Amsterdam UMC
Universitair Medische Centra

70TH ESCVS CONGRESS
& 7TH IMAD MEETING

20 I 23 JUNE 2022

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Dekker Senior Clinical Scientist Fellow
2019T065

The Role of Smooth Muscle Cells in Aortic Aneurysms

Associate Prof. Dr. Kak Khee Yeung, MD, PhD, FEBVS
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Disclosures

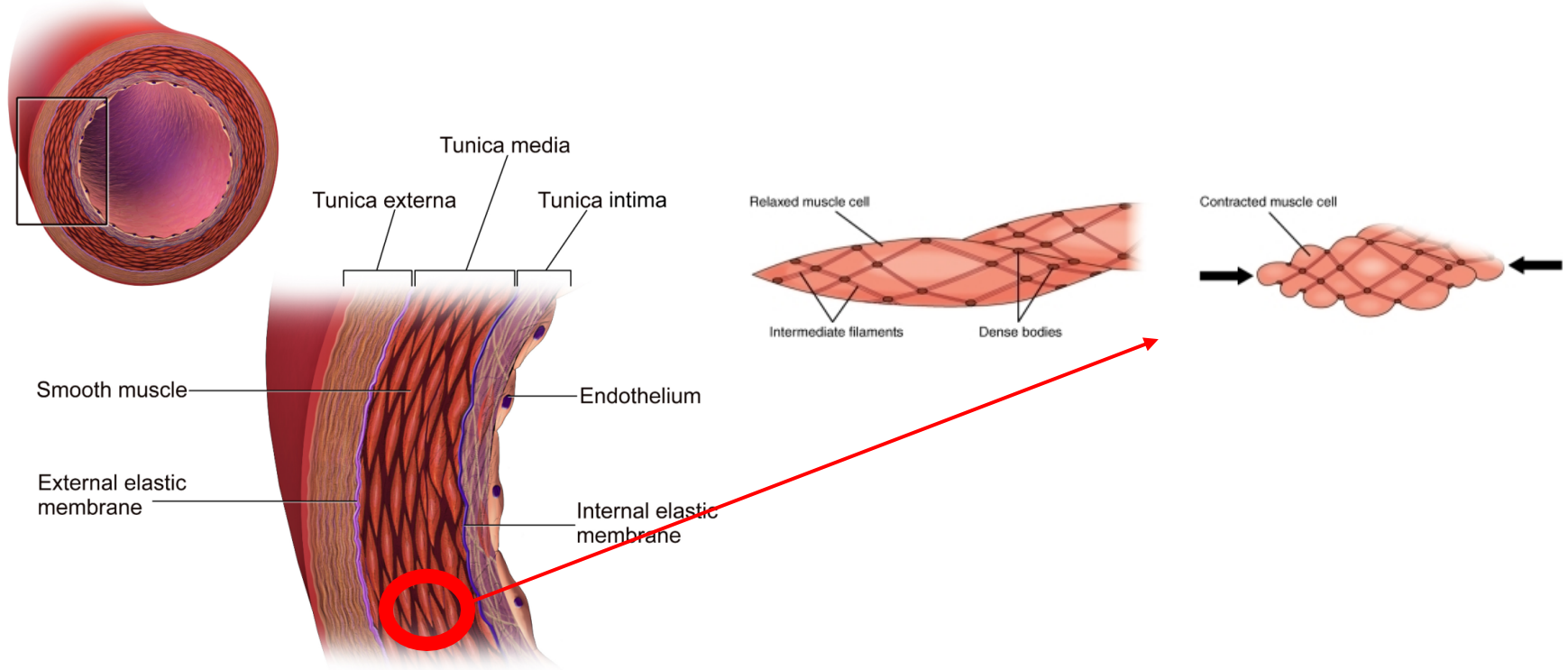
- Dutch Heart Foundation
- Unrestricted grant of W.L. Gore & Associates
- Patients: AMC Foundation and Stichting VUmc

Problem

- Still not able to predict Rupture or Growth rates of Aneurysms and Dissections
- Endovascular repair → 30% reintervention rates → AAA progression
- Are we able to select high risk patients for treatment?
- Personalised treatment
- Do we get to a medical treatment?

New insights from cell specific analysis

Vascular Smooth Muscle Cells (SMC)

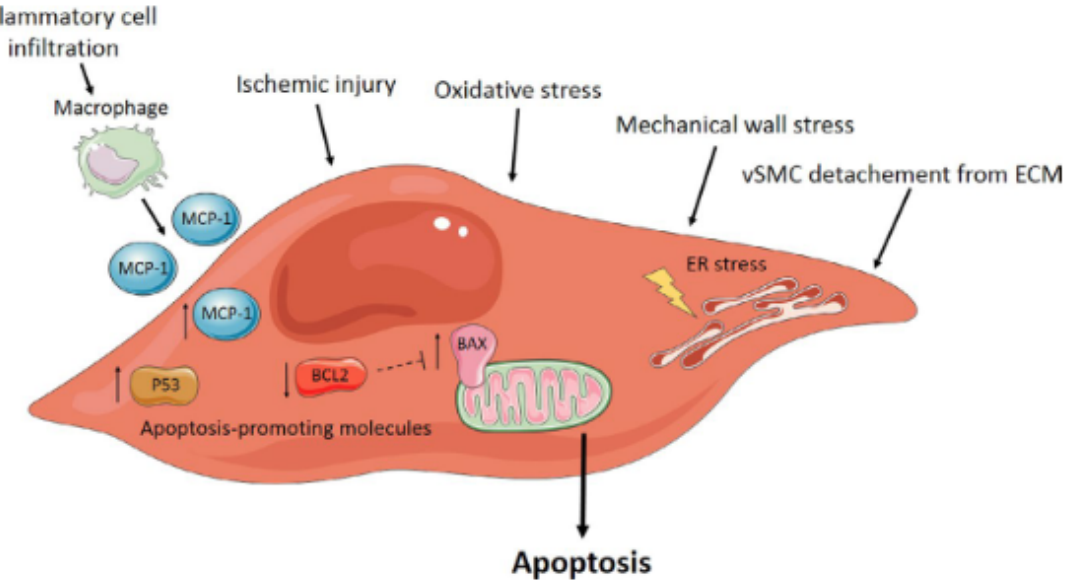


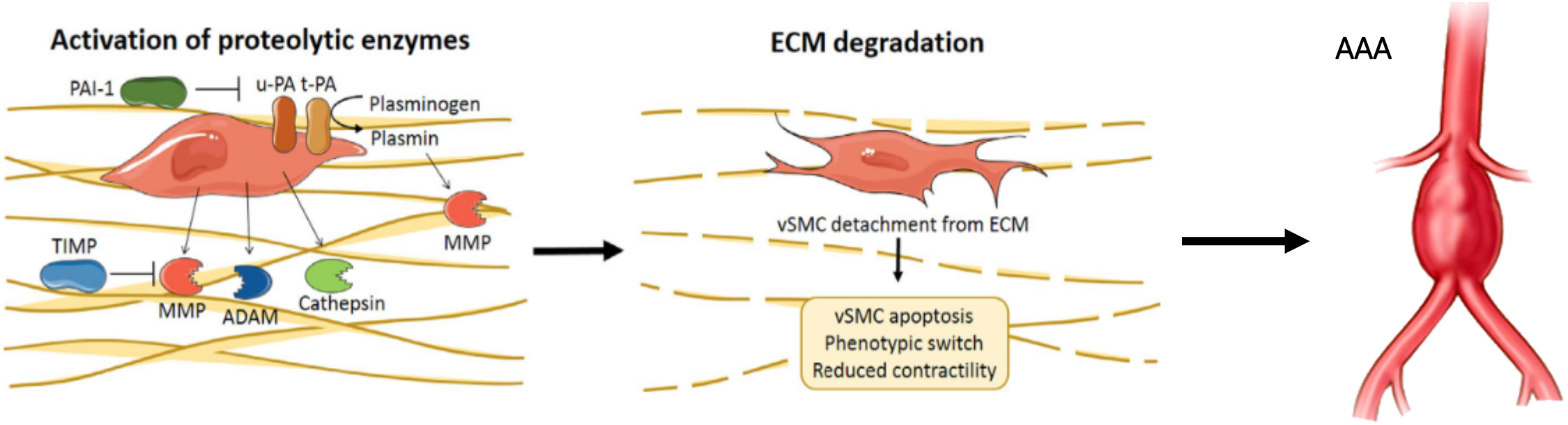
REVIEW *2021, Eur J Clin Invest*

WILEY

The role of vascular smooth muscle cells in the development of aortic aneurysms and dissections

