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The 10th international symposium on Biomechanics in Vascular Biology and Cardiovascular Disease

Thursday, April 23rd 2015

8:30 – 8:55 Registration and coffee

8:55 Opening Remarks
   Marina Senten, Dutch Heart Foundation

How to …
   Chairs: Francesco Migliavacca, Frank Gijsen

9:00 Julian Gunn (University of Sheffield)
   “Stenting coronary arteries - the clinician’s perspective ”

9:30 Steve White (University of Bristol)
   “How To Study Shear Stress Signalling In Endothelial Cells”

10:00 Matthieu de Beule (Gent University)
   “Cardiovascular device development from bench to bedside: a company perspective”

10:30 – 11:00 Coffee Break

Aneurysms
   Chair: Kristian Valen-Sendstad Lambert Speelman

11:00 Michael Walsh (University of Limerick)
   “On the role of calcifications in abdominal aortic aneurysm tissue behaviour”

11:30 Jaroslav Pelisek (Technische Universität München)
   “Biomechanics, extracellular matrix and gene expression in abdominal aortic aneurysm”

12:00 Gábor Janiga (University of Magdeburg)
   “Overview on the Computational Fluid Dynamics Challenge 2013 for rupture-prediction in two intracranial aneurysms”

12:15 Philipp Erhart (University Heidelberg)
   “Finite element analysis in asymptomatic, symptomatic and ruptured abdominal aortic aneurysms: In search of new rupture risk predictors”

12:30 Raoul Stevens (Eindhoven University of Technology)
   “Biomechanical Changes During Abdominal Aortic Aneurysm Growth”

12:45 – 14.30 Lunch and poster session

Keynote NHS Lecture
   Chair: Ton van der Steen

14:30 Prof. Dr. Chun Yuan
   University of Washington, Seattle, USA
   “On Biomechanical Conditions that Predispose Vasa Vasorum to Rupture and Cause Intraplaque Hemorrhage: An In Vivo Magnetic Resonance Imaging Study”

NHS Lecture: sponsored by the Dutch Heart Foundation, NL
The 10th international symposium on Biomechanics in Vascular Biology and Cardiovascular Disease

Vulnerable Plaques
Chair: Jolanda Wentzel, Peter Stone

15:15 Frank Gijsen (Erasmus MC)
“The stable plaque paradigm: reliably identifying thick-cap, low-stress, stable carotid fibroatheromas”

15:45 Rob Krams (Imperial College London)
“Mechanosensitive signalling pathways adapt during TCFA formation”

16:15 David de Wilde (Gent University)
“A novel application of gold particle enhanced CT: detection and quantification of vulnerable atherosclerotic plaques in mice”

16:30– 16:45 Coffee Break

Signalling
Chairs: Stéphanie Lehoux, Brenda Kwak

16:45 Hanjoong Jo (Georgia Institute of Technology and Emory University)
“Mechano-sensitive microRNAs in atherosclerosis – From Mechanobiology to Nanomedicine”

17:15 Linfang Zeng (King’s College London)
“Shear Stress and Histone Deacetylases: Maintenance of Endothelium Homeostasis”

17:45 Marwa Mahmoud (University of Sheffield)
“Disturbed flow promotes atherogenesis through the activation of endothelial-mesenchymal transition

18:00 Martin Harmsen (University of Groningen)
“Shear stress regulates the phenotype of endothelial cells by mediating the cross-talk between redox signalling and TAK1 signalling”

19:30– 21:30 Conference Dinner

Friday, April 24th 2015

Imaging
Chair: Chun Yuan, Jonathan Gillard

9:00 Aart Nederveen (Academisch Medisch Centrum Amsterdam)
“MRI based assessment of wall shear stress: is it good enough?”

9:30 Jonathan Gillard (University of Cambridge)
“MRI based assessment of structural plaque stress: assessing risk ”
10:00    Bram Trachet (Gent University)
“The role of suprarenal side branches in dissecting abdominal aortic aneurysm formation in angiotensin II-infused mice”

10:15    Pim van Ooij (Academisch Medisch Centrum Amsterdam)
“Bicuspid Valve Morphology Determines the Position of Elevated Velocity and WSS”

10:30 – 11:00 Coffee Break

Signalling
Chair: Hanjoong Jo, Paul Evans

11:00    Justin Mason (Imperial College London)
“Shear Stress, Accelerated Atherosclerosis and Therapeutic Manipulation of Cytoprotective Genes in the Vasculature”

11:30    Stéphanie Lehoux (McGill University)
“Neural guidance cues and atherosclerosis ”

12:00    Jean-François Denis (University of Geneva)
“Connexin40 regulates shear stress-induced endothelial NF-κB activation through interaction with IκBa”

12:15    Nina Rol (VU University Medical Center)
“The role of TGF-β/BMP signaling in microvascular endothelial cells in Pulmonary Arterial Hypertension”

12:30– 13:30 Lunch

Keynote Lecture
Chair: Frank Gijsen

13:30    Prof. Francesco Migliavacca,
Politecnico di Milano, Milan, IT
“Stenting and computer modeling”

14:15– 14:30 Coffee Break

Vulnerable Plaques
Chair: Rob Krams, Ton van der Steen

14:30    Peter Stone (Harvard Medical School)
“Natural History of CAD and Plaque Disruption: Implications for In-vivo Assessment”

15:00    Alfons Hoekstra (University of Amsterdam)
“Computational Modelling of the Effect of a Functional Endothelium on the Development of In-Stent Restenosis”
15:30  Ryan Pedigri (Imperial College London)
“Inducing Persistent Low Shear Stress Promotes Thin Cap Fibroatheroma Development in Hypercholesterolemic Minipigs”

15:45  Tommy Maas (Maastricht University)
“A predictive modeling framework to support vascular access surgery in a multicenter randomized clinical trial”

16:00–17:00  Awards, closing and drinks
How To: Study Shear Stress Signalling In Endothelial Cells

Steve White
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Introduction
Endothelial cells are mechanosensitive. They sense both shear stress and cyclic strain, triggering changes that profoundly affect their behaviour. Arterial segments that experience disturbed blood flow patterns are predileceted to develop atherosclerosis, and endothelial dysfuntion in response to disturbed flow is postulated to be largely responsible. This has prompted a large amount of work trying to understand the molecular basis of flow responses and how they impact cardiovascular disease.

Methods
There have been numerous systems adapted to investigate the response of endothelial cells to shear stress. These include rheostat/cone-and-plate systems, parallel plate systems and orbital shakers to induce movement of media and application of shear stress. Systems to simultaneously look at endothelial-smooth muscle interactions under flow have also been developed. In vivo analyses of endothelial function have largely focussed on the inner and outer curvature of the aortic arch and modulation of the flow in the carotid artery through application of a stenotic cast or by partial ligation. Individual components of the mechano-responsive machinery have been investigated by directly applying force to individual mechanoreceptors or by overexpressing components of the regulatory machinery, such as the transcription factor KLF2.

Results
There are two main objectives of this research, the first of which aims to identify the cellular machinery involved in the conversion of shear stress into a biological response. These experiments have frequently been performed with short-term exposure to shear stress or using direct application of force to specific mechanoreceptors. A second, slightly distinct aim is to understand how this relates to the development of atherosclerosis. This requires long-term exposure to shear stress as it takes at least 24 hours for the cells to fully adopt the phenotype of that shear environment. Additionally, the type of endothelial cell is important, as cells from a venous origin respond more aggressively to the application of shear stress compared to cells of an arterial origin. As with any experimental system, there needs to be an awareness of what the system can tell you, and what can it not. It has been shown that interaction with other cells, particularly smooth muscle cells affects the response of endothelial cells to shear stress. In addition, most systems only allow the study of shear stress and not cyclic strain, both of which affect endothelial function. Therefore it is important to at least acknowledge these caveats and look to translate in vitro findings using in vivo samples wherever possible.

Conclusions
A lot of progress has been made to understand the key pathways involved in the shear sensing mechanisms within endothelial cells and how this impacts cardiovascular disease. There is, however, a significant amount of work still needed to translate this research to beneficially impact cardiovascular disease.
On the role of calcifications in abdominal aortic aneurysm tissue behaviour

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b Department of Biomedical Engineering, Khalifa University of Science, Technology & Research (KUSTAR), P.O.Box: 127788, AbuDhabi, UAE

c Vascular Engineering, Intelligent Systems for Medicine Laboratory, School of Mechanical and Chemical Engineering ; The University of Western Australia, Perth, WA6009, Australia

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Introduction
Despite recent evidence that the incidence of abdominal aortic aneurysms may de declining, the number of deaths caused by AAA each year remains high. As rupture of the aneurysmal wall is a mechanical event it is appropriate that characterisation of aneurysmal tissue is carried out to better inform pre-operative treatment strategies and, in particular, to facilitate rupture prediction. The three main contributors to the biomechanical behaviour of AAA tissue in vivo are intraluminal thrombus, the wall tissue itself and the presence of calcifications with the tissue.

Methods
Aneurysmal tissue was collected from the anterior region of AAAs from 31 patients who underwent open surgical repair at University Hospital Limerick, Ireland. From these tissues 79 test samples were prepared and subjected to tensile testing. Fourier Transform InfraRed spectroscopy was carried out prior to testing to determine the presence of calcifications in the tissue. Preconditioning was carried out to 10% gauge length at a rate of 20% gauge-length/min for 5 cycles. Scanning electron microscopy and energy dispersive X-ray spectroscopy were employed to characterise the morphology and assess the chemical composition of the cross sectional area of the failure locations of the test samples.

Results
Characteristics of the patient cohort include 24/31 being male, age was 71.59±6.53 years, maximum diameter was 6.62±1.55cm and wall thickness was 1.24±0.07cm. 10 tests were excluded from the results as the samples either slipped in the clamps or failed near the clamps. Of the 69 samples included in the analysis 38 were identified as containing calcification within the gauge length. A statistically significant difference was found between the fibrous and partially calcified group for failure stress (p=0.01) i.e. 1.20±0.33 MPa vs. 0.87±0.32 MPa and failure stretch (p=0.04) i.e. 1.31±0.09 vs.1.25±0.04. Statistical significance was not achieved between the groups for measures of failure tension (p=0.27) i.e. 12.10±5.85 N/cm vs. 9.96±3.19 N/cm, however, the average trend was similar with the fibrous group exhibiting higher values than the partially calcified group. SEM images of the fibrous tissue’s failure line, following uniaxial tension tests, showed evidence of tissue delamination, severe thinning and prolonged extension of fibres whereas images of the partially calcified tissue group revealed large calcification deposits, embedded in the intima-medial layer at or in close proximity to the fracture line in all cases examined.

Conclusions
The failure properties of AAA wall tissue that contains calcifications are significantly reduced, supporting the hypothesis that a mismatch in compliance between the calcifications and the surrounding fibrous tissue may increase the rupture potential of the aneurysm.
Biomechanics, extracellular matrix and gene expression in abdominal aortic aneurysm

Jaroslav Pelisek¹, Christian Reeps¹, Sebastian Kehl², Fadwa Tanios¹, Jonas Biehler³, Wolfgang A. Wall², Hans-Henning Eckstein¹, Michael W. Gee³

¹Department of Vascular and Endovascular Surgery, ²Mechanics & High Performance Computing Group, ³Institute for Computational Mechanics; Technische Universität München, Germany

Introduction
The interaction of mechanical and biological factors in the pathogenesis of human abdominal aortic aneurysm (AAA) is widely accepted but so far purely explored. The aim of the study was therefore to analyse underlying relationships between local material properties of AAA wall and the concomitance of extracellular matrix components as well as gene expression of destabilising inflammatory, proteolytic, and structural factors.

