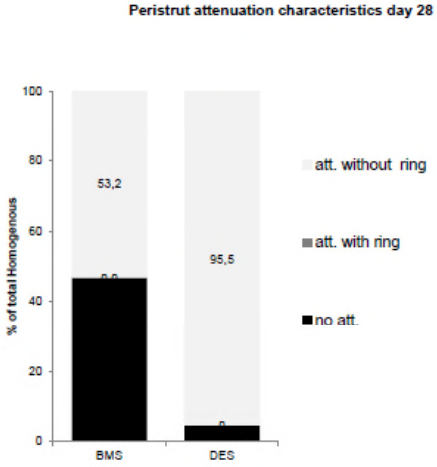
Results

Comparative analysis of the neointima by OCT

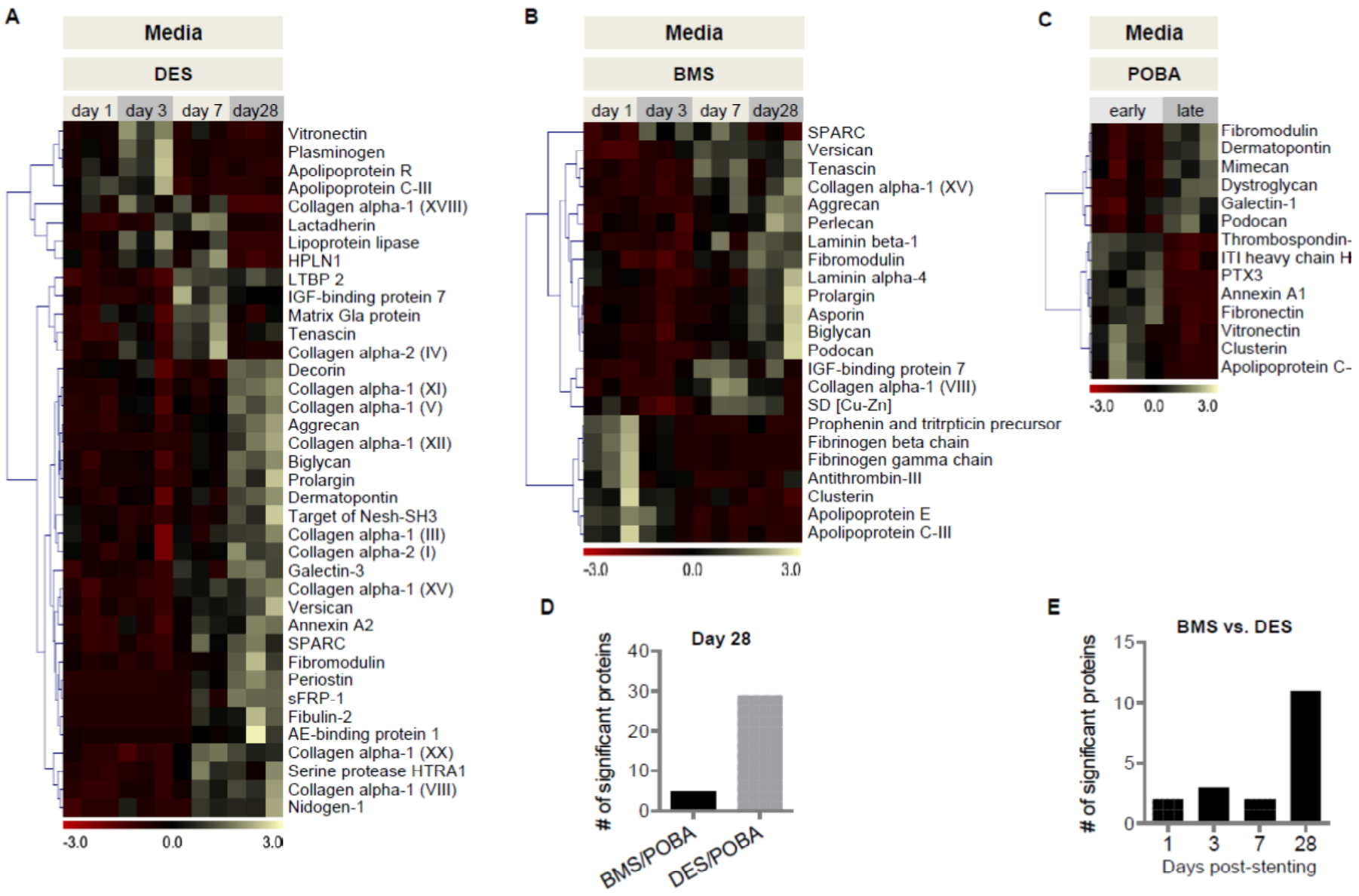






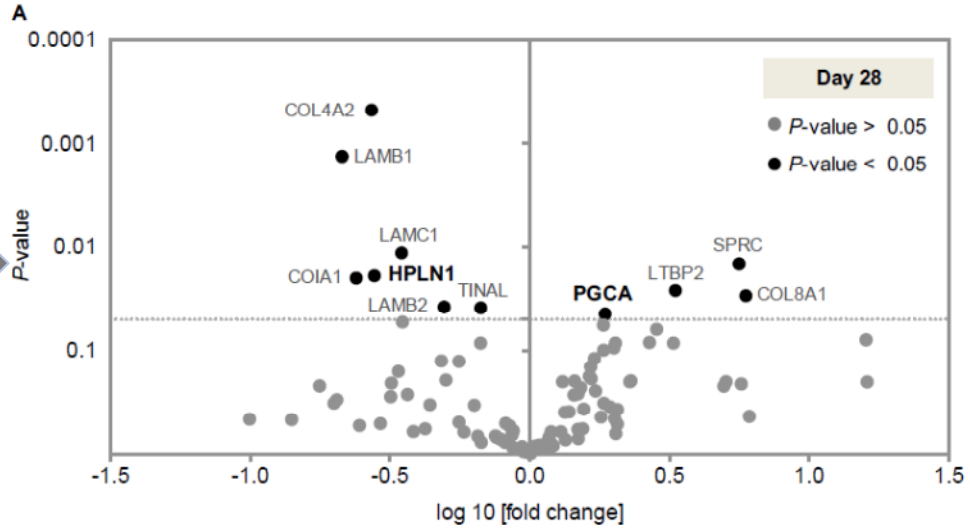


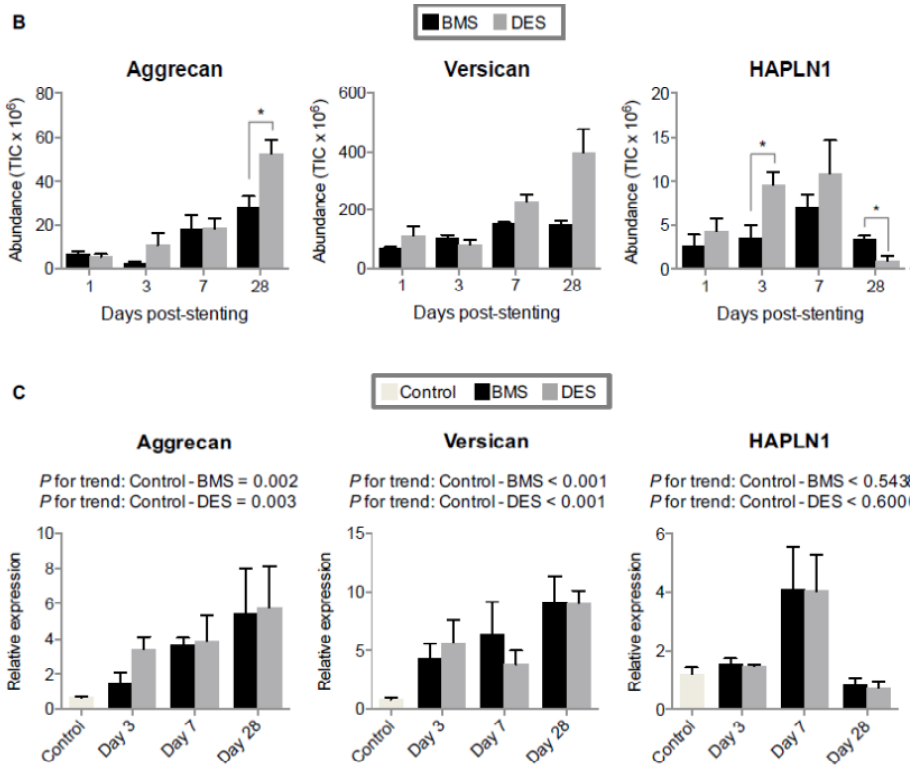
Results


A
Media
DES
day 1
day 3
day 7
day28
Vitronectin
Plasminogen
Apolipoprotein R
Apolipoprotein C-III
Collagen alpha-1 (XVIII)
Lactadherin
Lipoprotein lipase
HPLN1
LTBP 2
IGF-binding protein 7
Matrix Gla protein
Tenascin
Collagen alpha-2 (IV)
Decorin
Collagen alpha-1 (XI)
Collagen alpha-1 (V)
Aggrecan
Collagen alpha-1 (XII)
Biglycan