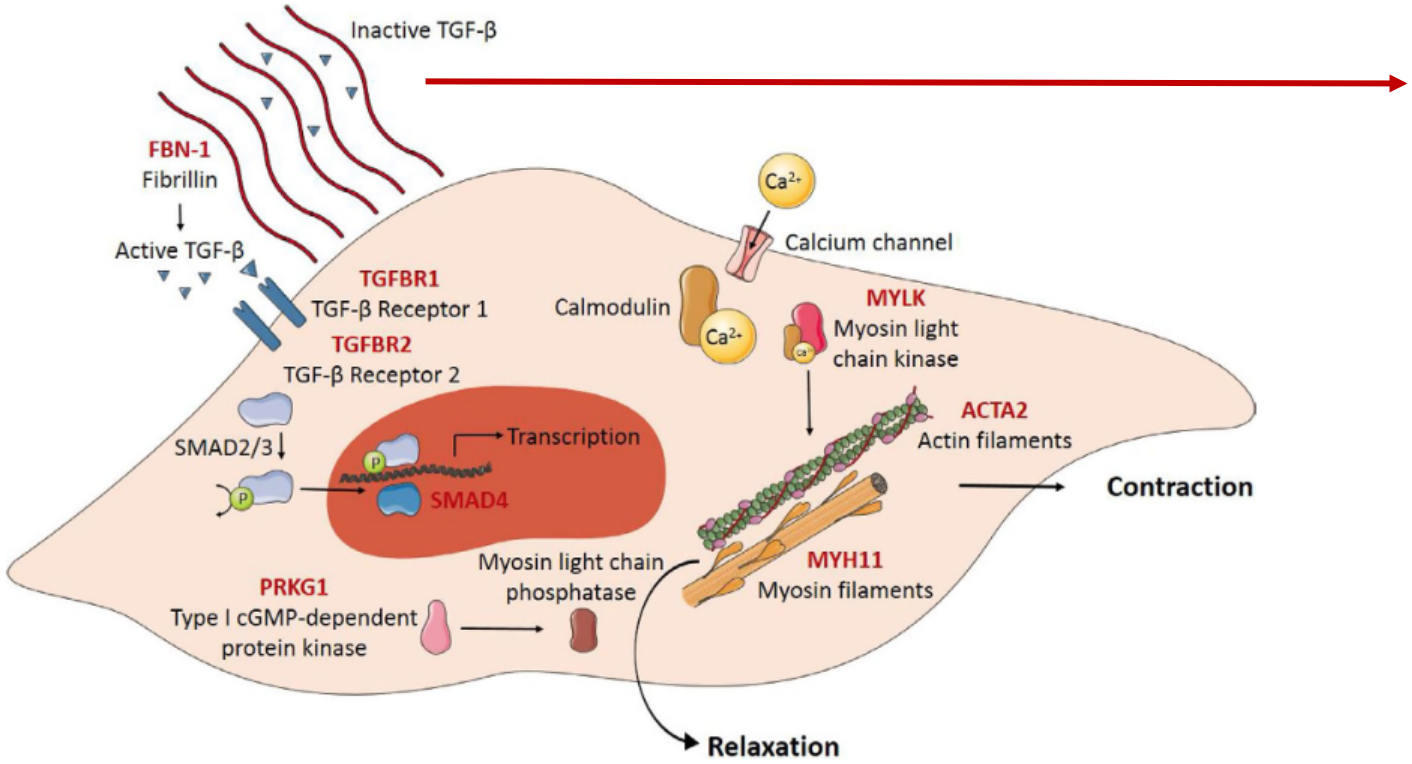
Karlijn B. Rombouts[1,2] | **Tara A. R. van Merrienboer**[1,2] | **Johannes C. F. Ket**[3] | **Natalija Bogunovic**[1,2,4] | **Jolanda van der Velden**[2] | **Kak Khee Yeung**[1,2]





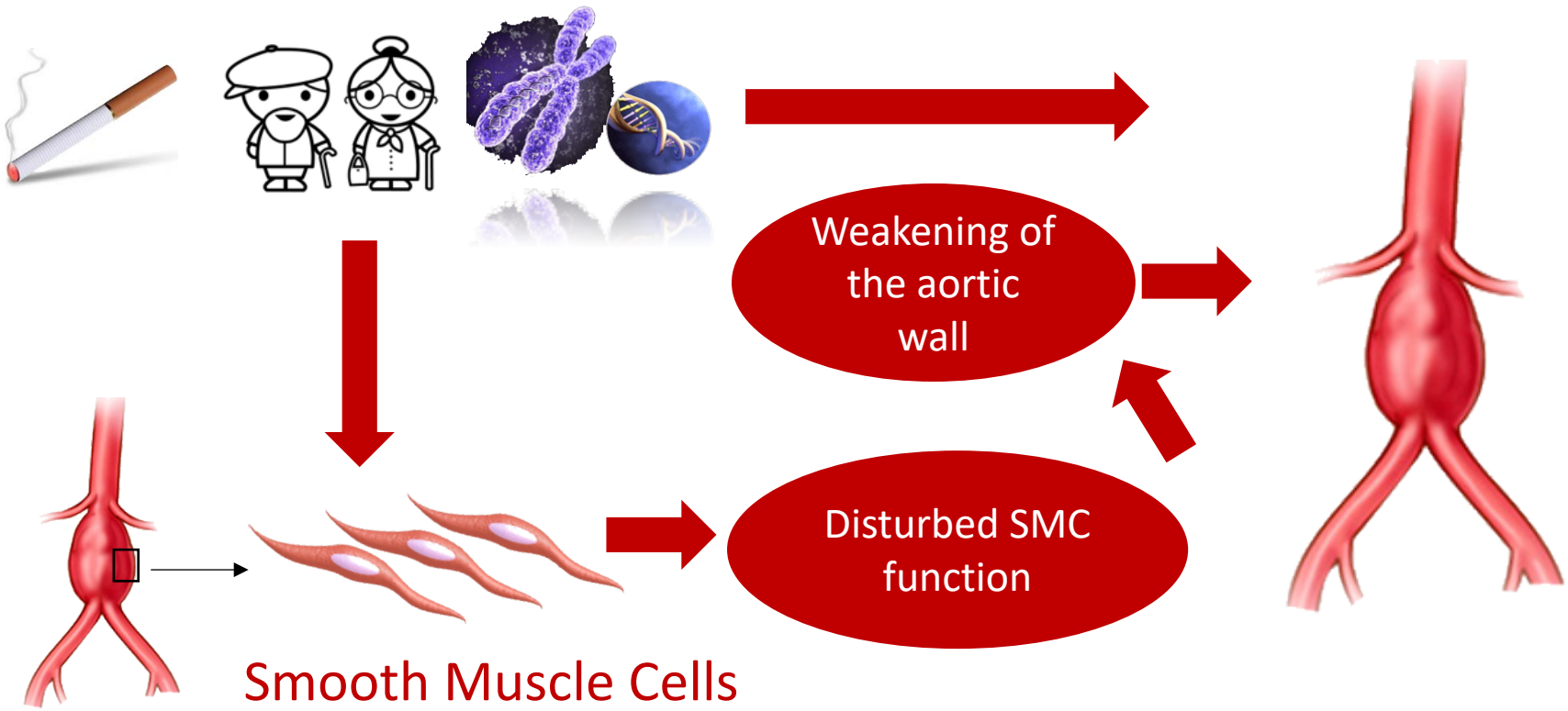
Genetic mutations involving SMC

- Mutations in genes of the mechano-transduction complex: smooth muscle cells + environment



20% Familial thoracic aneurysms

Hypothesis

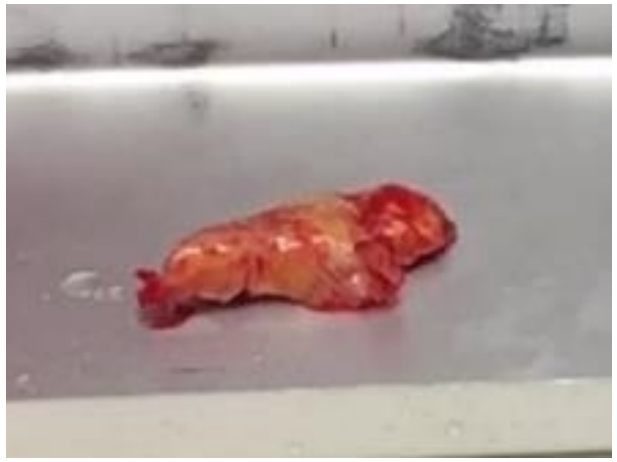

Weakening of the aortic wall
Disturbed SMC function
Smooth Muscle Cells

SMC isolation from AAA biopsies

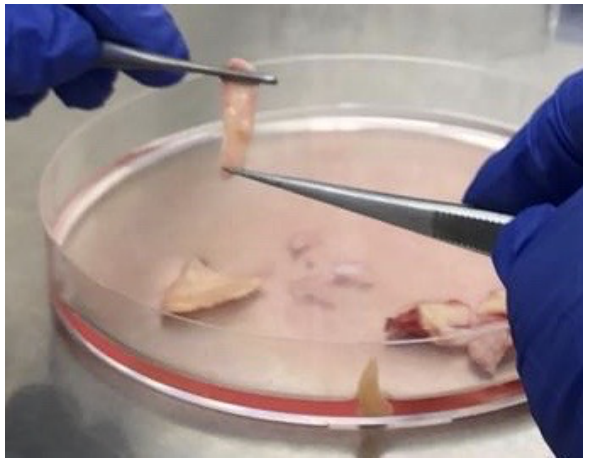
EXCLUSIVE Biobank
imagebank
databank
national: PAREL AAA (>700)

AAA open repair surgery

Aortic biopsy



Finding the right layer

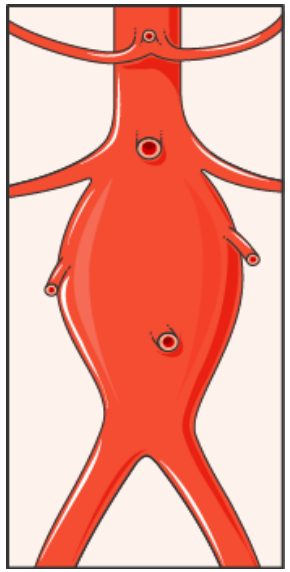


Cutting into pieces

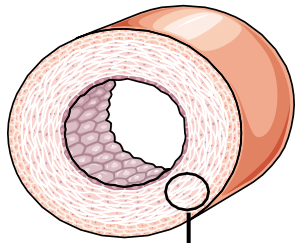
LIVE Biobank:

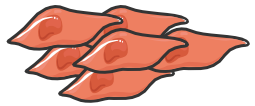
- Blood
- Tissue
- Urine
- Perivascular fat
- Fibroblasts isolation from the skin
- Thrombus

Overview Project

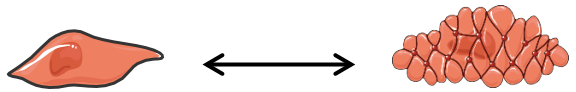


Abdominal Aortic Aneurysm

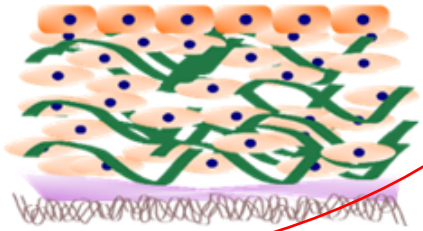




Smooth muscle cells



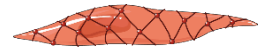
1. Impaired SMC contractility



2. SMC + EC 3D model

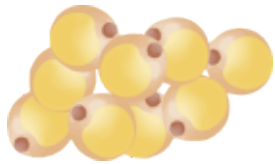
3. Transdifferentiation

Dermal fibroblast



SMC like cell

4. Perivascular fat inflammation



5. Personalised prediction + medication testing (+Imaging)

Insights in the pathophysiology of AAA can contribute to finding new targets to develop new non-invasive treatment options