Methods
Numerical simulation, tensile tests, histological and gene expression analyses were performed on 51 tissue samples from 31 patients, who underwent elective open surgical repair of AAA. Pre-surgical CT-data were used for 3D-segmentation of AAA and calculation of mechanical conditions by advanced finite element analysis (FEA). AAA wall stiffness, strength and failure tension were assessed by repeated cyclic sinusoidal and destructive tensile testing procedures. Collagen I and III, total collagen, elastin, and proteoglycans were quantified by computational image analyses following appropriate histological staining. Gene expression of collagen I and III, inflammatory factors CD45, MSR1, proteolytic enzymes MMP-2, -9, and their inhibitor TIMP-1 were analysed by quantitative RT-PCR.

Results
In AAA tissue samples, higher content of collagen I, III and total collagen were significantly associated with increased local wall stress ($r = 0.405, 0.323$ and $0.296$, $p = 0.002, 0.017$ and $0.030$, respectively) and likewise with von Mises strain ($r = 0.406, 0.338$ and $0.315$, $p = 0.002, 0.012$ and $0.020$, respectively). AAA wall failure tension exhibited significant positive correlation with collagen I, total collagen and proteoglycans ($r = 0.302, 0.301$ and $0.329$, $p = 0.037, 0.038$ and $0.022$, respectively). Aortic wall elasticity determined by $\alpha$-stiffness correlated with collagen I, III and total collagen ($r = 0.361, 0.298$ and $0.374$, $p = 0.011, 0.038$ and $0.008$, respectively), while $\beta$-stiffness was associated only with the level of proteoglycans ($r = 0.313, p = 0.028$). Furthermore, local AAA diameter was negatively correlated with elastin ($r = -0.361, p = 0.007$) and increased thrombus thickness was indirect proportional associated with collagen I, III and total collagen ($r = -0.399, -0.315$ and $-0.331$, $p = 0.003, 0.020$ and $0.015$, respectively). Regarding the expression of various destabilising factors of AAA and mechanical properties of the diseased aortic wall, expression of collagen III correlated with $\alpha$-stiffness ($r = -0.348, p = 0.017$). MMP-2 correlated with $\beta$-stiffness and wall strength ($r = -0.438$ and $-0.593$, $p = 0.005$ and $<0.001$, respectively). Furthermore, significant relationships were observed between local AAA diameter and the expression of CD45, MSR1 and TIMP-1 ($r = 0.285, 0.551$ and $0.328$; $p < 0.05$). However, no interrelation between local wall stress or strain and gene expression of the analysed factors was observed.

Conclusions
Our results indicate that in AAA wall increased locally acting biomechanical forces involve increased synthesis and expression of different components of extracellular matrix, especially collagens and proteoglycans. These findings confirm the presence of adaptive biological processes to maintain the mechanical stability of AAA.
Overview on the Computational Fluid Dynamics Challenge 2013 for rupture-prediction in two intracranial aneurysms

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Introduction
In the recent years various simulation challenges have been organized in order to check the applicability of computational hemodynamics for intracranial aneurysms. E.g., Steinman et al. [1] compared the velocity and pressure values obtained by various groups in a giant aneurysm. Nevertheless, one of the main difficulties when performing computational fluid dynamics (CFD) for patient-specific cases is the prediction of the rupture probability. Therefore, a computational challenge has been announced in 2013 [2].

Methods
The Computational Fluid Dynamics Challenge 2013 for rupture-prediction involved two patient-specific MCA-aneurysms for CFD analysis (Fig. 1). One of the provided cases was a ruptured, the other one was considered as unruptured aneurysm. The documented ruptured site of the aneurysm was not communicated in advance. The reconstructed geometries were provided to the participants as surface triangulation mesh files.

26 groups from 15 countries submitted a short abstract describing the details of their computations and their rupture predictions, representing a worldwide snapshot in computational hemodynamics of intracranial aneurysms.

During Phase I, each group had the freedom to choose any desired volume mesh with arbitrary types of elements and resolution, flow/velocity boundary conditions, etc. The objective of Phase I was to predict the ruptured aneurysm, and if possible the rupture site as well.

In Phase II, boundary conditions were prescribed. Here, the computational results have been compared in order to evaluate the variability.

Results
21 of 26 groups (81%), predicted the ruptured aneurysm correctly in Phase I. Various criteria have been applied in order to predict the rupture site. The documented rupture site was mostly associated with low time-averaged wall-shear stress (WSS) and high oscillatory shear index (OSI). However, this combined criteria is more pronounced at several locations outside of the documented rupture site. This study has thus shown that the exact rupture prediction remains quite challenging.

Conclusions
Around 80% of the participating groups were able to predict the rupture status from two intracranial aneurysms on the basis of their computational results. However, the rupture-site predictions were distributed throughout the entire aneurysm sac, showing the complexity of such a prediction based on CFD computations.

References
Finite element analysis in asymptomatic, symptomatic and ruptured abdominal aortic aneurysms: In search of new rupture risk predictors

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Introduction:
Finite element analysis (FEA) might be a complement diagnostic tool to predict abdominal aortic aneurysm (AAA) rupture risk. This study compared biomechanical rupture risk parameters of asymptomatic, symptomatic and ruptured AAAs.*

Methods:
From 2011 to 2013 computed tomography angiography (CTA) data from 30 asymptomatic, 15 symptomatic, and 15 ruptured AAAs were collected consecutively. FEA was performed according to the successive steps of AAA vessel reconstruction, segmentation and finite element computation. Biomechanical parameters like Peak Wall Rupture Risk Index (PWRI), Peak Wall Stress (PWS), and Rupture Risk Equivalent Diameter (RRED) were compared among the three subgroups.

Results:
PWRI differentiated between asymptomatic and symptomatic AAAs (p <.0004) better than PWS (p <.1453). PWRI-dependent RRED was higher in the symptomatic subgroup compared with the asymptomatic subgroup (p <.0004). Maximum AAA external diameters were comparable between the two groups (p <.1355). Ruptured AAAs showed the highest values for external diameter, total intraluminal thrombus volume, PWS, RRED and PWRI compared with asymptomatic and symptomatic AAAs. In contrast with symptomatic and ruptured AAAs none of the asymptomatic patients had a PWRI value >1.0. This threshold value might identify patients at imminent risk of rupture.

Conclusions:
From different FEA derived parameters, PWRI distinguished most precisely between asymptomatic and symptomatic AAAs. If elevated, this value may represent a negative prognostic factor for asymptomatic AAAs.

References:
Biomechanical Changes During Abdominal Aortic Aneurysm Growth

Raoul Stevens¹, Andrii Grytsan², Jacopo Biasetti², Joy Roy³, Moritz Lindquist Liljeqvist³, T. Christian Gasser²

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Introduction
Abdominal Aortic Aneurysms (AAAs) are local dilations of the abdominal aorta, frequently seen in the elderly population. Most of the AAAs contain an Intra-Luminal Thrombus (ILT), and despite that it plays an important role in AAA progression, its exact role is still unclear. With a high risk of rupture and mortality rates, accurate rupture risk assessment of AAAs is important. Nowadays, the maximum diameter and the growth rate are clinically used to predict the average rupture risk. However, both indices lack the power to describe the rupture risk of individual patients. In order to find improved risk indicators biomechanical changes during aneurysm growth are investigated.

Methods
Four patients with a total of 23 Computer Tomography-Angiography (CT-A) scans at different time points were analyzed. Patient-specific AAA geometries were reconstructed and structurally analyzed using semiautomatic diagnostic software (A4clinics Research Edition, VASCOPS GmbH, Austria). Specifically, key geometrical and structural properties as well as growth parameters, i.e. the change between two consecutive scans, were determined. To assess the hemodynamical parameters, five heart cycles with physiologically realistic boundary conditions were analyzed using rigid-wall Computational Fluid Dynamics (CFD) simulations (ANSYS 15.0, ANSYS Inc., US). Each AAA lumen was represented by approximately 300k finite volume elements and the non-Newtonian blood behaviour was modelled using the Carreau-Yasuda model. The flow field was analyzed using the λ²-method and the CFD simulations were qualitatively validated by reviewing the vortices and the Wall Shear Stress (WSS) [1]. Statistical correlation analyses were carried out to examine possible links amongst the investigated parameters.

Results
Correlations between the change in ILT volume and the maximum Wall Shear Stress (WSS) (p=0.002) as well as the blood's minimum shear rate (p=0.003) were observed. In addition, the change in ILT volume did correlate with the Oscillatory Shear Index (OSI) (p=0.028) and the predicted biomechanical rupture risk, i.e. the Peak Wall Rupture Index (PWRI) (p=0.008).

Conclusions
The finding that the change in ILT volume correlated with the PWRI might indicate that fast ILT growth defines an elevated risk for AAA rupture. This conclusion has also been drawn previously from purely clinical observations [2]. Consequently, especially in aneurysm surveillance programs, the change in ILT volume may serve as an additional AAA risk indication parameter, which however, needs to be further validated in clinical studies.

References
On Biomechanical Conditions that Predispose Vasa Vasorum to Rupture and Cause Intraplaque Hemorrhage: An In Vivo Magnetic Resonance Imaging Study

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Introduction
Intraplaque hemorrhage (IPH) is a common finding in human atherosclerotic plaques, which is implicated in rapid plaque progression and plaque rupture [1-2]. Despite its high clinical relevance, effective prevention and management strategies of IPH remain elusive due to our limited understanding of its etiology and contributing factors. A notable observation in this regard is the colocalization of IPH and vasa vasorum seen in both histopathological and imaging studies [3-4]. However, specific biomechanical conditions that may predispose vasa vasorum to rupture and cause IPH are unknown. This study aims to test if hemodynamic parameters of the cardiovascular system could be associated with IPH.

Methods
In a cohort study on the natural history of carotid atherosclerosis, subjects with 16-79% stenosis on carotid ultrasound are scanned with 3-dimensional MRI. Individual plaques (thickness>1.5 mm) in bilateral carotid arteries are identified through a large-coverage, isotropic, motion-sensitized driven equilibrium prepared rapid gradient echo (3D-MERGE) sequence [5]. The determination of IPH presence in each plaque is through a phase sensitive sequence referred to as simultaneous noncontrast angiography and intraplaque haemorrhage (SNAP) [6], which shares the same coverage and resolution as 3D-MERGE and can be flexibly reconstructed to highlight IPH or flowing blood as needed. The same MRI protocol is performed at 1-year follow-up to monitor plaque progression.

Results
Subject recruitment and image analysis are ongoing. This study provides an opportunity to evaluate the association between IPH and clinical variables that influence systemic hemodynamical conditions including heart rate and blood pressures in a contemporary cohort with distinct carotid plaques.

Conclusions
Our interim data as well as data from the Rotterdam study [7] have suggested a role of hemodynamical forces in the pathophysiology of IPH. Specific biomechanical conditions of plaques with IPH and their effect on vasa vasorum warrant in-depth investigations.

References
The stable plaque paradigm: reliably identifying thick-cap, low-stress, stable carotid fibroatheromas

H. Nieuwstadt, J. Wentzel, H. Verhagen, A. van der Lugt, A. van der Steen, F. Gijsen

Erasmus MC, Rotterdam, the Netherlands

Introduction: The peak cap stress is a promising biomechanical marker for plaque rupture risk, and current focus lies on identifying high-stress plaques. However, our recent study showed that MRI-based finite element analysis (FEA) stress computations are highly unreliable for thin-cap, high-stress carotid plaques [2]. Thick-cap, low-stress plaques yielded a reliable segmentation and stress computation. Can we better identify low-stress, histologically-stable carotid lesions to minimize unnecessary surgeries?