Prolargin
Dermatopontin
Target of Nesh-SH3
Collagen alpha-1 (III)
Collagen alpha-2 (I)
Galectin-3
Collagen alpha-1 (XV)
Versican
Annexin A2
SPARC
Fibromodulin
Periostin
sFRP-1
Fibulin-2
AE-binding protein 1
Collagen alpha-1 (XX)
Serine protease HTRA1
Collagen alpha-1 (VIII)
Nidogen-1
-3.0
0.0
3.0
B
Media
BMS
day 1
day 3
day 7
day28
SPARC
Versican
Tenascin
Collagen alpha-1 (XV)
Aggrecan
Perlecan
Laminin beta-1
Fibromodulin
Laminin alpha-4
Prolargin
Asporin
Biglycan
Podocan
IGF-binding protein 7
Collagen alpha-1 (VIII)
SD [Cu-Zn]
Prophenin and tritrpticin precursor
Fibrinogen beta chain
Fibrinogen gamma chain
Antithrombin-III
Clusterin
Apolipoprotein E
Apolipoprotein C-III
-3.0
0.0
3.0
C
Media
POBA
early
late
Fibromodulin
Dermatopontin
Mimecan
Dystroglycan
Galectin-1
Podocan
Thrombospondin-
ITI heavy chain H
PTX3
Annexin A1
Fibronectin
Vitronectin
Clusterin
Apolipoprotein C-
-3.0
0.0
3.0
D
Day 28
# of significant proteins
40
30
20
10
0
BMS/POBA
DES/POBA
E
BMS vs. DES
# of significant proteins
15
10
5
0
1
3
7
28
Days post-stenting

Results

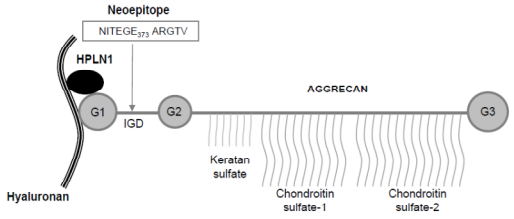
ECM composition in DES and BMS in the media