Measuring SMC contraction using ECIS

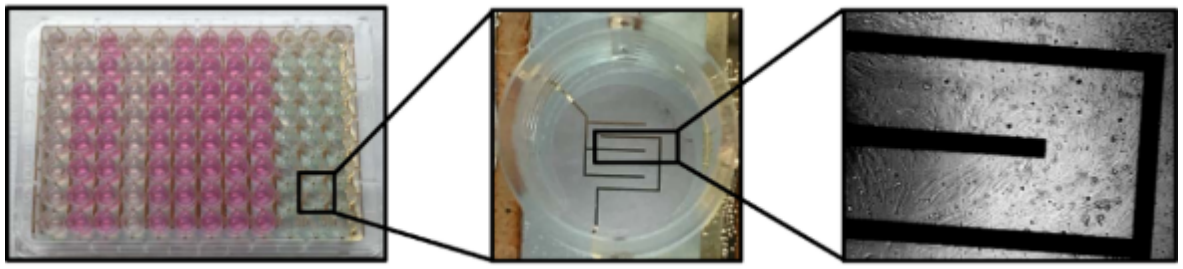
jove *2022*

Isolation of Primary Patient-specific Aortic Smooth Muscle Cells and Semiquantitative Real-time Contraction Measurements *In Vitro*

Natalija Bogunovic[*,1,2,3], **Karlijn B. Rombouts**[*,1,2], **Kak Khee Yeung**[1,2]

[1] Department of Vascular Surgery, Amsterdam UMC, Vrije Universiteit Amsterdam [2] Department of Physiology, Amsterdam Cardiovascular Sciences, Amsterdam UMC, Vrije Universiteit Amsterdam [3] Laboratory of Experimental Cardiology, Department of Cardiology, Leiden University Medical Center

[*] These authors contributed equally

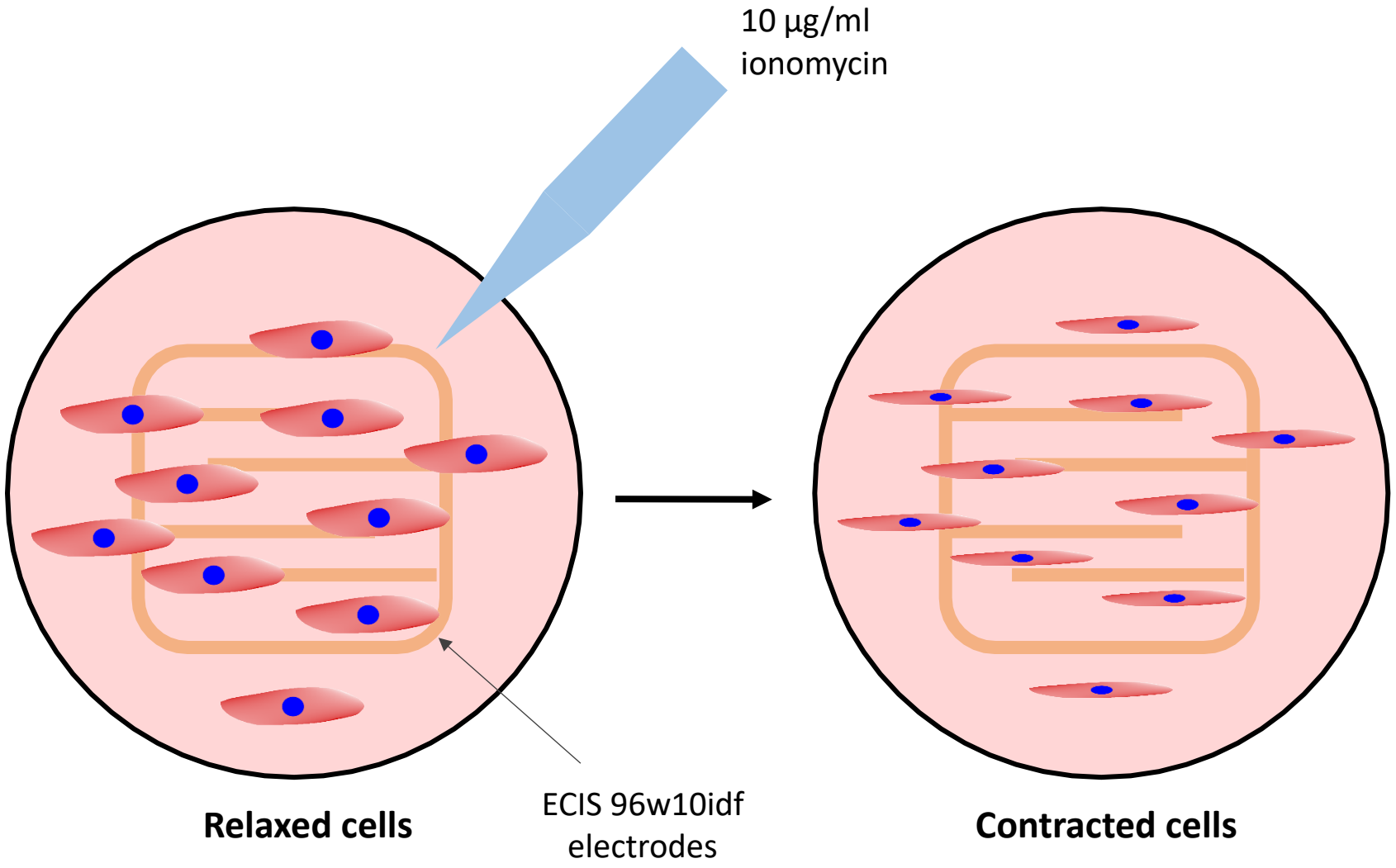


ECIS cell culture plate | Well with electrodes | SMC covering electrodes

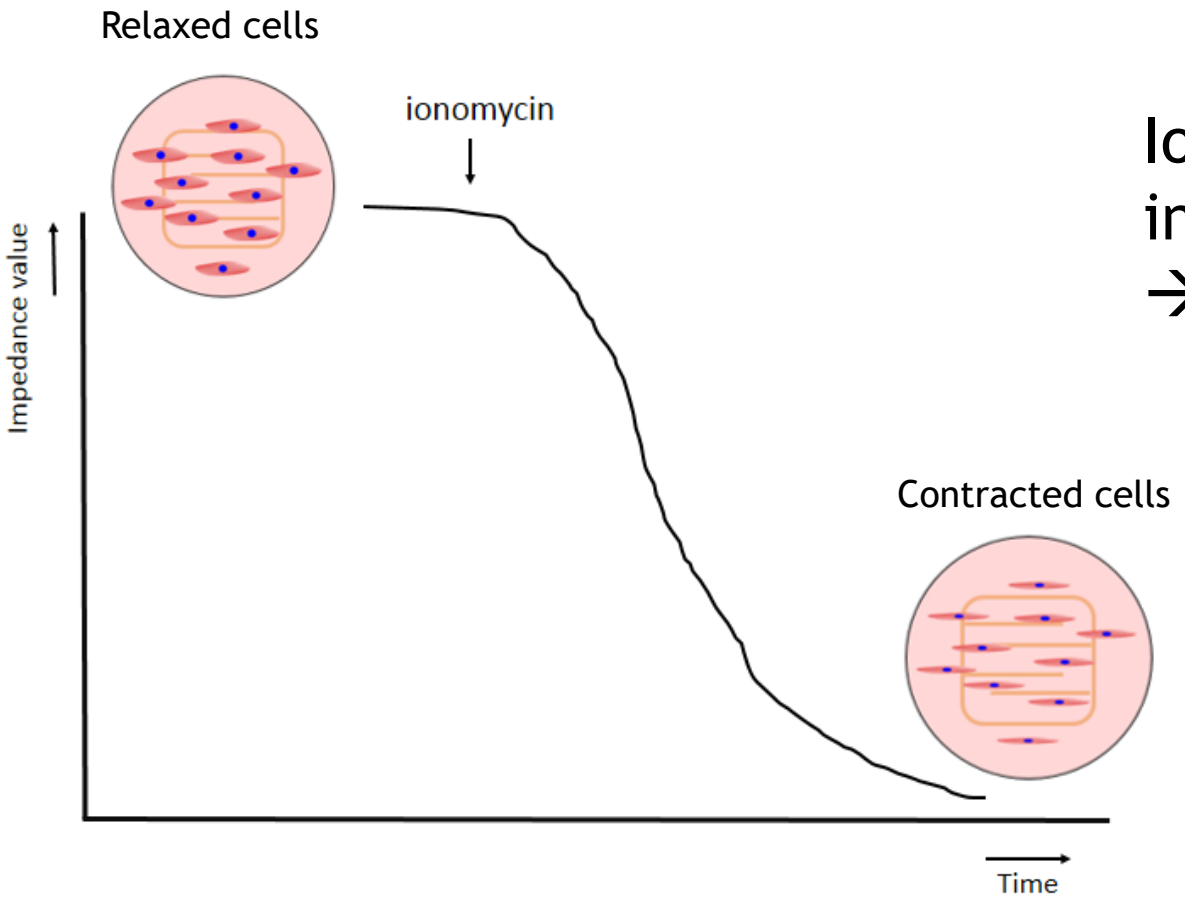
Figure from Bogunovic et al., 2019

Electric Cell-substrate Impedance Sensing (ECIS) measures impedance value based on electrode coverage