Methods: We obtained 32 carotid histological plaque cross-sections from 12 patients with >70% stenosis and classified the lesions according to a histological classification scheme [1]. We indentified 8 plaques as pathologic intimal thickening (Type 2), 14 as fibrous cap atheromas (Type 3), and 10 as thin fibrous cap atheromas (TCFA) (Type 4) with a cap thickness <200 microns. Type 2 and 3 plaques are generally considered stable while TCFA are acknowledged rupture-prone, vulnerable plaques. The peak cap stress in the cross-sections was computed with Abaqus 6.11. To mimic in vivo carotid MRI, we subjected all plaques to numerical MRI simulations (JEMRIS). This provided a fully-controlled setting enabling a submillimeter-scale comparison with the underlying morphology/stress [2]. MRI segmentations were subjected to FEA for the MRI-based plaque model stress.

Results: One example and all results are shown in the Figure. We found that the peak cap stress in Type 2 plaques was on average the lowest, followed by Type 3 plaques. Type 4 plaques had the highest average stress values. Importantly, not all Type 4 plaques had a peak cap stress higher than a fibrous cap atheroma (Type 3). This disagreement between histological classification and FEA outcome suggests the likely added value of biomechanical stress analysis over merely histology-based classification. As shown in the table, only the selection of the lowest stress plaques (25% quartile) guaranteed agreement with a histologically-classified stable plaque. MRI-based plaque FEA resulted in an inaccurate, underestimated peak cap stress, yet it still yielded consistent agreement with histological classification for only the lowest-stress (25% quartile), stable plaques.

Conclusions: With current MRI technology, a large fraction of high-stress, vulnerable carotid plaques cannot be reliably identified due to stress underestimation and disagreement with histological classification. Low-stress plaques based on MRI FEA were always histologically classified as stable. Therefore, identifying solely the lowest-stress carotid plaques could be a more effective approach to reduce unnecessary surgeries.

Mechanosensitive signalling pathways adapt during TCFA formation

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Introduction
A surge of recent studies have highlighted the role of blood flow in determining plaque growth and plaque composition, both in animal studies and recent clinical trials. Despite these compelling data the underlying mechanism for flow-induced plaque formation is currently unknown.

Methods
A platform consists of state-of-the-art ultra-high resolution, small animal imaging (µCT, µMRI and US) coupled to finite element methods to determine the endothelial shear stress and strain fields during the development of the vulnerable plaque. These maps will subsequently drive a robot-driven laser-capture machine (Zeiss, PALM microbeam) which will isolate groups of endothelial cells exposed to a certain shear and strain field during plaque development. Next, RNA will be isolated, amplified with linear amplification kits, and libraries are prepared for deep RNA sequencing (Illumina Hiseq 2500). After this, in-house developed bioinformatics tools will be applied to decipher gene networks and gene modules.

Results
New shear metrics have been identified that predict/drive advanced plaque formation\(^1,2\). A low shear index was capable of predicting both small animal (mice) and large animals (pigs) TCFA formation and shown to be superior to other indices to predict TCFA formation and location. Single shear metrics were incapable of predicting TCFA formation sufficiently and a combination of metrics appeared essential to predict TCFA formation over time accurately\(^1,2\).

These metrics are now incorporated in a platform where endothelial cells are captured on basis of shear metrics and RNA is isolated, and cDNA is generated. This allows performing RNAseq and to decipher gene networks that are involved in progression of plaques to advanced plaques.

Conclusions
A simple low shear index was dominant in driving advanced plaque formation, including TCFA in mice and pigs. This metric will be used to identify small groups of endothelial cells for further genomic analysis.

References
A novel application of gold particle enhanced CT: detection and quantification of vulnerable atherosclerotic plaques in mice

David De Wilde (1), Bram Trachet (1,2), Carole Van der Donckt (3), Bert Vandeghinste (1), Benedicte Descamps (1), Christian Vanhove (1), Guido R. Y. De Meyer (3), Patrick Segers (1)

(1) Department of Electronics and Information Systems, Ghent University – iMinds -iBiTech, Gent, Belgium
(2) Institute for Bioengineering, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland
(3) Division of Physiopharmacology, University of Antwerp, Wilrijk, Belgium

Introduction

In-vivo differentiation between vulnerable plaques, prone to rupture, and stable plaques, which do not rupture, is challenging. One of the indicators of plaque vulnerability is the presence of macrophages. In this abstract we describe a novel method to visualize and quantify the presence of macrophages in atherosclerotic plaques, based on the uptake of nanoparticles in in-vivo contrast-enhanced micro CT. The methodology has been applied to a group of mice known to develop vulnerable plaques (ApoE−/− Fbn1C1039G+/−) and a group of mice developing a more stable plaque phenotype (ApoE−/−).

Methods

Female, 6-weeks-old ApoE−/− (n=9) and ApoE−/−Fbn1C1039G+/− (n=14) mice were put on a western type diet for 20 weeks. At weeks 10, 15 and 20 after the start of the diet they underwent a 50 μm micro-CT imaging protocol (Triumph-II, Trifoil Imaging, CA, USA) involving, at each time point, one scan prior to and one scan after contrast injection. As contrast agent, we used AuroVist (Nanoprobes, Yaphank, NY). Both carotid bifurcations were segmented using VMTK (www.vmtk.org), based on the angiographic images. At weeks 15 and 20, contrast accumulations (from previous contrast injections) were visible within the arterial wall on the pre-contrast scans. Metrics were subsequently developed to quantify the amount of accumulated contrast, (> 350 HU) with a threshold of 0.3 mm into the vessel wall, along its normal. The resulting value was mapped onto the luminal surface. The contrast-enhanced area fraction [CAF, expressed in %area] was calculated along the three branches of the carotid bifurcation: the external (ECA), internal (ICA) and common (CCA) carotid arteries. The CAF quantifies the area percentage of each branch that was affected by contrast accumulations and as such it serves as an indirect measure for the extent of plaque development. Animals were sacrificed after 20 weeks and tissue samples were collected for histological analysis.

Results

Histology confirmed the hypothesis that AuroVist was taken up by macrophages. The CAF values (figure 1) show a clear growth over time of the plaque between weeks 15 and 20 (p<0.01) and higher values for the ApoE−/− Fbn1C1039G+/− mice compared to the ApoE−/− mice (p<0.05). The highest amount of accumulation occurred in the ECA. No statistical difference in uptake between the left and right carotid bifurcation was detected.

Conclusions

We proposed a novel methodology to quantify in-vivo macrophage infiltration in atherosclerotic plaques, and validated it with histology. The method detected a significant difference between mice with vulnerable plaques and mice with stable plaques.

Acknowledgements

David De Wilde and Carole Van der Donckt are recipients of research grants of the IWT. Christian Vanhove is supported by the GROUP-ID consortium.

Figure 1: The contrast enhanced area fraction (CAF) showing the area where contrast is present at the three branches of both strains of mice.
Mechano-sensitive microRNAs in atherosclerosis - From Mechanobiology To Nanomedicine

Hanjoong Jo, PhD, FAHA, FAPS, FBMES

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Atherosclerosis is the major underlying cause of myocardial infarction and stroke and preferentially occurs in arterial regions exposed to disturbed flow (d-flow) by mechanisms involving broad changes in gene expression. We have shown that D-flow rapidly induces atherosclerosis in vivo using a mouse partial carotid ligation model. In addition, we developed a novel intimal RNA preparation method using this animal model and identified numerous mechanosensitive endothelial genes and epigenetic DNA methylome that change in response to d-flow. We showed that flow robustly regulate expression of microRNAs (miRNAs). I will discuss the role of mechanosensitive miRNAs in endothelial dysfunction and atherosclerosis. Our results suggest that targeting mechanosensitive genes and "athero-miR" with anti-miR-based approaches may provide a new treatment paradigm in atherosclerosis. I will also discuss how we use nanotechnology to develop endothelial-targeted delivery of miRNA inhibitors to treat atherosclerosis using mouse models.
Shear Stress and Histone Deacetylases: Maintenance of Endothelium Homeostasis

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Introduction
Dysfunction of endothelium is the initial step of cardiovascular disease development. Along the arterial tree, endothelial cells (ECs) sense and respond to different flow patterns due to the variation of local geography. Shear stress imposed by blood flow plays an essential role in the maintenance of vascular homeostasis.

Methods
Laminar flow was applied to ECs or pre-differentiated embryonic stem cells (ESCs) cultured on glass slide with a rectangular chamber system, while disturbed flow was applied to ECs cultured in 75ml flask using a shaker at 90rpm with a 7º angle, which created an oscillatory flow with 4.7dyne/cm² shear stress.

Results
Laminar flow on mature ECs increased HDAC1 stabilization and activation through a VEGF receptor/PI3K pathway. The activated HDAC1 in turn deacetylated p53 protein at Lys320 site, leading to the transcription of p21waf1. The overall effect is to increase cell quiescence and survival. Laminar flow on pre-differentiated ESCs increased HDAC3 stabilization and activation through a VEGF receptor/PI3K pathway. The activated HDAC3 in turn deacetylated p53 protein at Lys317 site, leading to the transcription of p21waf1. The overall effect is to increase ESC differentiation toward EC lineage, leading to the repair of damaged blood vessel. Knockdown of HDAC3 or p21waf1 attenuated laminar flow or VEGF-induced EC differentiation. Disturbed flow on mature ECs stabilized and activated HDAC3 through VEGF receptor/PI3K pathway. The activated HDAC3 formed a complex with mTOR, the unsliced X-box binding protein 1 and Akt1, leading to Akt phosphorylation, suggesting a feed forward mechanism for Akt activation. The active Akt1 upregulated heme oxygenase-1 transcription, which antagonized disturbed flow-induced oxidative stress. Knockdown of HDAC3 in ECs increased endothelium damage and neointima formation. Recent study revealed that mouse HDAC3 underwent unconventional splicing, contributing to endothelial-to-mesenchymal transition and cardiac fibrosis in transverse aortic constriction mouse model.

Conclusions
Shear stress maintains the endothelium homeostasis through modulating functional class I HDACs expression and activation. Targeting HDACs may provide therapeutic strategies to intervene cardiovascular diseases.

References
Disturbed flow promotes atherogenesis through the activation of endothelial-mesenchymal transition

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Introduction
Atherosclerosis is influenced by local blood flow patterns, which exert wall shear stress (WSS) on endothelial cells (EC). Low, oscillatory WSS promotes atherosclerosis by inducing EC proliferation and permeability, while high WSS is athero-protective. We recently used microarray technology coupled to computational fluid dynamics to study the transcriptome of EC at regions of the porcine aorta exposed to low, oscillatory or high, unidirectional WSS. The study revealed differential expression of GATA4 and Twist1. These transcription factors promote endothelial-mesenchymal transition (EndMT), a process that involves altered vascular endothelial (VE)-cadherin function and enhanced EC proliferation. Here we tested the hypothesis that GATA4 and Twist1 may promote atherogenesis at sites of disturbed flow by inducing EndMT.

Methods
The expression of GATA4, Twist1 and downstream effectors of EndMT was measured at inner (low WSS) and outer (high WSS) sites of the porcine and murine aorta by qRT-PCR or by en face immunofluorescent staining and confocal microscopy. To establish a causal link between flow and EndMT, WSS was modified in murine carotid arteries using a constrictive cuff. Twist1 function was determined by conditional deletion in vascular EC (Tie2-KO). Porcine aortic EC (PAEC) or human umbilical veins EC (HUVEC) were exposed to flow using an orbiting 6-well plate (210 rpm) which generates low, oscillatory WSS (centre) and high, unidirectional WSS (periphery). The expression of GATA4, Twist1 and EndMT genes was determined by qRT-PCR and immunostaining. Chromatin Immunoprecipitation (ChIP) was used to assess GATA4 promoter interaction with Twist1 and Snail. The expression of GATA4, Twist1 and Snail was silenced in cultured EC using siRNA and the subsequent effects on proliferation (Ki67 immunofluorescence) and permeability (Transwell assay) was determined.