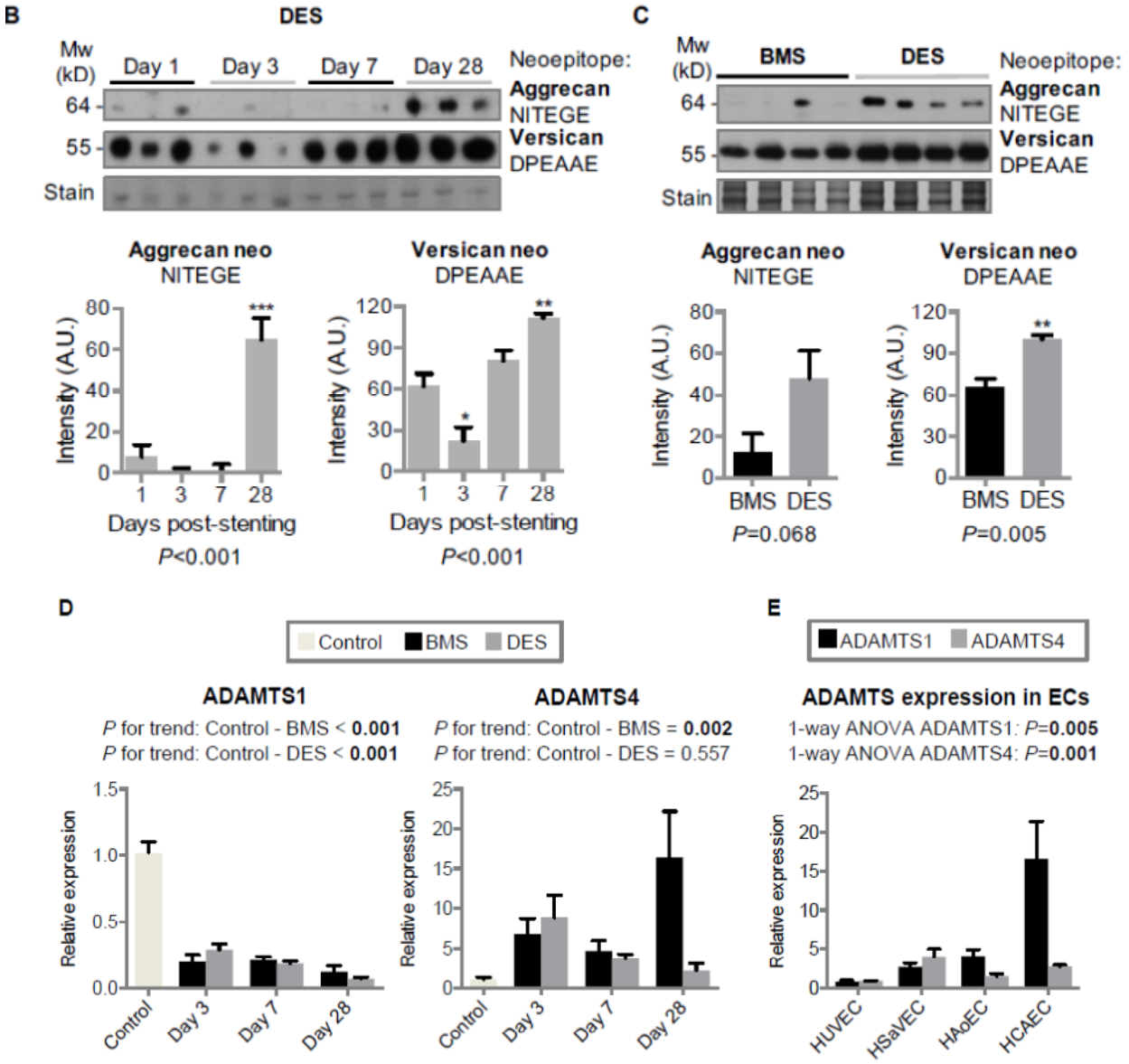




Results

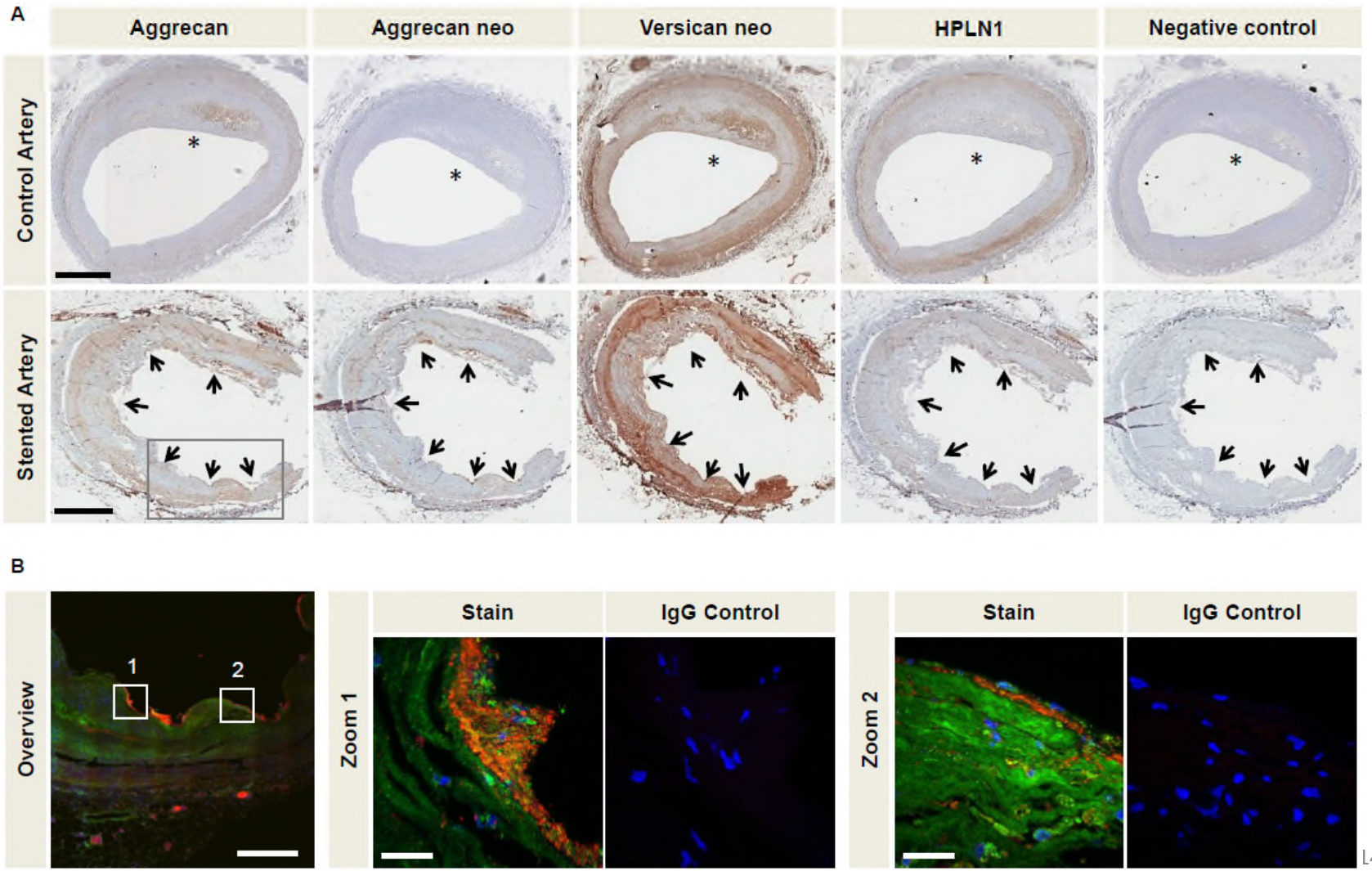
Aggrecanase Expression in DES and BMS and Endothelial Cells