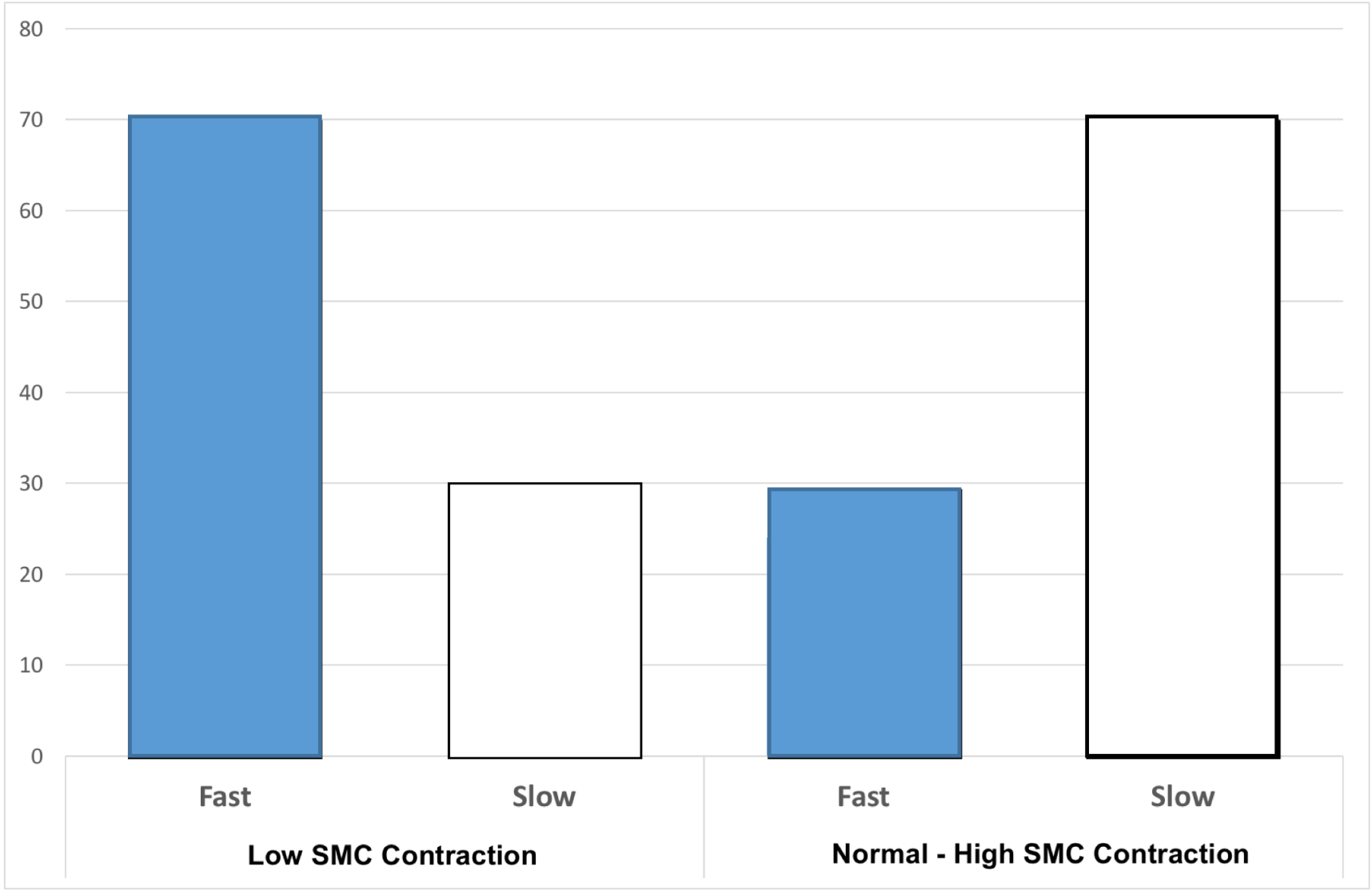
Methods – Stimulation with ionomycin



Measuring SMC contraction using ECIS



Ionomycin stimulus (Ca^{2+} ionophore inducing influx of Ca^{2+}) → SMC contraction → Drop in impedance value



Impaired smooth muscle cell contractility as a novel concept of abdominal aortic aneurysm pathophysiology

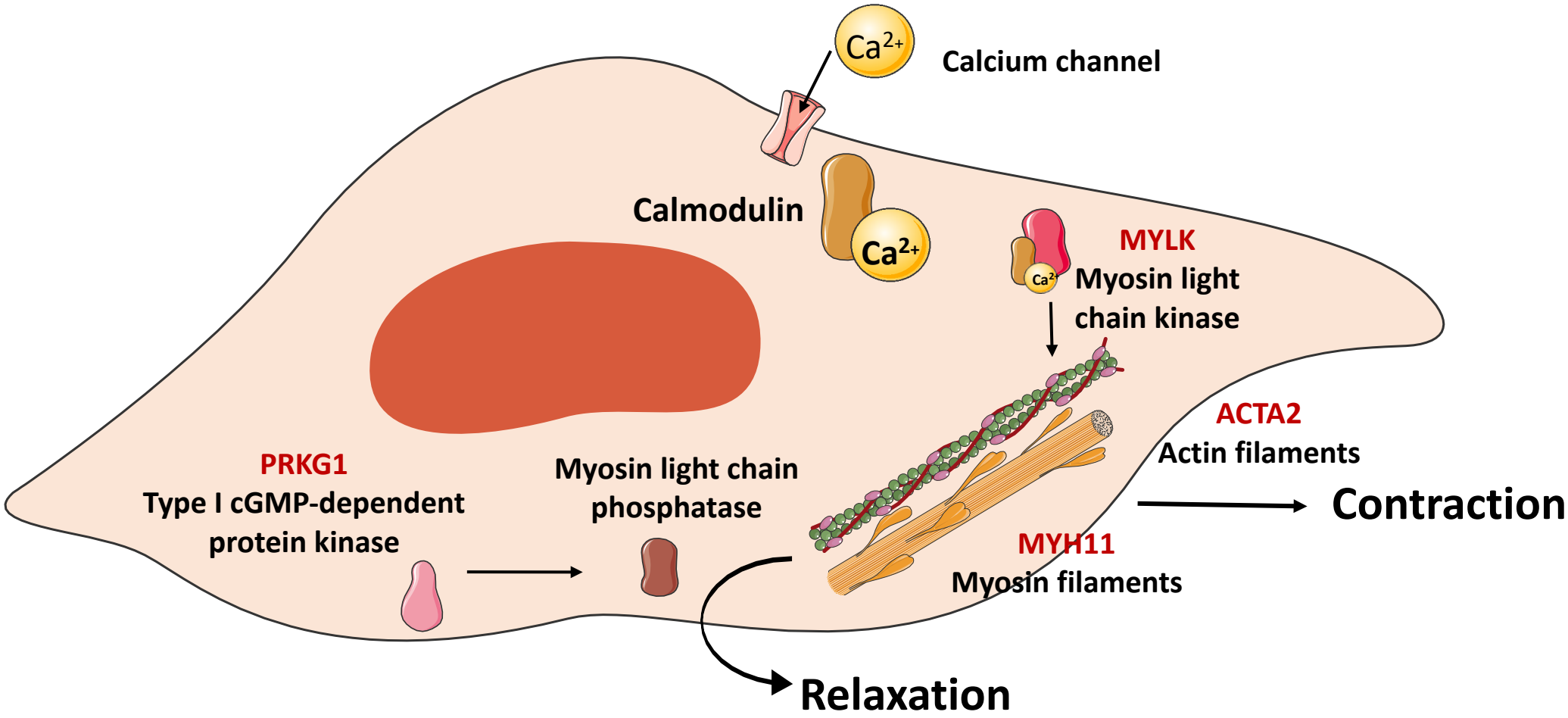
Natalija Bogunovic[1,2,3], Jorn P. Meekel[1,2], Dimitra Micha[3], Jan D. Blankensteijn[1], Peter L. Hordijk[2] & Kak K. Yeung[1,2]

AAA patients (open repair) n = 39
Control n = 18

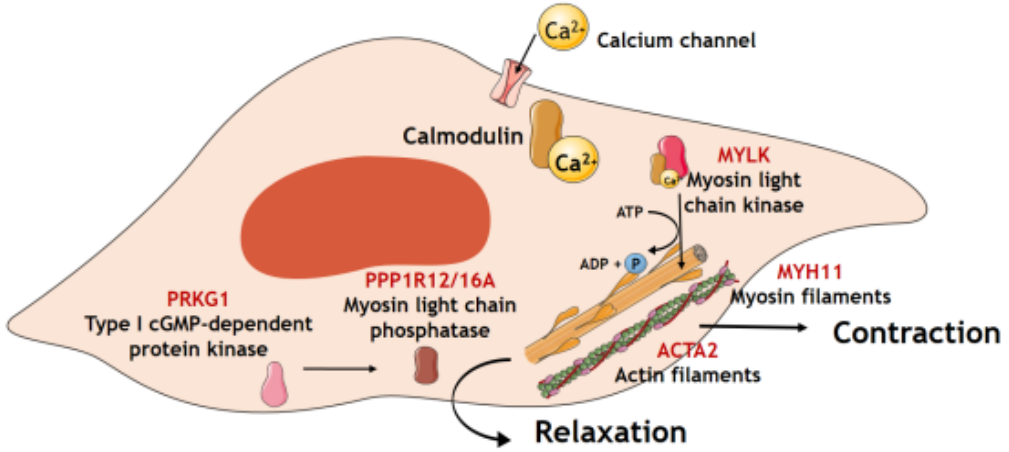
No reference for normal SMC contraction: Mean controls ± 2SD

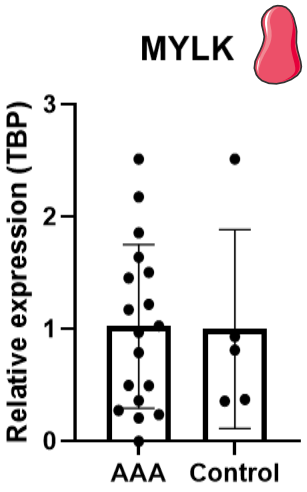
N. Bogunovic et al, J Vis Exp 2022

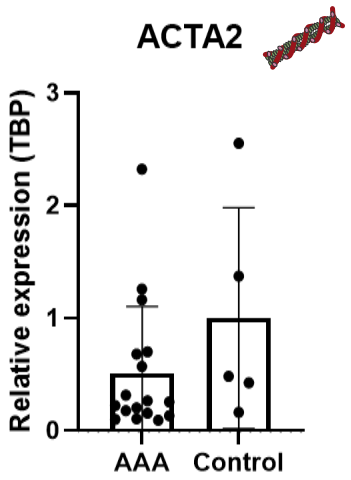
SMC contraction dysfunction

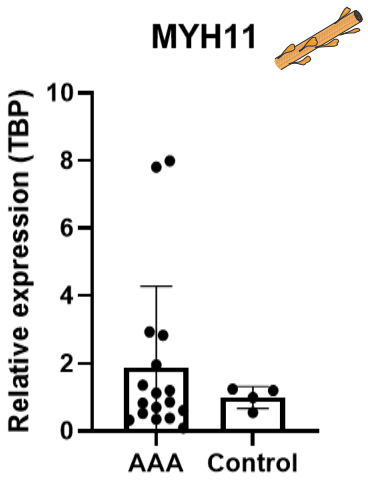


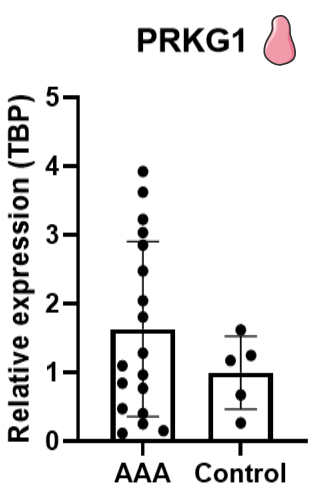
Gene expression of proteins involved in contraction