Results
The expression of GATA4, Twist1 and EndMT effector genes (Snail, Slug, N-cadherin) was enhanced at the inner compared to the outer curvature in porcine (p<0.05) and murine (p<0.05) aortae. The application of a flow-modifying cast induced Twist1 at the low WSS site (proximal to stenosis) and induced GATA4 at the low, oscillatory WSS site (distal). Similarly, the expression of Twist1, GATA4, Snail, Slug and N-cadherin was significantly higher in HUVEC or PAEC exposed to low, oscillatory WSS compared to high, unidirectional WSS (all p<0.05). Gene silencing demonstrated that GATA4 and Twist1 are required for Snail induction in EC exposed to low, oscillatory WSS, and ChIP revealed GATA4 interaction with promoter regions of Twist1 and Snail, suggesting that disturbed flow induces EndMT via a GATA4-dependent transcriptional process. Low, oscillatory WSS promoted several changes that are characteristic of EndMT including N-cadherin induction, VE-cadherin disorganization and enhanced proliferation. Silencing of GATA4, Twist1 and Snail significantly reduced these processes and limited EC permeability (all p<0.05) in EC exposed to low, oscillatory WSS, indicating that these transcription factors are positive regulators of EndMT in cells exposed to disturbed flow. Finally, Snail expression at the inner curvature of the murine aorta was reduced by deletion of Twist1 in EC (Tie-2 Twist1-KO) compared to controls (Twist1-KO, p<0.05), demonstrating that Twist1 promotes Snail expression at a low WSS site in vivo.

Conclusions
We conclude that low WSS induces EndMT and subsequent EC proliferation and permeability through the induction of GATA4 and Twist1. Our observations illuminate for the first time, the role of EndMT in arterial biomechanics and injury. Future studies should define the role of EndMT in focal atherosclerosis.
Shear stress regulates the phenotype of endothelial cells by mediating the cross-talk between redox signalling and TAK1 signalling.

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Introduction
Severe alterations in endothelial phenotype cause endothelial dysfunction. This is featured by reduced bioavailability of nitric oxide (NO), and accumulation of reactive oxygen species (ROS) and links to TGFβ signaling in particular TAK1. In total it underlies vascular disorders such as atherosclerosis, neointimal formation, pulmonary arterial hypertension, peripheral and coronary artery disease. Released NO is anti-inflammatory effect on endothelial cells, while NO production is promoted by fluid shear stress. We hypothesized that high shear stress rescues endothelial cells from dysfunctional phenotype through regulation of redox signaling and TAK1 signaling.

Methods
Human umbilical vein endothelial cells (HUVECs) were cultured in bare basal medium or supplemented with either endothelial cell growth factor and/or TGF-β1. The HUVEC were exposed to high shear stress (20 dynes/cm²) or none. The influence of specific pathways such as ERK5, TAK1, p38, NFκB and JNK was investigated by using retroviral overexpression or specific inhibitors. Data were obtained by Western blotting, ELISA, quantitative real time PCR and high-performance liquid chromatography (HPLC)-based techniques for measurement of nitrite and nitrate compounds.

Results
In vitro, NO rescued the dysfunctional endothelial phenotype through suppression of the expression of cytokine-stimulated adhesion molecules and chemoattractants. We continued to investigate the combined influence of oxidative stress (ROS) and TGF-β1, both of which affect the redox state and induce endothelial dysfunction. TGF-β1 promoted the oxidative stress-induced expression of VCAM-1 and IL-8. The induction of VCAM-1 and IL-8 was suppressed by treatment with TAK1 inhibitor, 5Z-7-oxozeaenol, which suggests that the TAK1 pathway is involved in endothelial inflammatory activation. Upon stimulation with TGFβ1, TAK1 downstream activated both NFκB and p38, as induction of VCAM-1 and IL-8 was suppressed by inhibitors to these pathways. However, JNK was not involved. Next, we found that the ROS/TGFβ-stimulated expression of VCAM-1 and IL-8, was suppressed by high shear stress. As expected, high shear stress activated ERK5, which in turn induced expression of eNOS and production of NO. This coincided with a suppression of the NFκB and p38 pathways via inactivation of the TAK1 signaling. It would suggest that FSS-activated ERK5 suppressed TAK1 signaling. However, in statically cultured MEK5D-transduced HUVECs, in which ERK5 pathway is constitutively active, VCAM-1 expression was not suppressed. This indicates that TAK1 signaling is sensitive to FSS by other pathways than MEK5/ERK5.

Conclusions
Taken together, our in vitro study showed that high shear stress rescued endothelial cells from a dysfunctional phenotype through regulation of the redox signaling and repression of the TAK1 signaling.
MRI based assessment of wall shear stress: is it good enough?

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Introduction
Wall shear stress (WSS) is the tangential force exerted by the flowing blood on the vascular endothelium and has been proposed to be a critical determinant of vascular disease progression. Both low and oscillating WSS patterns were shown to correlate with atherosclerosis and aortic dilatation, whereas high WSS was reported in aortas with bicuspid valves. In addition, high WSS is believed to induce physiological remodeling of the vessel wall and maintain an atheroprotective phenotype. Spatial WSS gradients have been suggested to relate to intracranial aneurysm progression and rupture risk. 4D flow MRI [1] can measure time resolved velocity vectors in vivo and thus in principle allows for the calculation of WSS. In this presentation the reliability of this approach for assessing information on magnitude, direction and spatial and temporal gradients of WSS will be discussed.

Methods
Over the past years we have investigated the validity of MRI based wall shear stress assessment using state-of-the-art 4D flow sequences [2-5]. An algorithm was developed that facilitates volumetric assessment of WSS in both vessels and aneurysms. The accuracy and precision of this approach was assessed both in software phantoms and in vivo by comparing MRI based estimations with CFD and by varying the spatial resolution of the MRI acquisition. The trade off between temporal and spatial resolution of 4D flow measurements and scan time was studied in a carotid flow phantom. The developed algorithm has been applied in patients suffering from vascular disease in both carotids, aortas and intracranial aneurysms.

Results
We found that at least 8 voxels across the diameter of a vessel are required to obtain a WSS accuracy of 5% and a precision of 20%. Systematic WSS quantification errors up to 40% were detected in the case of segmentation errors. Although WSS magnitude based on MRI was lower than WSS based on CFD, the spatial WSS patterns at diastole, which are more relevant to the vascular biology, were similar. In patients suffering from atherosclerosis the inverse relationship between WSS and vessel wall thickness could be confirmed. In the aorta cohort-averaged systolic WSS maps were successfully created. In a dilation cohort significantly lower WSS was found in 7% of the ascending aorta surface, whereas in a stenosis cohort significantly higher WSS in 34% of the ascending aorta surface was reported. In cerebral aneurysms the quantitative agreement between MRI and CFD-based WSS estimations was moderate. There was qualitative agreement between MRI and CFD, i.e. WSS vector direction was similar for both modalities.

Conclusions
WSS estimation based on 4D flow MRI still has several limitations but these do not prohibit its use as method for probing biological relevant changes in patients suffering from vascular disease.

References
The role of suprarenal side branches in dissecting abdominal aortic aneurysm formation in angiotensin II-infused mice

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Introduction
While angiotensin II-infused mouse models are often used to study abdominal aortic aneurysm (AAA), their large variation in shape (from eccentric to polymorphic) and their suprarenal (rather than infrarenal) location have never been fully understood [1]. In this work we used a novel ex vivo imaging technique to unveil the anatomy and micro-structure of the so-called dissecting AAAs in these mice.

Methods
Dissecting AAAs were provoked by a combined administration of angiotensin II and anti-TGF-β antibody in n=20 male normolipidemic C57BL6 mice (age 12 weeks) [2]. The animals were followed up in vivo with high-frequency ultrasound (Vevo 2100, VisualSonics) and contrast-enhanced micro-CT (Quantum FX, Caliper Life Sciences). N=9 intact dissecting AAAs were sacrificed 2 to 15 days after aneurysm induction and n=6 samples were obtained post mortem from animals with aortic rupture. All dissecting AAA samples were scanned ex vivo using phase contrast X-ray tomographic microscopy (PCXTM) at the Tomcat beamline of the Swiss Light Source at the Paul Scherrer Institute in Villigen. These images combine a detailed soft tissue contrast with a superior isotropic image resolution of 6.5 micron. After the ex vivo scans the samples were embedded in paraffin. We subsequently developed a novel technique called PCXTM-guided histology, which allowed us to cut paraffin slices exactly at the rupture sites observed on the PCXTM images. The organization of the lesion (H&E), and the distribution of elastic lamellae (Millar stain), collagen fibers (Sirius Red), smooth muscle cells (alpha-actin), endothelial cells (CD31) and fibrin (MSB) could thus be studied with unprecedented accuracy.

Results
We visualized that two independent phenomena caused the aortic abdominal lesions. First, a tear was detected in the abdominal tunica media of all aneurysmatic mice (n=15/15), either caudal or left to the ostium of the celiac artery. Second, local ruptures of the tunica media were observed near the ostium of small suprarenal side branches. These branch-related ruptures led to a dissection of the tunica adventitia and to the formation of an intramural hematoma in n=13/15 mice. In vivo images showed how the apparent luminal dilatation, often reported in literature, was directly related to a false channel that resulted from the tear near the celiac artery. Similarly, PCXTM-guided histology showed how the size and number of branch-related ruptures was directly related to the suprarenal location and the large variation in shape between different dissecting AAAs.

Conclusions
Introducing PCXTM and PCXTM-guided histology, we visualized how suprarenal branch ruptures, apparent luminal dilatation and intramural hematoma formation can explain the location and the variation in shape of dissecting abdominal aortic aneurysms in mice.

References
Bicuspid Valve Morphology Determines the Position of Elevated Velocity and WSS

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Introduction: Bicuspid aortic valve (BAV) disease occurs in 1-2% of all newborns and is associated with two dominant patterns of aortic dilation, that is: a ‘type 1’ pattern in which the dilation involves the root and proximal portion of the tubular ascending aorta (AAo); or a ‘type 2’ in which the distal AAo and arch are dilated. There is recent evidence that aortopathy may be related to hemodynamic changes (i.e. outflow patterns and wall shear stress [WSS]) due to the orientation of the valve leaflets1. This study uses a volumetric technique to investigate the valve morphology and hemodynamic hypothesis in a cohort of 202 4D flow MRI exams.

Methods: 4D flow MRI exams were performed in 140 BAV patients and 62 healthy controls on 1.5 and 3T MAGNETOM Avanto, Espree, Aera and Skyra MRI systems (Siemens Healthcare, Erlangen, Germany). The BAV morphology was assessed on balanced steady state free precession images at the level of the valve and classified according to the Sievers scheme, with Sievers type 0 AP and LAT indicating BAV with no raphe, and opening in anterior-posterior and lateral direction, respectively. Sievers type 1 RL and RN indicate BAV with a raphe, with fusion of the right-left coronary valve (and opening in AP direction) and fusion of the right-non-coronary valve (and opening in LAT direction), respectively. All subjects had no stenosis (peak velocity <2m/s). Sinus of Valsalva (SOV) and mid-ascending aortic (MAA) diameters were measured from contrast-enhanced MRA data. Two methods were used to determine the difference in peak systolic aortic velocity and WSS between different BAV morphology configurations: 1) cohort-averaged velocity and WSS maps using a previously published methodology2, and 2) P-value maps2 delineating significantly higher or lower velocity and WSS (Wilcoxon rank sum test, P<0.05 was considered significant) for all patient cohorts, as compared to the healthy age-matched control groups.