Results

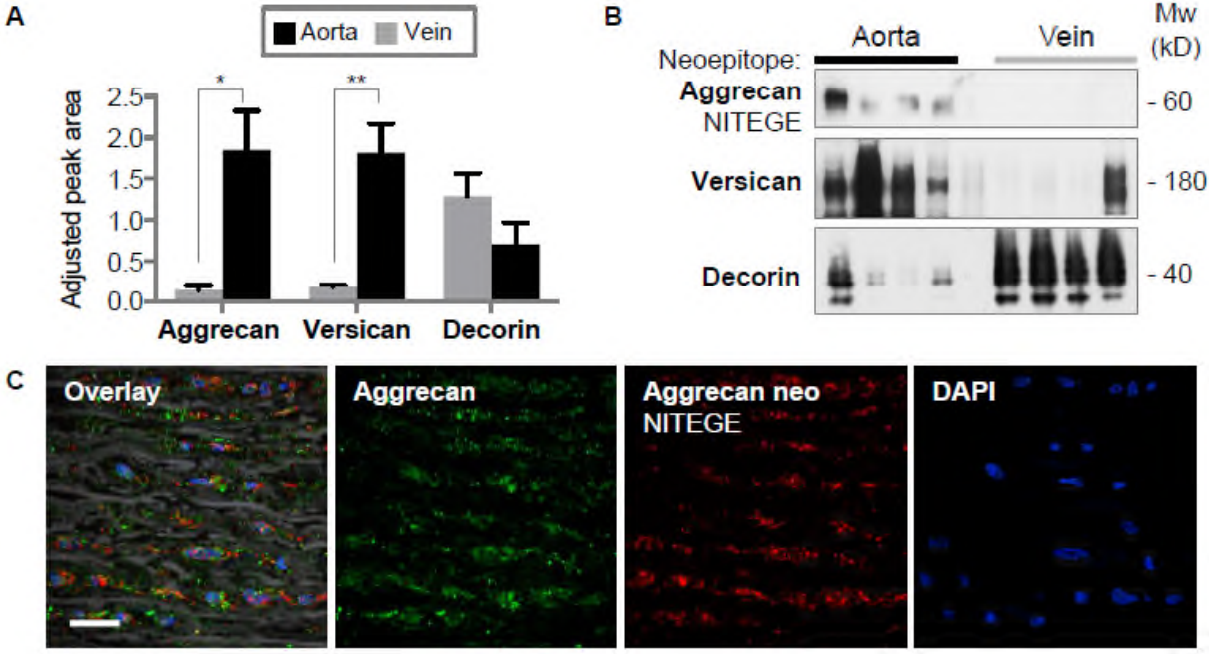
Human coronary arteries

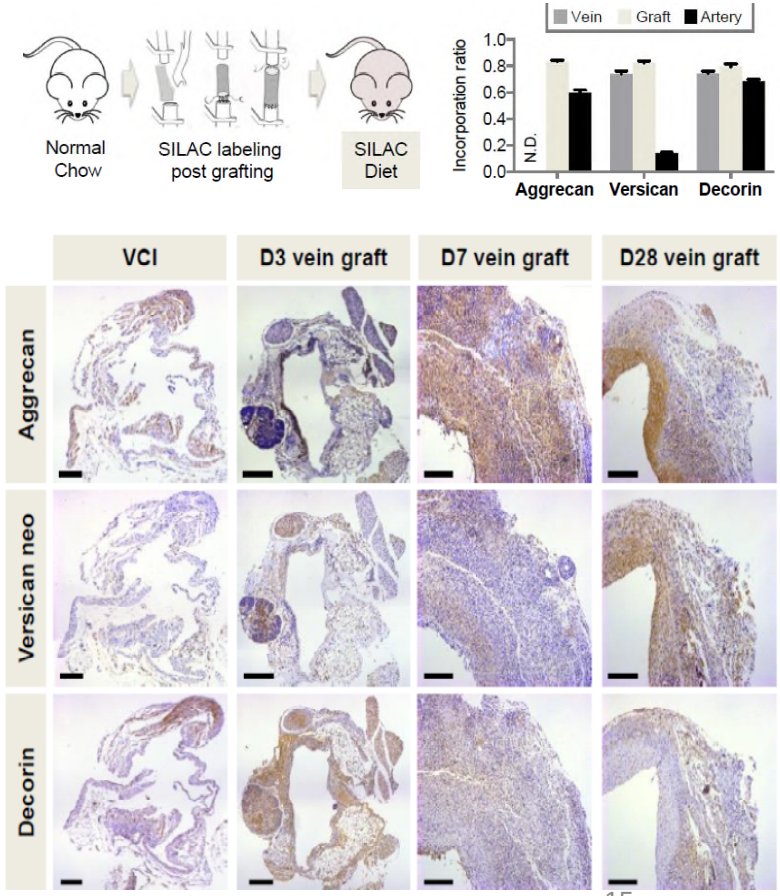


Results

Aggrecan in the vasculature

Role of mechanical force





Conclusions

- This is the first proteomics analysis of ECM remodeling in stented coronary arteries
- OCT showed similar early healing pattern of DES and BMS
- The effects of DES go beyond inhibition of SMC, but have a wider impact on vascular remodelling by altering the composition of the ECM
  - reduction in basement membrane proteins and decreased cellularity
  - increased production of large proteoglycans like aggrecan and versican (adaptation to increased wall stretch)
- Proteomics approach to identify extracellular matrix protein changes in response to BMS and DES revealed differential expression of aggrecan, a proteoglycan that is usually associated with articular cartilage degradation in RA and OA.
- Aggrecanase activity is an integral component of the vascular injury response post stenting
  - potential new drug targets to alter extracellular matrix remodeling in the vasculature.

Gonca Suna, Wojtek Wojakowski, Marc Lynch, Javier Barallobre-Barreiro, Xiaoke Yin, Ursula Mayr, Ferheen Baig, Ruifang Lu, Sophie Kwan, Chris Molenaar, Stephen J. White, Tomasz Roleder, Krzysztof P. Milewski, Pawel Gasior, Piotr P. Buszman, Pawel Buszman, Marjan Jahangiri, Jonathan Hill, Manuel Mayr

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