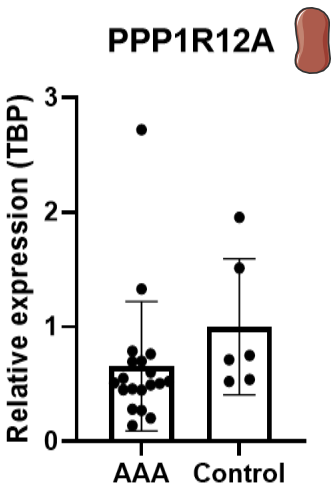


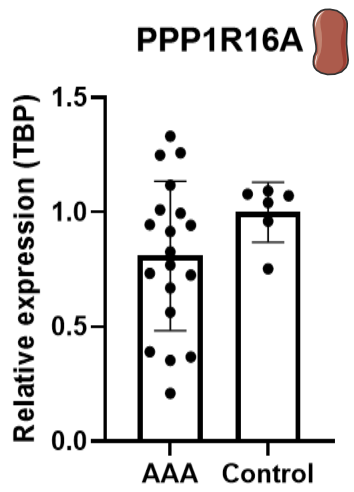






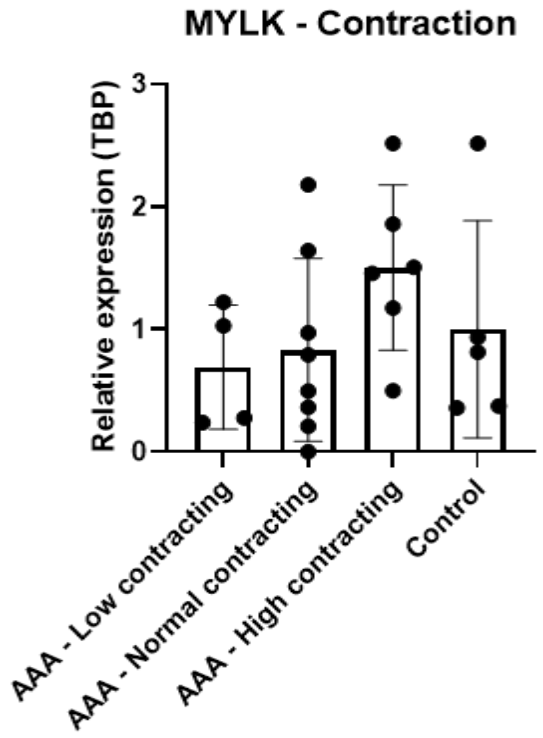


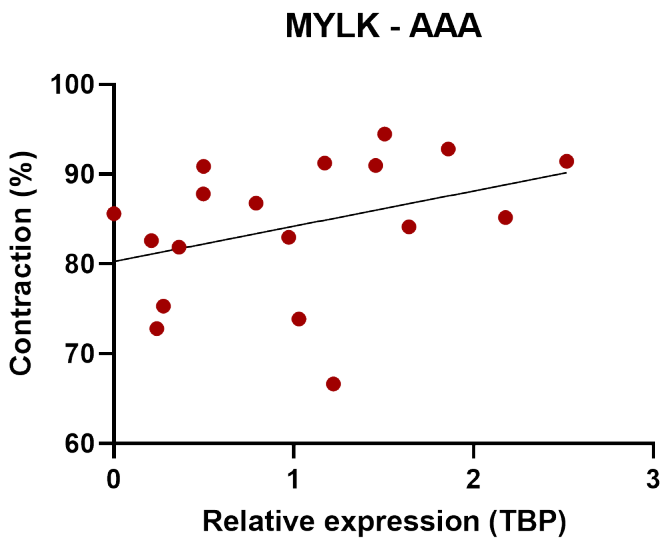




AAA-SMC subdivided based on contraction and gene expression correlated to SMC contraction

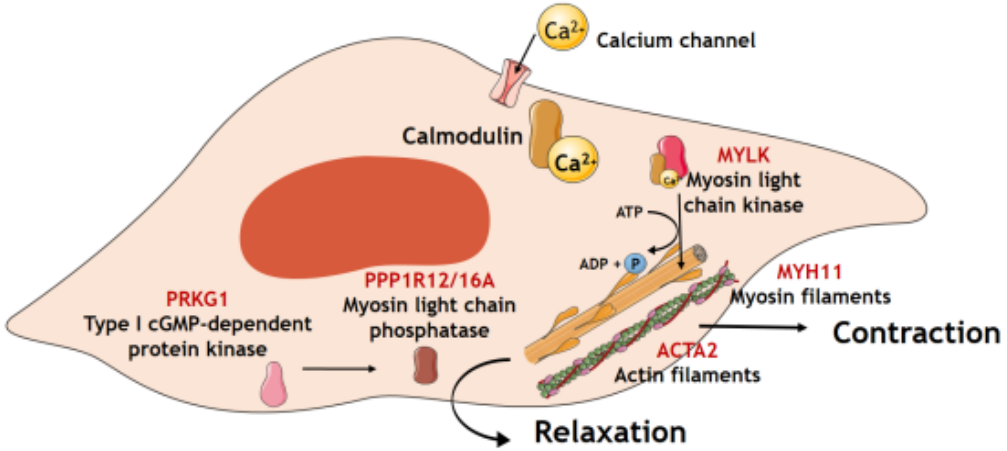
MYLK expression subdivided based on SMC contraction



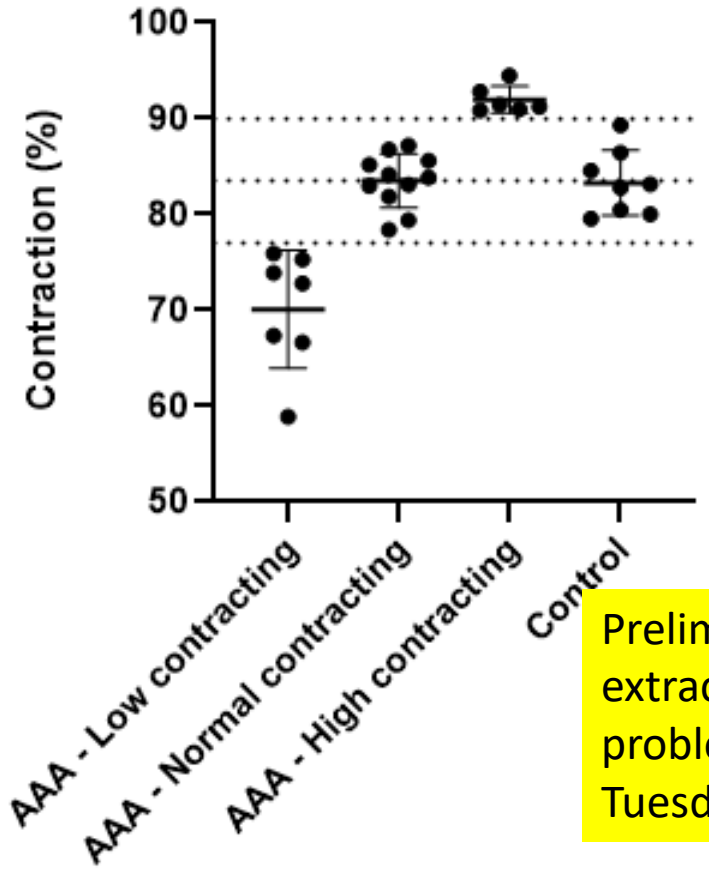


p=0,044

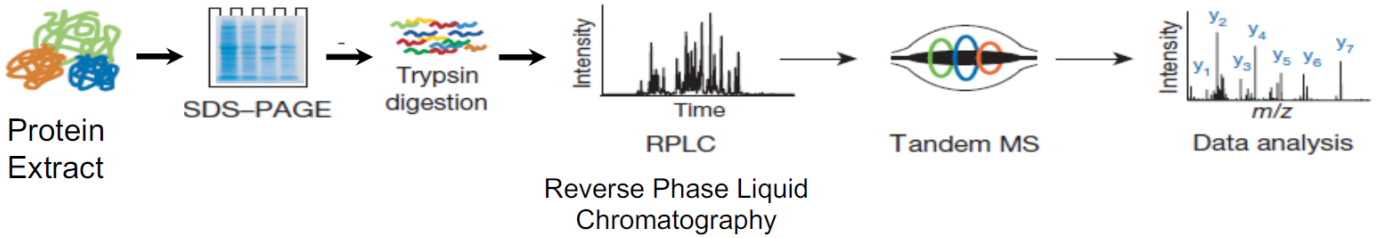
AAA patients



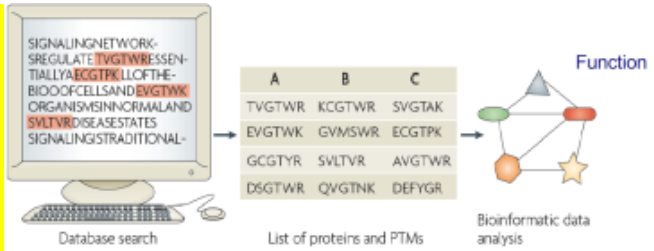
Phosphoproteomics screen to explain differences in SMC contraction



- (Phospho)proteomics samples of only SMC and control cellines:
  - AAA-SMC Low contracting n=7
  - AAA-SMC Normal contracting =11
  - AAA-SMC High contracting n=6
  - Control n=8
- Data of all proteins expressed and phosphorylation of these proteins (pathways on/off?) in these cellines