Results: Subject demographics and aortic diameters are given in table 1. In Fig 1 it can be seen that results were very similar for valves opening in AP direction (Sievers 0 AP and Sievers 1 RL) and for valves opening in LAT direction (Sievers 0 LAT and Siever 1 RN). Fig 1b shows that the average outflow jet for Sievers 0 AP and Sievers 1 RL subjects is directed more towards the anterior part of the proximal aorta, whereas for Sievers 0 LAT and Sievers 1 RN, the outflow jet is directed more towards the posterior part of the proximal aorta and impinges on the outer curvature of the aorta at a more distal location. Accordingly, the velocity P-value maps in Fig 1c for Sievers 0 AP and Sievers 1 RL show a larger volume of significantly higher velocity in the proximal and anterior aorta than Sievers 0 LAT and Sievers 1 RN. The highest WSS for Sievers 0 AP and Sievers 1 RL is found at the level of the right pulmonary artery, whereas for Sievers 0 LAT and Sievers 1 RN, the highest WSS is found more distally (Fig 1d). For the P-value WSS maps in Fig 1e, significantly lower WSS than controls on the aortic root can be distinguished on the Sievers 0 LAT map and the Sievers 1 RN map, whereas the proximal aorta of the Sievers 0 AP map and the Sievers 1 RL map shows significantly elevated WSS compared to controls.

Conclusions: The cohort-averaged and P-value maps revealed important differences in aortic velocity and WSS for AP- and LAT-opening BAV patients. It is intriguing that these WSS differences match with the reported differences in aortic dilation expression for the different BAV-fusion patterns. Larger studies with longer patient follow-up (for detection of aortic growth) are necessary to evaluate the influence of outflow patterns and WSS expression on the pattern of aortic dilation.


Table 1. Subject demographics

<table>
<thead>
<tr>
<th>BAV type</th>
<th>Number of subjects</th>
<th>Age (years)</th>
<th>SOV (cm)</th>
<th>MAA (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sievers0 AP</td>
<td>27M, 6W</td>
<td>40±13</td>
<td>4.0±0.6</td>
<td>3.7±0.8</td>
</tr>
<tr>
<td>Sievers0 LAT</td>
<td>6M, 10W</td>
<td>39±9</td>
<td>3.9±0.6</td>
<td>3.9±0.7</td>
</tr>
<tr>
<td>Sievers1 RL</td>
<td>65M, 21W</td>
<td>42±13</td>
<td>3.9±0.3</td>
<td>3.8±0.7</td>
</tr>
<tr>
<td>Sievers1 RN</td>
<td>3M, 2W</td>
<td>42±7</td>
<td>4.0±0.3</td>
<td>3.8±0.5</td>
</tr>
<tr>
<td>Controls</td>
<td>42M, 20W</td>
<td>44±12</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Fig 1. a) Schematic of the BAV valve types under investigation. LCA and RCA indicate the left and right coronary artery. Thick lines indicate raphes. b) Cohort-averaged velocity maps. c) P-value maps for velocity. d) Cohort-averaged WSS maps. d) P-value maps for WSS
Arteritis, Shear Stress, Accelerated Atherosclerosis and Therapeutic Manipulation of Cytoprotective Genes in the Vasculature

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Introduction
Large vessel vasculitis leads to distorted arterial anatomy, aortic stiffness, disrupted patterns of shear stress and endothelial cell (EC) dysfunction, so predisposing to premature atherogenesis. Thus, EC dysfunction represents a potential therapeutic target.

Methods
We use a combination of in vitro and in vivo, gain and loss of function studies in the vascular endothelium, alongside clinical translational investigation of large vessel vasculitis patients. Our aim is to investigate effects of different shear stress patterns on cytoprotective gene regulation, the generation of microparticles and endothelial responses to exogenous factors and therapeutic agents.

Results
Study of patients with Takayasu arteritis (TA) (a disease characterised by stenosis of the aorta, subclavian and carotid arteries) and matched healthy controls, demonstrated an increase in total microparticles (MP) and both platelet and endothelial-derived MPs in TA (p<0.01). CT angiography revealed coronary artery stenoses and premature atherosclerosis in up to 50% of patients. Study of potential vasculoprotective pathways demonstrated that oscillatory shear (OSS, +/- 5 dynes/cm²) activated PKCζ, while in contrast laminar shear (LSS, 20 dynes/cm²) activated PKCε. Activity of PKCζ is pro-inflammatory and pro-atherogenic. Targetted activation of PKCε modulated NF-κB signalling to induce protective genes (A20, Bcl-2, eNOS), while suppressing inflammatory pathways. In addition, PKCε activated CREB and Nrf2 to induce HO-1, so increasing EC resistance to inflammation and apoptosis. Differential regulation of cytoprotective genes was further illustrated by attenuated expression of the complement inhibitory protein CD59 at OSS flow sites compared to LSS (p<0.05). Using HO-1 expression as a model, we found differential induction by atorvastatin in atheroresistant compared to atheroprone sites of the murine aorta. Synergy was observed between laminar shear stress and atorvastatin, resulting in optimal expression of HO-1 and resistance to oxidative stress, a response inhibited by HO-1 siRNA. Mechanistically, synergy required Akt phosphorylation, activation of KLF2, Nrf2 and increased nitric oxide synthase activity. In contrast, HO-1 induction by atorvastatin in EC exposed to oscillatory flow was markedly attenuated.

Conclusions
Endothelial responses to shear stress influence MP generation and susceptibility to atherogenesis, which may be exacerbated by arterial injury. Moreover, biomechanical signalling contributes significantly to endothelial responsiveness to pharmacological agents. Detailed understanding of EC cytoprotective signalling will allow development of novel targeted therapeutic approaches to condition vascular endothelium and reverse endothelial dysfunction.
Connexin40 regulates shear stress-induced endothelial NF-κB activation through interaction with IκBα

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Introduction
Endothelial cells (ECs) of healthy arteries express high levels of connexin 40 (Cx40), but this expression is lost in ECs overlying atherosclerotic plaques. Low/oscillatory shear stress observed in bends and bifurcations of arteries is known to be atherogenic partly through activation of the pro-inflammatory NF-κB pathway in ECs. Here, we investigate the relation between shear stress, Cx40 and NF-κB.

Methods
Shear stress-modifying casts were placed around the right common carotid artery of mice expressing eGFP under the Cx40 promoter. Subsequently, Cx40 and eGFP expression in response to flow were assessed by en face immunofluorescence in heterozygous Cx40/eGFP mice. Furthermore, identification of potential binding partners for the regulatory intracellular C-terminus of Cx40 was performed by phage display. In vitro binding of the potential binding partners was investigated using crosslinking and en face proximity ligation assay on carotid arteries.

Results
Cx40 expression was down-regulated in regions of oscillatory flow (0.5±0.1, P<0.05), but was conserved in regions of high and low laminar flow (1.1±0.2 and 0.9±0.1, respectively, N=10). Furthermore, using high-throughput phage display we retrieved a consensus-binding motif for the C-terminus of Cx40, i.e. HS[I,L,V][K,R]. One of the retrieved peptides (HSLRPEWRMPGP) showed a 58.3% homology with amino acids 5 to 16 of IκBα, a member of the family of inhibitory proteins that control the translocation of NF-κB. In vitro binding of this peptide was confirmed by crosslinking and by en face proximity ligation assay on rat carotids. Finally, stimulation with 10 ng/ml TNFα induced a modest NFκB translocation to the nucleus of mouse ECs (bEnd.3). This translocation was significantly enhanced in ECs in which Cx40 expression was inhibited by siRNA, illustrating a functional implication of Cx40 in the NF-κB signaling pathway.

Conclusion
Our data show a novel functional interaction between IκBα and Cx40 that may be relevant for the control of NF-κB activation by shear stress.
The role of TGF-β/BMP signaling in microvascular endothelial cells in Pulmonary Arterial Hypertension

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Introduction
Hemodynamic alterations and increased shear stress are known contributors to the vascular remodeling in Pulmonary Arterial Hypertension (PAH), a deadly lung disease associated with mutations in the Transforming Growth Factor beta (TGFβ) –Superfamily. Specifically, a mutation of bone morphogenetic protein receptor 2 (BMPR2) and the increased expression of TGFβ support the idea for a central role of TGFβ/BMP disbalance in the disease progression. Usually shear stress induces phosphorylation of their downstream regulator proteins Smad1/5 and Smad2/3, whereafter these proteins form a complex with Smad4 and translocate into the nucleus. To date, the relation between the TGFβ/BMP pathway and shear stress are unknown in PAH. Therefore we investigated whether shear stress induces dysfunctional TGFβ/BMP responses in pulmonary microvascular endothelial cells (MVEC) isolated from PAH patients.

Methods
Control MVECs from lobectomies of cancer patients were compared to PAH MVECs isolated from autopsy and lung transplantation biopsies. The study was approved by the IRB of the VU University Medical Center. Confluent cells were stimulated with TGFβ1 (0.5ng/mL) or BMP9 (0.1ng/mL) and subjected to different levels of fluid shear stress (2,5 and 15 dynes/cm²) for 1 and 24 hours. The TGFβ/BMP pathway activation was quantified by phosphorylation and localization of Smad2/3 and Smad1/5. Involvement of TGFβ and BMP in the shear stress induced responses was tested by adding TGFβ neutralizing antibody 1D11 (1.25ug/mL) or the selective inhibitor of the BMP type I receptor kinases LDN-193189 (250nM) to the medium and study their effect on ligand and shear stress induced cellular responses.

Results
Both control and PAH MVECs showed increased phosphorylation of Smad2/3 upon TGFβ1 and shear stress stimulation, which was blocked by 1D11. Surprisingly, BMPR2 mediated responses appear to be intact as demonstrated by increased phosphorylation of Smad1/5 when exposed to BMP9 and shear stress. This response is inhibited in presence of LDN-193189, indicating the involvement of an activated BMP type I receptor kinase. Shear stress induced a transient activation in control and PAH MVECs of Smad2/3 and Smad1/5 protein 1 hour after high shear exposure (15 dynes/cm²), which returned to baseline values after 24 hours. Low shear stress (2.5 dynes/cm²) shows the same pattern for Smad1/5 phosphorylation, albeit less prominent.

Conclusions
Under the in vitro conditions used, both TGFβ and BMP mediated signaling is intact in PAH MVECs when compared to control cells. When MVECs are exposed to shear stress, or ligand stimulation, no differences are found in downstream signaling of the TGF-β/BMP pathway between control and PAH MVECs. The effective inhibition of Smad phosphorylation in response to shear stress by, respectively 1D11 and LDN-193189 shows the involvement of TGFβ ligands and the importance of BMPRI with regards to shear adaptation in MVECs.
The study of the structure and function of biological systems by means of the methods of mechanics is widely accepted as definition of “biomechanics”. In the study of pathologies of the cardiovascular system, biomechanics has always been considered an important science or methodology to provide a deeper knowledge. In particular, the most favourite adopted methodology is undoubtedly the numerical or computer simulation.

In mini-invasive cardiology, the first numerical study related to the stent world appeared at the end of the nineties.

This talk will cover how the numerical models applied to stenting have changed since then, in terms of anatomies reconstructions from medical images, stenting design, influence of material properties on the stenting performance, translational impact on clinical practice, etc…

The potentials of the numerical models will be described including the new applications of traditional stenting to percutaneous valve and degradable scaffolds, in which a deep description of the verification and validation process of the numerical model will be provided.

Figure – From medical images to prediction of stent deployment and associated fluid dynamics in a coronary bifurcation model.
Inducing Persistent Low Shear Stress Promotes Thin Cap Fibroatheroma Development in Hypercholesterolemic Minipigs

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Introduction
The precise environmental cues that lead plaques towards an advanced phenotype are yet to be fully elucidated, but disturbed flow is thought to play a central role. Naturally occurring regions of disturbed flow within coronary arteries has been correlated to regions of atherosclerosis in vivo, but no study has demonstrated causality in these vessels. Herein, we test the hypothesis that inducing low shear stress within coronary arteries causes the development of thin-cap fibroatheroma (TCFA).