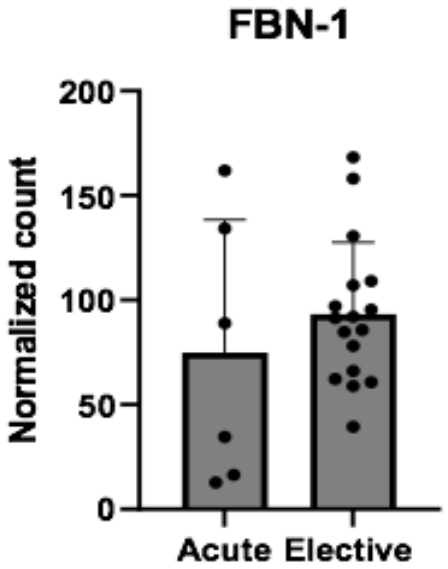


Preliminary results:
extracellular matrix and cytoskelet problems.
Tuesday 16.30h session Karlijn Rombouts



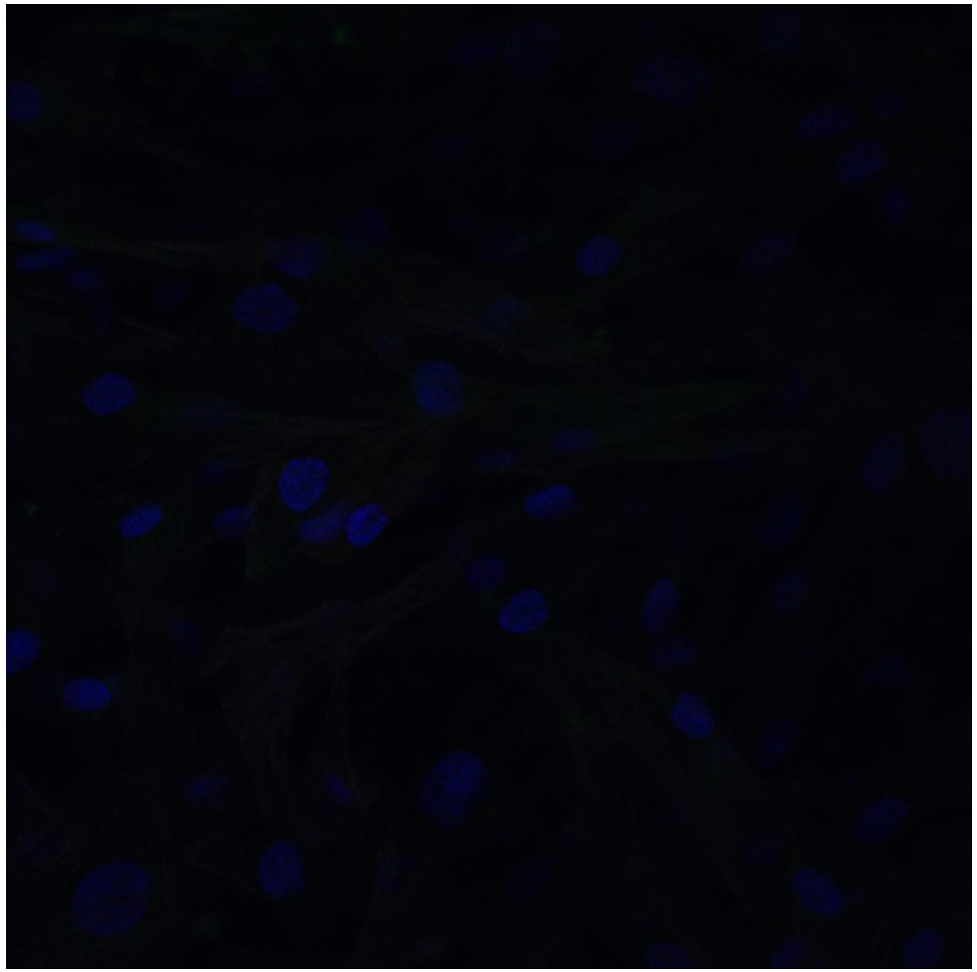
- We aim to identify proteins and pathway differences between AAA-SMC and c-SMC and proteins and pathways involved in altered SMC contraction

Also in non-familial AAA vSMC differences were found, for example FBN-1



ECM production problem?

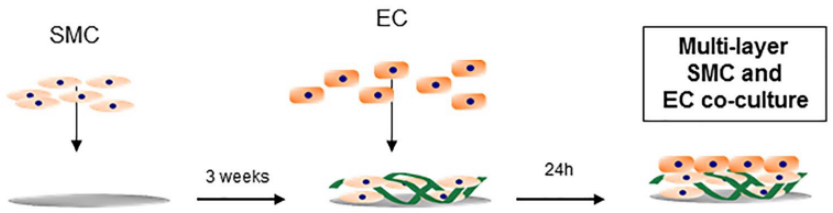
Project 2: SMC + EC 3D model



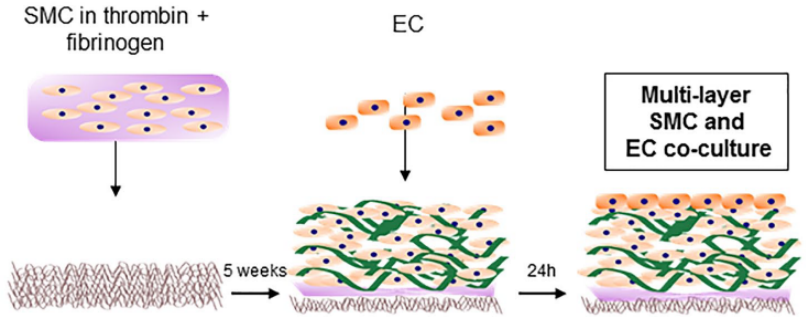
co-culture

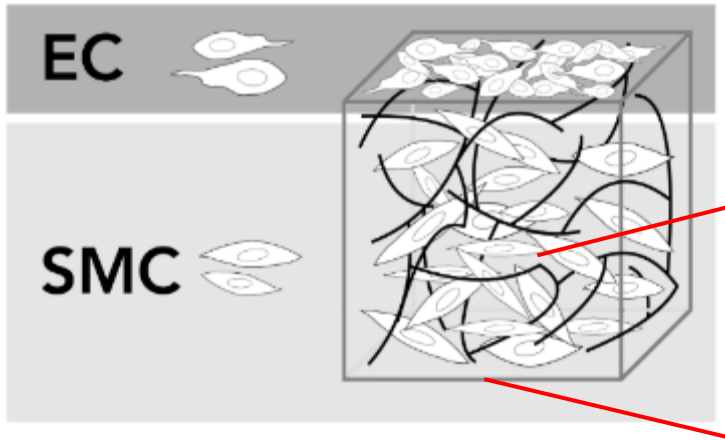
Smooth muscle cells

Endothelial cells



Our construct





Cell suspension: gel that mimics blood clotting

Scaffold: biodegradable PLGA +micropatterns to direct cell growth

Experimental Investigation

JOURNAL OF ENDOVASCULAR THERAPY

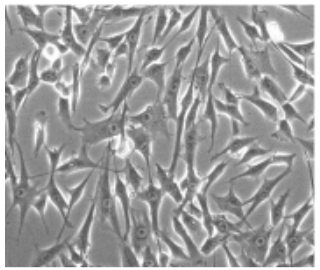
Journal of Endovascular Therapy
1–10

DOI: 10.1177/15266028211009272
www.jevt.org
SAGE

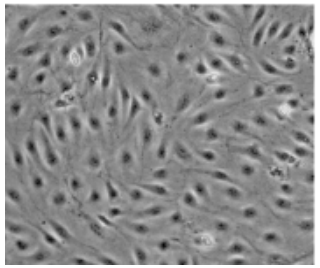
Patient-Specific 3-Dimensional Model of Smooth Muscle Cell and Extracellular Matrix Dysfunction for the Study of Aortic Aneurysms

Natalija Bogunovic, PhD[1,2,3], Jorn P. Meekel, MD[1,2], Jisca Majolée, MSc[2], Marije Hekhuis, BSc[3], Jakob Pyszkowski, MSc[4], Stefan Jockenhövel, PhD[5,6], Magnus Kruse, PhD[6,7], Elise Riesebos, MSc[3], Dimitra Micha, PhD[3], Jan D. Blankensteijn, MD, PhD[1], Peter L. Hordijk, PhD[2], Samaneh Ghazanfari, PhD[5,6*], and Kak K. Yeung, MD, PhD[1,2*]

Study the SMC and ECM in 3D

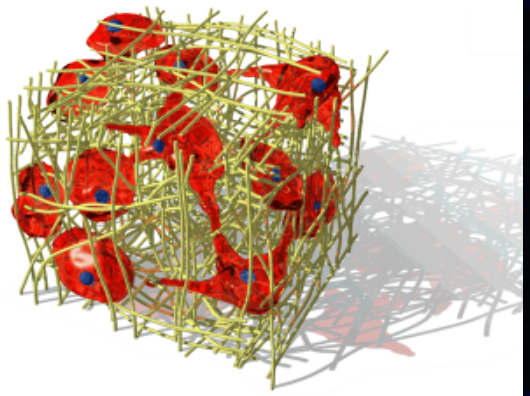


SMC from:
AAA patients
Healthy controls

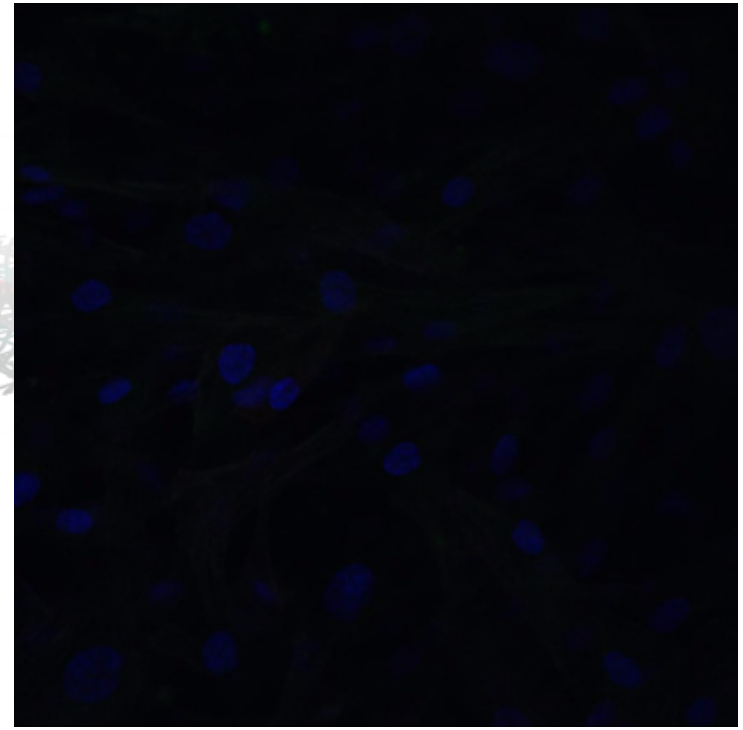


EC from:
Healthy controls

Degradable 3D printed scaffold

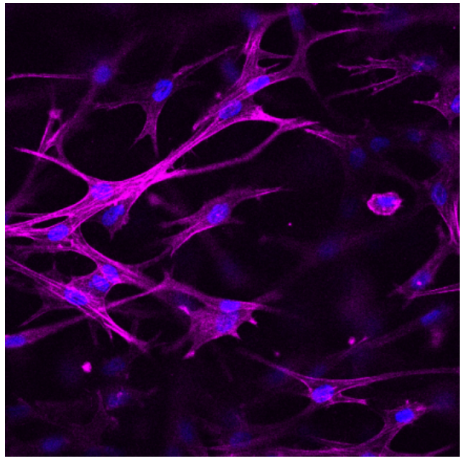


Cell growth degrades the scaffold, leading to the production of an original extracellular matrix