Methods
Shear-modifying stents (SMS) were implanted into either the left anterior descending or left circumflex arteries of hypercholesterolemic D374Y PCSK9 transgenic minipigs (N=5), with the unstented artery serving as a control. Intracoronary frequency domain optical coherence tomography (FD-OCT) and Doppler flow velocity measurements were obtained at baseline, 0, 19, and 34 weeks. The 3-D geometry of each artery was reconstructed and computational fluid dynamics was performed to compute standard and custom shear metrics of disturbed flow. Plaque type was assessed at 60 µm intervals over 2.5 mm vessel segments (~4.5 mm in vivo) excised immediately upstream and downstream of the SMS and in the control. The segments were serially sectioned and alternating sections were stained with hematoxylin and eosin (H&E) and picrosirius red. Stains were used to characterize plaque type as TCFA, fibrous cap atheroma (FCA), pathological intimal thickening (PIT), xanthomata (XA), intimal thickening (IT), or normal vessel wall (NOR) and a color coding system was used to delineate plaque type on a point-to-point basis within each H&E-stained section. This histology data from each pig at the final study time point was co-registered to the in vivo 3-D coronary artery reconstructions of that pig over all time points to evaluate locally the time-course of change in magnitude of each shear metric within each plaque type.

Results
The SMS caused a flow-limiting stenosis of 55 ± 7% immediately after implantation to 80 ± 2% at 34 weeks (p < 0.05). The stenosis severity resulted in an overall reduction of mean blood flow velocity from 147.8 ± 18.3 at baseline to 90.7 ± 11.1 mm/sec at 34 weeks (p < 0.01), which corresponds to an overall reduction in time averaged shear stress. In addition, the region downstream of the SMS exhibited accelerated blood flow directed towards the outer curvature of the vessel in a concentrated stream, causing regions of increased shear stress on the outer curvature and large regions of low and multidirectional shear stress on the inner curvature. As a result of these flow disturbances, plaque burden was observed to be ~3-fold higher in the segment downstream of the SMS compared to both the SMS upstream and control artery segments (p < 0.0001). TCFA only occurred in the downstream instrumented vessel segment. Over the duration of the study, TCFA regions demonstrated statistically increased low shear index (LSI, which was used to quantify low shear stress) compared to both FCA and PIT (p < 0.0001), as well as early plaque types and the normal vessel wall (p < 0.0001). FCA and PIT also exhibited increased LSI compared to regions containing early lesions or normal wall (p < 0.001).

Conclusions
We are the first to show that inducing low shear stress within the coronary artery of a hypercholesterolemic minipig accelerates atherogenesis and causes TCFA formation, which aligns with previous work from our group in the mouse carotid artery.2

References
A predictive modeling framework to support vascular access surgery in a multicenter randomized clinical trial

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Introduction
Patients suffering from end-stage renal disease have an irreversible loss of their kidney function and therefore depend on renal replacement therapy. The most frequently used therapy is hemodialysis. To perform this therapy a vascular access (VA) needs to be created. This VA has to provide a sufficient high blood flow rate (>600 ml/min) and needs to be easily accessible. An arteriovenous fistula (AVF; surgically created by directly connecting an artery and a vein) is preferred over arteriovenous grafts or central venous catheters. Better long-term patency rates, fewer complications and interventions once fully matured (adequate flow, vessel wall thickness and diameter) characterize AVFs. Although an AVF is considered the best option and the surgeon selects the optimal location for an AVF based on an extensive pre-operative ultrasonography (US) vessel examination, it still suffers from complications (e.g., non-maturation and high-flow) due to a complex interplay of patient characteristics. To support the surgeon by selecting the optimal AVF location a computational tool was recently developed (ARCH-project [1]). This model, fed with patient-specific US-data, was able to adequately predict post-operative blood flows for individual patients [1,2]. Moreover, the model allowed for pre-operative evaluation of expected flows in different AVF configurations, hereby providing the surgeon with additional information. It should, however, be assessed if this additional flow information will improve clinical decision-making in AVF surgery planning by conducting a large randomized clinical trial (RCT). Recently, we have obtained funding for the initiation of a multicenter RCT. However, before the RCT can be started, a modeling framework needs to be developed that is robust and has clinically acceptable calculation times. Moreover, the framework should allow for model personalization based on standardized input data from different centers and should produce a prediction of the post-operative flows including a proper uncertainty quantification. The aim of this study was to develop such a modeling framework.

Methods
A modeling framework is developed that allows for patient-specific flow predictions of different AVF configurations. The model that is used within the framework is based on [1,3] and extended with vascular adaptation as shown in [2]. Monte Carlo simulations (N=400) are performed to estimate the uncertainty in the flow predictions for each AVF configuration due to uncertainties in the input data. The framework is designed so that routinely acquired data (ultrasound and pressure measurements) can serve as input data. To demonstrate the framework, we have retrospectively applied the framework to 25 patients and compared predicted flows with measured flows six weeks after surgery.

Results
The framework provides post-operative flows including output uncertainty of three different AVF configurations within 60 minutes and allows for easy model personalization. The output uncertainty due to uncertainty in the input was approximately 20%. The predicted flows after six weeks showed 80% overlap with the measured flows.

Conclusions
The developed model framework shows adequate flow predictions, provides additional information within a clinically acceptable time frame and allows for easy model personalization. Therefore, it will now be used in the RCT.

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Towards the Development of a Stratification Parameter to Predict Atherosclerotic Plaque Mechanical Response to Endovascular Treatment

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Introduction
Results following endovascular treatment of atherosclerotic lesions vary according to vascular location. One of the most pronounced variances is the incidence of restenosis. Specifically, restenosis rates are approximately four times higher in the femoral arteries than in the carotid arteries. This causes high re-intervention rates for lower extremity revascularisation that drive up treatment costs. Numerous studies have investigated the biological and clinical factors contributing to this variance. However, no study has compared the mechanical properties of the atherosclerotic tissue at these two locations as potential contributors to the varying restenotic response. The mechanical properties that influence restenotic response are: the propensity of the tissue to undergo stretch injury¹, the ability of the tissue to bear elevated stress levels², and tissue stiffness³. This study characterises these parameters in a group of excised carotid and femoral human atherosclerotic plaques to elucidate differences in mechanical properties that may be exploited to explain the variance in clinically observed restenosis rates. Furthermore, the indicators of this heightened restenosis rate remain unclear and independent predictors are required to assess patient suitability for endovascular intervention. A predictor of the target lesion’s mechanical properties may be an appropriate surrogate predictor of restenotic response due to the previously demonstrated correlation between these two variables. To develop such a predictor the biological composition of each plaque sample is characterised and correlated with mechanical properties.

Methods
Mechanical properties were characterised using uniaxial circumferential extension tests to failure on 24 carotid and 15 femoral plaque samples. This method applies circumferential stretch to the samples in a manner comparable to endovascular device deployment. From the resulting stress-stretch plots; the stretch that the plaque tissue can withstand prior to ultimate tissue failure (λF), the corresponding stress induced in the tissue at this point (σF), and also the high stiffness response (EHi) of the samples was obtained. Biological composition of these samples was characterised using Fourier Transform InfrRed (FTIR) spectroscopy to identify biological parameters potentially capable of predicting the mechanical properties. From this analysis the ratios of collagen (Col), lipid (Li), and calcification (Ca) to the total measureable content (Tot) are presented. This results in three parameters: Col:Tot, Li:Tot and Ca:Tot.

Results
Significant differences in carotid and femoral mechanical properties were identified. The femoral group displays significantly lower mean values of λF, σF and EHi compared to the carotid group (p=0.02, p<0.001 and p<0.001, respectively). This aids in explaining the increased restenotic response clinically observed in femoral vessels. Bivariate correlation and regression analysis reveal that the ratio of calcified tissue to lipid content (Ca:Li) is the most effective parameter for predicting the mechanical response of atherosclerotic tissue to circumferential stretch.

Conclusions
Both calcified tissue fraction and lipid content can be identified by existing clinical imaging tools. This indicates that the parameter Ca:Li may be a suitable and clinically available surrogate predictor of restenotic response following endovascular intervention. This may allow clinicians to pre-operatively stratify patients into different treatment strategies therefore ensuring that patients at increased risk of developing restenosis following endovascular intervention, based on the predicted mechanical response of the atherosclerotic tissue, receive open surgery or a monitoring protocol.

References
Reconstruction of incomplete lipid pool geometry for stress calculations in atherosclerotic arteries

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Introduction
Determining stresses in the fibrous cap can aid in predicting plaque rupture. The only way to obtain in vivo plaque geometry and composition of coronary arteries is via intravascular imaging, for example by using intravascular ultrasound (IVUS) or Optical Coherence Tomography (OCT). With IVUS, the lumen, calcifications and the transition from media-adventitia can be observed. OCT has limited penetration depth but higher resolution, and can therefore visualize the front of the lipid pool and the lumen. Previous studies showed that in addition to the cap thickness also the lipid pool thickness affect the plaque stresses [1]-[3]. Therefore, missing the backside of the lipid pool would limit stress calculations. The goal of this study was to develop a method to reconstruct the lipid pool in case of incomplete data and to investigate the influence on peak cap stress.

Methods
Coronary segments were post-mortem obtained from thirteen arteries of seven patients and were used for histology. In total, 76 lipid pools were present in 54 cross sections. In this study all lipid pools were treated separately so that 76 plaques were investigated. A modified Movat pentachrome staining was used to manually delineate the lumen, lipid, intima, media and adventitia. Based on information of the lipid pool geometry, a correlation was derived between lipid pool thickness and geometric parameters with a Generalized Estimation Equation (GEE) model to account for dependencies among arteries. The GEE model correlates the relative lipid pool thickness (rLP) with known IVUS/OCT geometrical plaque parameters: cap thickness, lipid angle and vessel wall thickness (vessel wall thickness = distance from cap thickness to media-adventitia transition). The rLP was determined at three locations along the lipid pool surface: 50% and 25 / 75% of the lipid pool angle and used to reconstruct, the backside of the lipid pool. In order to quantitatively compare the reconstructed lipid pool geometry (LP_rec) with the lipid pool geometry from histology (LP_hist) the similarity index (SI) and relative area difference (ΔA%) were used. Stresses in the cap were calculated for the cross sections with the LP_rec and the LP_hist applying an intraluminal systolic blood pressure of 140 mmHg. The peak cap stress was defined as the maximum von Mises stress in the cap and the shoulders (15 degrees adjacent to the lipid pool). The peak cap stress found for the geometry with LP_hist was compared with the peak cap stress of the geometry in which LP_rec was used. Since all data were not normally distributed median and interquartiles are reported.

Results
The GEE model estimated rLP at 50% of the lipid angle as: 0.056 * lipid angle (rad)+4*10^{-4} * wall thickness (µm)-6*10^{-4} * cap thickness (µm)+0.15 (r^2=0.54 and p<0.05). Similarly, rLP at 25/75 % lipid angle = 0.11 * lipid angle (rad)+2.8*10^{-4} * wall thickness (µm)-4.4*10^{-4} * cap thickness (µm)+0.11 (r^2=0.50 and p<0.05). Quantitative analysis is performed on 72 lipid pools: the LP_rec matched the LP_hist well with a SI and ΔA% of 0.89 (0.85-0.92) and 13.4% (6.6-25.0), respectively. The peak stresses of LP_rec were highly correlated to the peak stress of LP_hist (r^2=0.95). The difference in peak cap stresses were 5 (1–12) %.