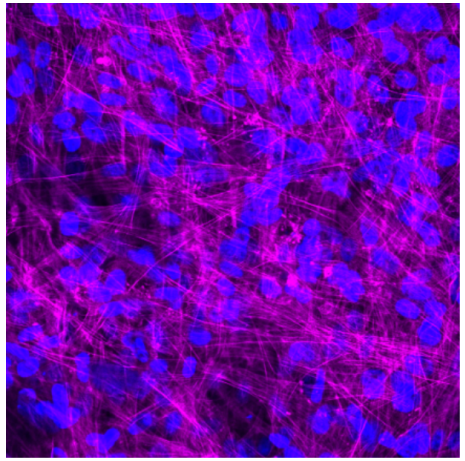


BIO-ENGINEERED VESSEL: ANISOTROPY
a parameter of order in the system

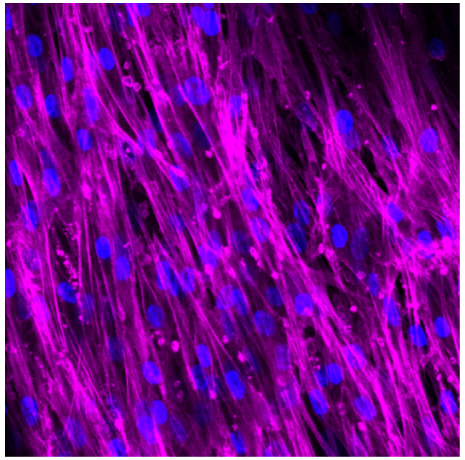
Scaffold - 5 days

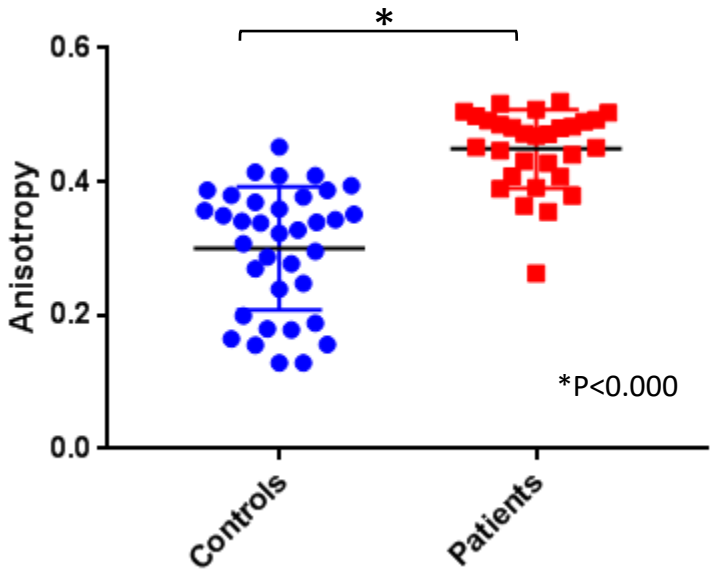


Scaffold - 2.5 weeks



Scaffold - 5 weeks

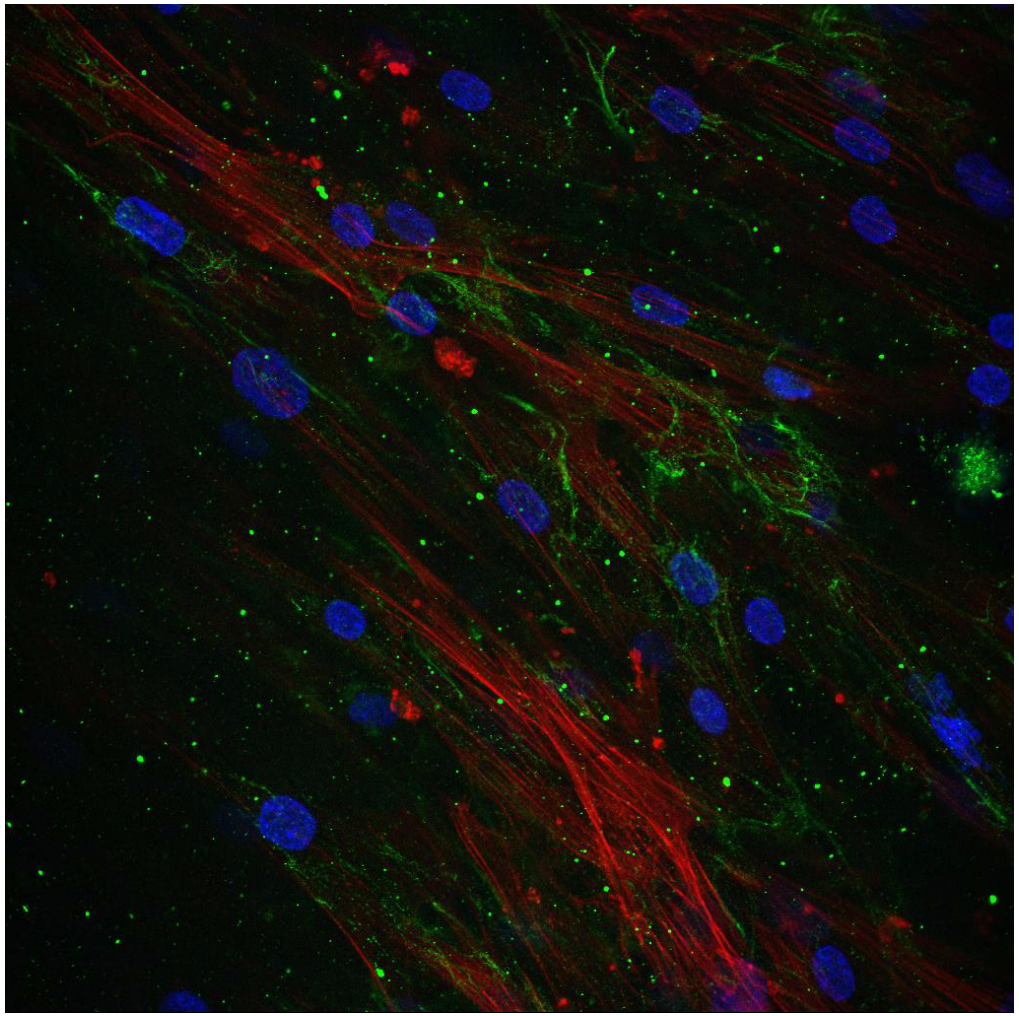




More straight matrix and
not a network of ECM in AAA patients

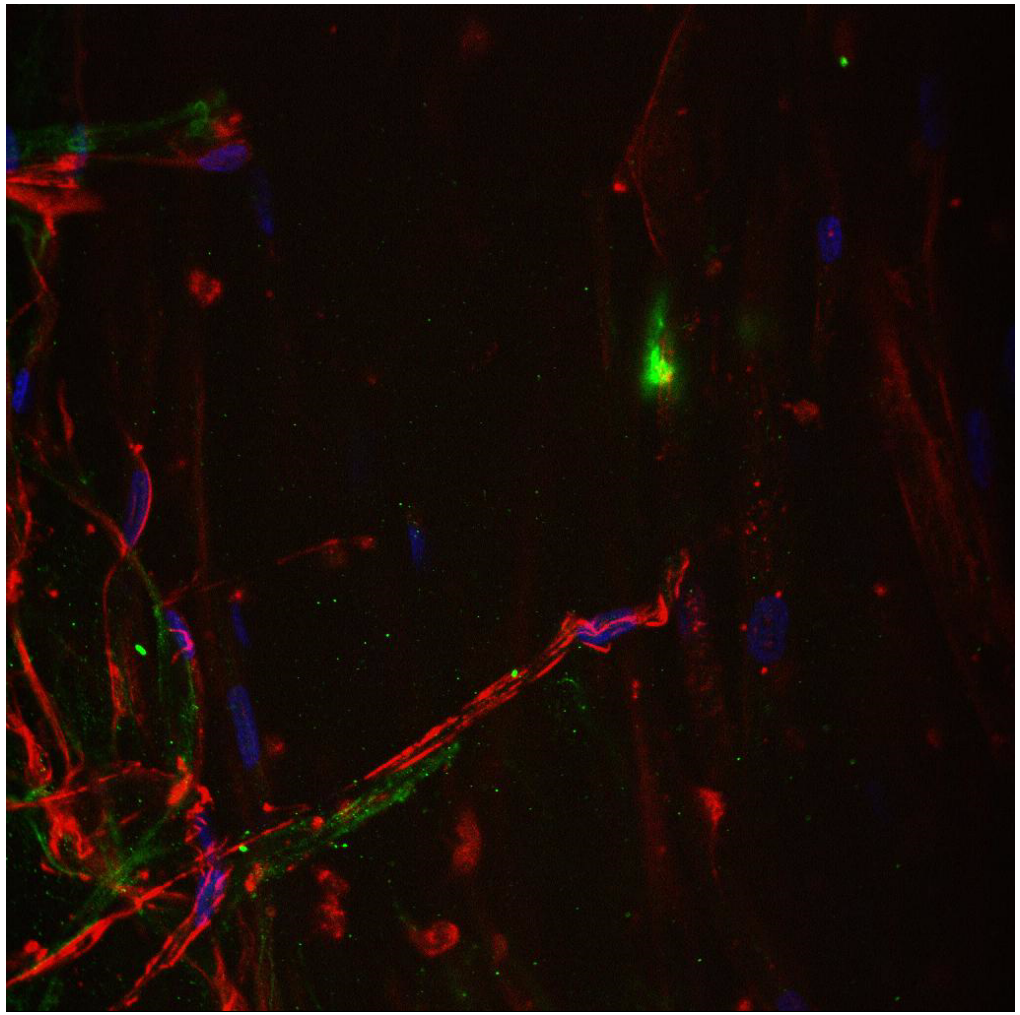
Extracellular matrix production -FBN1

CONTROL



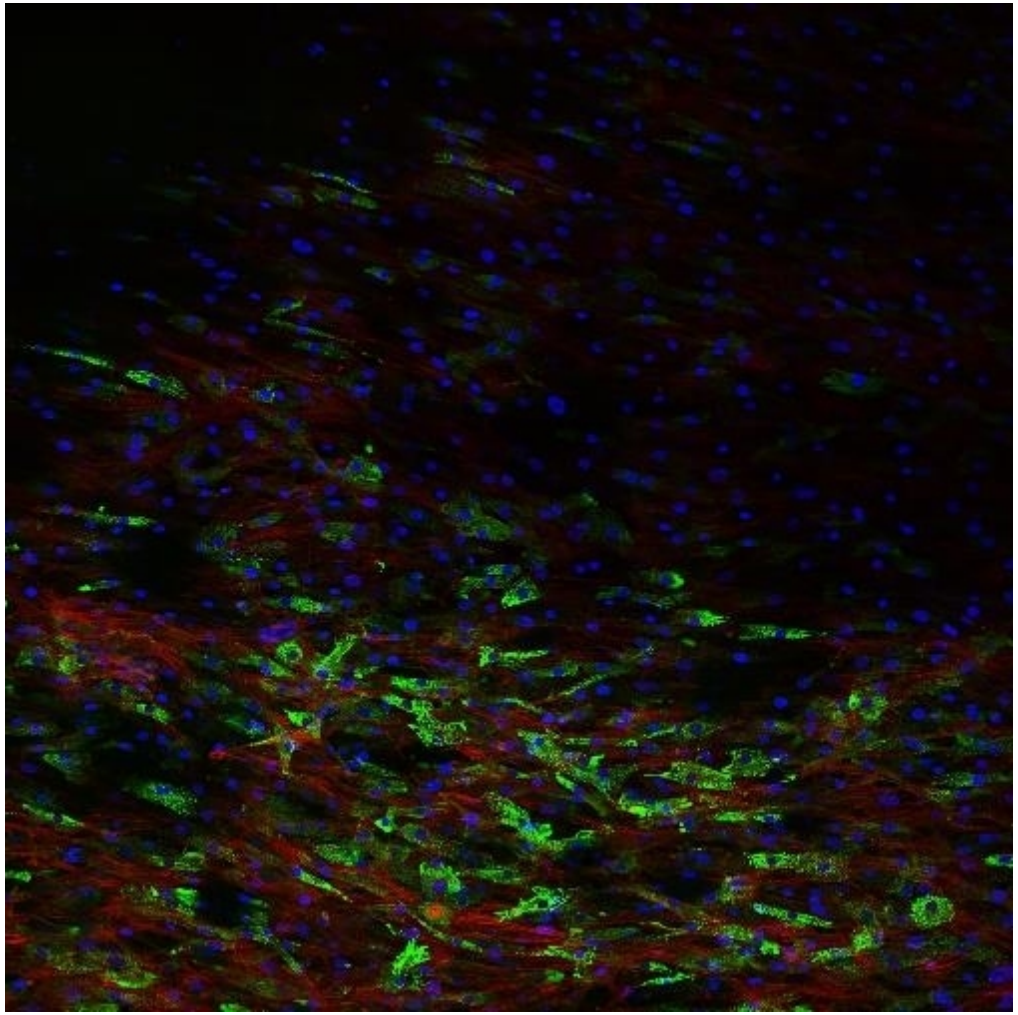
DAPI
FBN1
F-actin

PATIENT



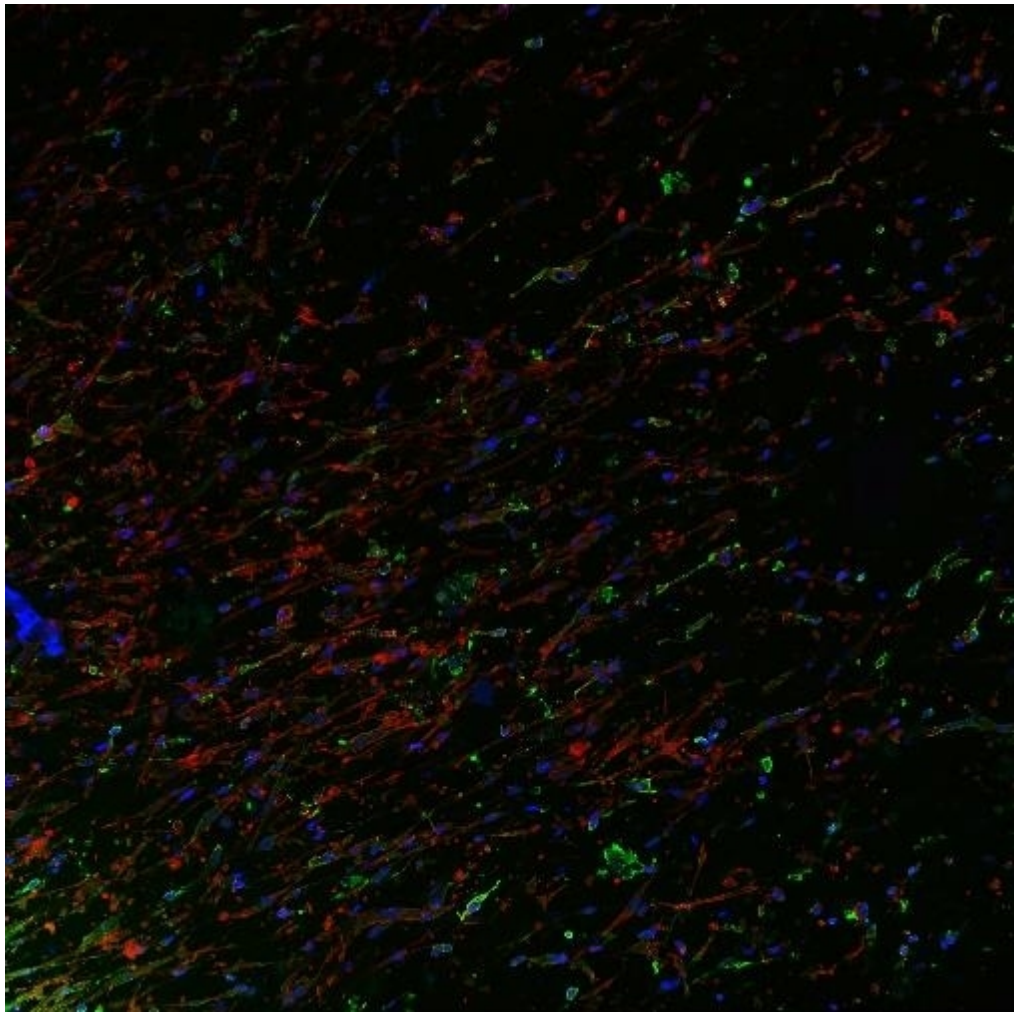
Extracellular matrix production -COL3

CONTROL

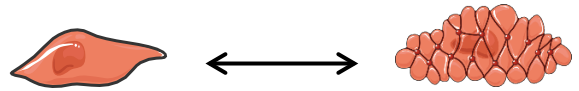


DAPI
COL3
F-actin

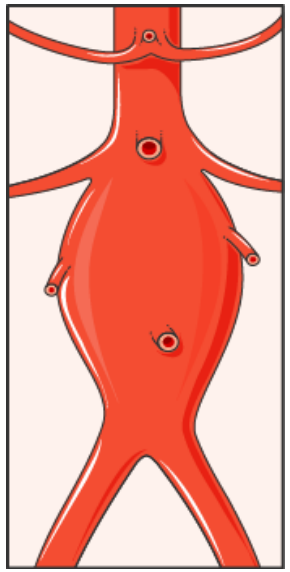
PATIENT



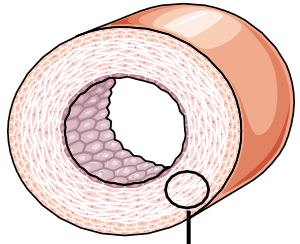
Summary

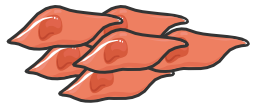


1. Impaired SMC contractility



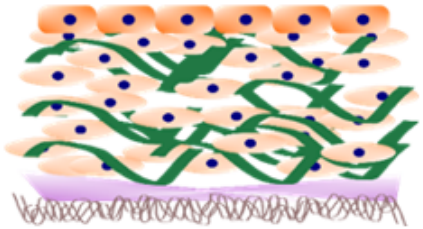
Abdominal Aortic Aneurysm





Smooth muscle cells

2. SMC + EC 3D model
→ differences in ECM

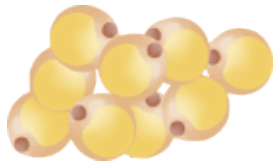


3. Transdifferentiation

Dermal fibroblast

SMC like cell

4. Perivascular fat inflammation



5. Personalised prediction + medication testing (+Imaging)

Gene therapy
Natalija Bounovic 16.30 Tuesday

Insights in the pathophysiology of AAA can contribute to finding new targets to develop new non-invasive treatment options

Amsterdam UMC
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