Conclusions
Peak cap stress can be accurately calculated in vessel wall geometries in which information on the backside of the lipid pool is incomplete. However information on cap geometry and wall thickness (e.g. derived from OCT and IVUS) is crucial for reconstruction of the backside of the lipid pool.

References
Novel technique for assessment of mechanical properties of carotid arteries

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Introduction
Vulnerable plaques in carotid arteries are potentially lethal or at least cause high risk of stroke when they rupture. Currently the severity of the stenosis is used to estimate the risk of plaque rupture. However, plaque rupture occurs when the mechanical stresses in the cap of the plaque exceed the local tissue strength. To determine the risk of rupture, mechanical properties of plaque components are needed. In this study, we model a slice of artery or arterial plaque as a 2D object, and load it in a physiological way. A finite element model is used to determine mechanical properties.

Methods
Slices (0.3mm thickness) were cut from a fresh porcine carotid artery and slightly compressed between two glass plates (figure 1). Paraffin oil was injected into the lumen, to inflate the sample. A pressure sensor (St Jude Medical, Uppsala, Sweden) monitors the intraluminal pressure, while motion of the sample was measured using a high speed video camera, synchronized to the pressure measurement at a frequency of 200Hz. The images were processed using Matlab software (R2012b) to render diameter as a function of pressure.

To assess mechanical properties, the experiment was modelled in FEBio finite element code (v2.1.0), using quadratic hexahedral elements. To start, the vascular material was modelled as being neo-Hookean. The experiment was modelled and the shear modulus of the carotid sample was assessed during pressure increase. A least-squares approach was used to compare the experiment to the model results.

Results
Pressures between 10 – 150 mmHg were obtained in the lumen of the sample. The diameter of the carotid sample changed according to the variations in pressure. A shear modulus of 1 MPa was found to be the closest fit to the experimental data (figure 2). It was observed that in the experiment the pressure increases faster than the diameter.

Conclusions
The first results of this novel technique for assessment of mechanical properties of carotid arteries are promising. The synchronized pressure and diameter measurement in the experiment have been successfully implemented. The carotid sample used in this experiment was found to be stiff compared to other porcine carotids, which resulted in a higher shear modulus. Paraffin oil leaks between the sample and the glass, resulting in near frictionless movement of the sample between the microscope slides. The nonlinear behaviour of the tissue has been modelled with a linear material model, which may explain the difference in the pressure/diameter curves of the experiment and the model.

The main advantage of this technique is the physiological loading condition imposed in a quasi 2D setting. In future applications, heterogeneous properties, like in atherosclerotic plaque material, may be assessed as well, using vital staining techniques to distinguish different tissue components without affecting their mechanical behaviour.
Intima Heterogeneity in Atherosclerotic Plaque Stress Calculations

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Introduction
Atherosclerotic fibrous cap rupture occurs when the stresses in the cap exceed the strength of the tissue. Computational plaque mechanics to evaluate stresses in the cap is therefore a growing field of research. One of the unevaled assumptions in computational plaque mechanics is that each of the components (lipid core, intima, wall, and calcifications) has a homogeneous mechanical behavior. However, especially the intima generally has a very heterogeneous composition, including collagen fibers, smooth muscle cells, inflammatory cells, fatty material, (micro)calcifications, and extracellular matrix. In this study, we created a computational methodology that enables us to evaluate the influence of heterogeneity in material properties of the intima in stress calculations of atherosclerotic plaques.

Methods
As a reference, conventional homogeneous plaque finite element (FE) models were created by manual delineation of the lumen, lipid core, intima, and wall on histology plaque cross-sections. To create the new heterogeneous FE models, the intima section was subdivided in 4 clusters by means of a k-means clustering algorithm, based on histology image intensity. All clusters were assigned Young's moduli ranging from 6 to 891 kPa, which is the full range of stiffness values of the intima found by plaque indentation tests [1]. As it is unknown what the stiffness is of each cluster, a Latin hypercube sampling method was used to generate 80 combinations with different stiffness values for all clusters. Based on the resulting stiffness distribution of the intima, each element in the FE model was assigned its own element stiffness.

The stresses in the conventional and 80 heterogeneous FE models were computed and the 95 percentile cap stresses (95pCS) were determined. The variation in 95pCS of the heterogeneous models was related to the stiffness and distribution of the individual intima clusters. The method was evaluated with histological cross-sections of 12 coronary plaques.

Results
Element-wise assigning material properties resulted in 95pCS which were comparable to the conventional FE model 95pCS. For most plaques (10 out 12), the 95pCS of the conventional model was located within the interquartile 95pCS range of the 80 heterogeneous models. Generally, the relative standard deviation of the heterogeneous 95pCS was moderate (on average 32% of the mean 95pCS). There was no clear relation between the average 95pCS and the variation in 95pCS due to the intima heterogeneity. For most plaques, the stiffness of 1 or 2 dominant clusters showed a strong positive relation (r>0.6, p<0.01) with the 95pCS, especially when the (thin) cap mainly consisted of this cluster.

Conclusions
By assigning material properties element-wise to a FE model, stiffness heterogeneity was introduced in plaque stress analyses and the effect of intima heterogeneity on the stresses in the cap was evaluated. Due to the large range of Young’s moduli assigned to each cluster, we created a ‘worst-case scenario’ for the variation in peak cap stress. As the variation in 95pCS was not correlated to the average 95pCS, the cap stress variation cannot be anticipated based on the magnitude of cap stress itself. Preliminary analyses indicated that, depending on their size and distribution, not all clusters influenced the 95pCS. This is the first study to include intima stiffness heterogeneity in plaque stress analyses. This methodology will provide valuable insight in the uncertainty and variation of the calculated cap stress.

References
**Poster 5**

**3D Collagen Architecture of Human Atherosclerotic Carotid plaques**

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**Introduction**

The main cause for ischemic stroke and myocardial infarction is rupture of an atherosclerotic plaque. Therefore, predicting plaque rupture is desired to help preventing future strokes and heart attacks. Current methods, based on general plaque features such as stenosis degree and intima-media thickening, have been shown to be not reliable enough and plaque rupture prediction needs to be improved. Rupture occurs when the stresses in the plaque exceed the strength of the plaque. Biomechanical modelling, enabling stress analysis in atherosclerotic plaques is a promising tool to help improving plaque rupture risk assessment. However, the accuracy of these models depends strongly on the structural and material properties used as input for the models. Previous studies analysed the local anisotropic material properties of plaques. These studies focused on the mechanical properties of local collagen fibres, which are the load-bearing structures [1]. However, the benefit of these results, without knowing the general arrangement and alignment of collagen, is very limited. Therefore, the aim of this study was to visualise and analyse the global 3D collagen architecture of human atherosclerotic plaques.

**Methods**

Nine human atherosclerotic plaques obtained from carotid endarterectomy were imaged using Diffusion Tensor Imaging (DTI). DTI has been proven to be a fast and non-destructive method to visualise collagen fibre alignments in human arteries [2]. Carotid were embedded in type VII agarose. DTI was conducted with the embedded samples using a 9.4 T horizontal-bore MRI scanner. Longitudinal cross-sections were prepared for histological analyses.

**Results**

In 4 plaques, collagen fibers are deposited in a new layer in a different direction during the development of atherosclerosis (see figure). Two distinct layers of collagen fibers were found; an outer layer, where the collagen is aligned in the circumferential direction (14.5°±28.0°), similar to healthy arteries [2], and an inner layer where the collagen follows a longitudinal direction (77.4°±22.4°). The axial orientation of the collagen fibres at the luminal side was confirmed by histology. The data from the other 5 plaques were less conclusive, showing a more scattered fibre distribution.

**Conclusions**

These new results indicate that during plaque development a major reorientation of the collagen fibres occurs, with fibres aligned in the axial direction. These new results on the 3D collagen architecture of atherosclerotic plaques can be used in 3D FEM studies to improve the accuracy of biomechanical models of plaques. This will be another step towards reliable plaque rupture prediction and improve the prevention of atherosclerosis related cardiovascular events, such as strokes and heart attacks.

2) Ghazanfari et al., Biochemical and Biophysical Research Communications, 426(1), 2012.
Tissue prolapse and stresses in stented coronary arteries using a multi-layer computational model of atherosclerotic plaque
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Introduction
The association between tissue prolapse (TP) and the appearance of restenosis has been reported in several clinical studies. On the other hand, the plaque type in terms of the potential injury to a stented artery has been shown to have significant implications for in-stent restenosis. Moreover, high mechanical stresses in the thin fibrous cap during expansion can cause rupture if they exceed the ultimate strength and eventually arteries develop restenosis in response to that injury. Numerous computational studies have been focused on stent-artery interaction. Majority of these studies have concentrated on the impact of stent design parameters on the risk of restenosis. However, it has not been yet studied how the presence of plaque may affect the risk of restenosis in stented arteries. The current study investigates, for the first time, the impact of stenosis severity and plaque morphology on the prolapse in stented coronary arteries. A finite element method (FEM) is applied for the set of stent-plaque-artery to quantify the tissue prolapse and the corresponding stresses in stenosed coronary arteries. Plaque is modelled as a multilayered medium, with different thicknesses, attached to the single layer of arterial wall.

Methods
Two eccentric plaques with initial degrees of stenoses as 51% (P1) and 36% (P2) are considered. These plaques are classified as fibro-atheroma type while each having a plaque burden greater than 40%. The arterial wall (AW), fibrous cap (FC), and fibrous (F) layers of the plaque are assumed as incompressible hyperelastic material. The necrotic core (NC) layer is assumed as an isotropic, incompressible material.

Results
Figure 1 shows the total deformation of the vessel wall in one repeated unit of stent cell model. Figure 2 demonstrates the mean distribution of von Mises stresses (VMS) obtained for different layers of plaque.

Figure 1. Total deformation of the vascular tissue within stent cell model.

Figure 2. Average VMS (<VMS>) distribution in plaque layers and AW for varying thickness of FC and NC for the plaque P1 (a-d), and the plaque P2 (e-h).

Conclusions
The results of this study reveal that the morphological characteristics of atherosclerotic plaque affect on the risk of restenosis. This parameter needs to be considered in designing the stents to effectively prevent the restenosis.
On The Effect of Calcific Content on the Mechanical Behaviour of Carotid Plaque Tissue

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Introduction
The success of minimally invasive endovascular stenting is dictated by the combined response of pathological components within complex atherosclerotic plaque. Specifically, rigid calcifications have been linked to treatment failure by initiating regions of high stress, under the supra-physiological loading forces, causing mechanically induced tissue ruptures [1]. However, mechanical properties of plaque and how this varies with calcification is not fully understood. Therefore, a clear need exists to use more accurate methods of characterising the diseased tissue to better understand the effect these structural components have on plaque mechanical properties. The paucity of experimental mechanical data characterising plaque failure under the influence of calcification has driven the need for this experimental study which relates biological and structural characteristics of carotid plaque to mechanical properties.

Methods
Uniaxial circumferential stretching tests to failure were used to examine the mechanical properties of 17 human carotid plaque specimens in a manner that similarly conforms to the stretching conditions experienced during in vivo stent deployment. The macroscopic failures were tracked via noncontact videography and correlated with the corresponding mechanical data to characterise the tissue in failure and obtain the peak stress concentrations at the interface between the calcification and tissue matrix. Biochemical analysis, by Fourier Transform Infrared (FTIR), was used to identify key pathological tissue components and characterise the degree of plaque progression by identifying the functional groups associated with lipid, collagen and calcification. A failure analysis, by micro x-ray computed tomography (µX-CT) imaging and scanning electron microscopy (SEM) was performed to identify the cause of failure and examine the global internal morphology of each mechanically ruptured plaque.

Results
The average Cauchy stress and stretch at failure was 0.414±0.253 and 2.02±0.38 respectively. FTIR analysis identified a high concentration of calcification adjacent to the lumen surface in both vulnerable high lipid and advanced fibrotic plaque types. µX-CT imaging revealed the local heterogeneity of the plaques internal morphology with each specimen consisting of copious clusters of randomly dispersed micro-calcifications together with millimeter scale macro-calcifications of randomly oriented geometries throughout the tissue structure. The quantification of the overall calcification to plaque tissue volume ratio (calc:plaque) revealed two significantly distinct groups in this sample set. The two groups were classified as lightly calcified (0<calc:plaque<0.20) and heavily calcified (0.3<calc:plaque<0.55). Applying this classification to the mechanical data demonstrated that the calcification is strongly influencing the plaques ultimate strength. Ultimate plaque failure occurred along the calcification-plaque tissue interfacial boundary region for all plaques classified as heavily calcified and the analysis of individual inclusions explains the wide range in ultimate strength within this group. The weaker plaques failed as a result of single macro-calcification inclusions completely surrounded by compliant tissue whereas the plaques consisting of larger masses of agglomerated calcifications with fewer tissue interfaces failed at higher stresses. This suggests that plaque strength diminishes with an increasing interfacial area between calcification and plaque tissue matrix.

Conclusions
This study proposes that not only calcific content but the morphology and location of the content are important determinants of plaque mechanical properties which are required for accurately modelling the procedural risk associated with endovascular treatment. Furthermore, plaque assessment via imaging modalities should include calcification geometrical measures and interfacial boundary properties in order to accurately evaluate localised stress concentrations and rupture risk.

References
On-site coronary CT angiography derived FFR: Effect of coronary calcium deposits and CT image quality on performance, validated by invasive FFR.

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Introduction
Coronary CT angiography (CCTA) is an established examination with a strong ability to rule out significant coronary artery disease. Specificity of CCTA is relatively low, especially when validated with functional stenosis severity measurements. Fractional flow reserve (FFR) is currently regarded as the standard of reference for stenosis severity classification. The application of computational fluid dynamics onto CCTA dataset allows for the computation of FFR (CTA-FFR) by simulating the coronary blood flow. This study evaluates the effect of CCTA image quality and coronary artery calcium (CAC) score on an on-site CTA-FFR application and validates this by invasive FFR.

Methods
In 116 patients CTA-FFR was performed (cFFR, version 1.4; Siemens Healthcare, currently not commercially available), and validated with 203 invasive FFR measurements. Diagnostic performance of CCTA was evaluated for patients with a total Agatston CAC score <400, between 400-1000 and above 1000. CCTA image quality was scored per segment using a 4 point Likert scale.

Results
From the 203 vessel 90 (44%) were considered hemodynamically significant with an FFR ≤0.80. Overall performance of CTA-FFR was good with sensitivity, specificity and accuracy of respectfully 88% (CI: 79-94%), 65% (55-73%) and 75% (69-81%). No relationship was found between the diagnostic performance of CTA-FFR and image quality [figure 1], though this effect is dampened somewhat as patients with non-diagnostic CCTA image quality were excluded from this study (n=4). We found a non-significant reduction in specificity from 74%(60-85%) to 48%(26-70%) for increasing CAC scores.

Conclusions
On-site CTA-FFR performs well in this population with a high prevalence of significant hemodynamic coronary artery lesions. A non-significant decrease in specificity was found for increasing CAC scores. No relationship was found between CCTA image quality and CTA-FFR diagnostic performance, suggesting robust performance within the group of patients with diagnostic CCTA image quality.

Figure 1

Figure 1: Scatterplot with on the y-axes CTA-FFR values minus invasive FFR, a value of 0 would indicate an identical outcome for CTA-FFR and invasive FFR. On the x-axes mean CCTA image quality.
Comparison of MRI and CFD based wall shear stress and their relationship with wall thickening in human carotid arteries

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Introduction

Low wall shear stress (WSS) triggers endothelial dysfunction leading to atherosclerotic plaque formation in carotid arteries in the presence of risk factors. Assessment of WSS in-vivo is therefore essential for a better understanding of initiation and of the disease. WSS is generally calculated by computational fluid dynamics (CFD) simulations but this approach requires engineering skills, computational power and time. Alternatively WSS can be calculated by using in-vivo MRI flow measurements which is easily applicable to clinical use. However in the latter approach, the accuracy of the estimated WSS relies on the spatial resolution of the measurements. In this study, we aim to compare MRI and CFD based wall shear stress (WSSMRI and WSSCFD) in the carotid arteries of an elderly population with asymptomatic plaques and also to compare the association of WSSMRI with wall thickness (WT) and of WSSCFD with WT.

Methods

16 subjects (74±6y) underwent time averaged 3D flow MRI with 3D velocity encoding (resolution: 0.70x0.7x1.0mm, TR/TE: 13ms/4.3m) and PDw-EPI scans (resolution: 0.51x0.51x1.2mm, TR/TE: 12000ms/24.3ms, venc: 60cm/s) using 1.5T GE scanner. We segmented lumen and wall on PDw-EPI images manually. WSSMRI was calculated as suggested by Potters [1]. WSSCFD was calculated based on steady state CFD simulations performed with a commercial CFD software package FIDAP 8.7.4 (Ansys). For the CFD calculations, MRI measured flows at common carotid artery (CCA) and internal carotid artery (ICA) were used as boundary conditions. The blood density was set as 1.06 g/cm3 and viscosity was assumed to obey Carreau-Yasuda model. The results were analyzed for CCA and ICA. WSSMRI and WSSCFD values were divided into 3 categories representing low, medium and high WSS tertiles. WT of each tertile was compared by one-way ANOVA, post-hoc test.

Results

In ICA, mean WSSMRI (0.5±0.3Pa) was lower than mean WSSCFD (0.8±0.5Pa). In CCA, WSSMRI (0.3±0.7 Pa) was almost equal to WSSCFD (0.5±0.7 Pa). Underestimation of WSSMRI increased at higher WSS values in ICA. The difference between WSSMRI and WSSCFD was smaller in CCA. Figure 1 shows the mean WT for each WSSMRI and WSSCFD tertiles. In ICA, WT was inversely associated with both WSSMRI (p=0.001, Figure 1a) and WSSCFD (p=0.007, Figure 1b). In CCA, WT was inversely correlated with WSSMRI (p=0.005, Figure 1c) but not with WSSCFD (p=0.692, Figure 1d).

Conclusions

To our knowledge, the associations of wall thickness with WSSMRI and WT with WSSCFD have never been point to point compared [3]. In our study, we found that WSSMRI and WSSCFD patterns were similar in ICA despite underestimation and both were inversely associated with WT. The associations between WT and WSS in CCA were however contradictory. In conclusion, CFD and MRI can be used equally to associate wall characteristics with WSS in the ICA, but CCA has to be examined more carefully.

References:

Longitudinal study of changes in shear stress over developing atherosclerotic plaques in mice

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Introduction
Shear stress is the frictional force that blood flow exerts on the endothelium of the arterial wall. It is known to play a role in the initiation of atherosclerotic plaque growth. As disease advances, plaques can progress into two distinctive types: a stable or an unstable/vulnerable plaque. We hypothesize that shear stress plays a role in plaque progression and plaque stability. We aim to study this correlation using an atherosclerotic mouse model in which both a stable and a vulnerable plaque are present in the same vessel.

Methods
We placed a tapering cast around the right common carotid artery (RCCA) of ApoE^{-/-} mice fed a high fat diet. The cast manipulated the shear stress environment along the RCCA. Formation of atherosclerotic plaques was thus induced and imaged at 5, 7, 9 weeks after cast placement. In order to calculate shear stress, blood flow and detailed 3D geometry of the RCCA is required. Therefore, we first measured the blood flow velocity through the RCCA by Doppler Ultrasound. Next, we obtained detailed 3D geometry of the RCCA using contrast-enhanced micro-CT with a resolution at 40 µm. We previously created a standardized protocol to reconstruct 3D vessel geometry. Computational fluid dynamics was then applied to compute the velocity and shear stress distribution at three time points during plaque progression.

Results
We successfully obtained 3D shear stress maps of 5, 7, and 9 weeks after cast placement (Fig.1). Five weeks after cast placement, shear stress was low upstream of the cast, while maximum shear stress was observed inside the cast region where the lumen narrowed, corresponding to the designed geometry of the tapering cast. Downstream of the cast, an asymmetric shear stress pattern was found. Shear stress slightly increased proximal to the cast region at seven weeks after cast surgery. As the plaques progressed over time, shear stress patterns changed at nine weeks after cast placement. Increased shear stress regions were identified upstream of the RCCA, corresponding to areas with lumen narrowing. Meanwhile, elevated shear stress levels were revealed immediately downstream of the cast.

Conclusions
We demonstrated the possibility to reconstruct vessel geometry and determine shear stress patterns in a diseased mouse carotid artery over time. We analyzed the effect of plaque growth on shear stress by performing multiple imaging at 5, 7 and 9 weeks after cast placement. Next, we will perform histology analysis to characterize plaque composition. We can then correlate the changes in shear stress to plaque composition and thus plaque stability.
Contrast-enhanced micro-CT imaging for studying the relationship between shear stress and plaque composition in mice

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Introduction
Shear stress is the frictional force that blood flow exerts on the endothelium of the arterial wall. It is known to play a role in the initiation of atherosclerotic plaque growth. As disease advances, plaques can progress into two distinctive types: a stable or a vulnerable plaque. We hypothesize that shear stress plays a role in plaque progression and thus determines plaque composition. We aim to study this correlation using an atherosclerotic mouse model in which both a stable and a vulnerable plaque are present.

Methods
We placed a tapering cast around the right common carotid artery (RCCA) of ApoE-/- mice fed a high fat diet. The cast manipulated the shear stress environment along the RCCA. Formation of atherosclerotic plaques was thus induced within five weeks after the surgery. In order to calculate shear stress, blood flow and detailed 3D geometry of the RCCA is required. Therefore, we first measured the blood flow velocity through the RCCA by Doppler Ultrasound. Next, we obtained detailed 3D geometry of the RCCA using contrast-enhanced micro-CT with a resolution at 40 µm. We applied several approaches for lumen segmentation and established a standardized protocol to reconstruct 3D vessel geometry. Computational fluid dynamics was then applied to compute the velocity and shear stress distribution. The mice also underwent ultrasound and micro-CT imaging at 7 and 9 weeks after cast placement. Finally, we created 3D shear stress maps of the RCCA at three time points during plaque progression.

Results
Five weeks after cast placement, shear stress was low upstream of the cast, while maximum shear stress was observed inside the cast region where the lumen narrowed, corresponding to the designed geometry of the tapering cast. Downstream of the cast, an asymmetric shear stress pattern was found. Shear stress slightly increased proximal to the cast region at seven weeks after cast surgery. As the plaques progressed over time, shear stress pattern changed significantly at nine weeks after cast placement. Increased shear stress regions were identified upstream of the RCCA, corresponding to areas with lumen narrowing. Meanwhile, elevated shear stress levels were revealed immediately downstream of the cast.

Conclusions
From our study, we demonstrated the possibility to reconstruct vessel geometry and determine shear stress patterns in a diseased mouse carotid artery longitudinally. We analyzed the effect of shear stress on plaque progression over time, by performing multiple imaging at 5, 7 and 9 weeks after cast placement.
Mathematical bifurcation theory applied to atherosclerosis evolution

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Introduction

Mathematical models can be very helpful to gain insight in biochemical processes. Of particularly interest is the dependence of the model on parameter values. Even if actual values for the parameters are not known, bifurcation analysis can be used to obtain bounds for the values that the parameters can attain. Moreover, qualitative predictions for the behaviour described can be made. We apply this analysis to a simple model for the progression of atherosclerosis.

Methods and Results

We analyze two ordinary differential equation (ODE) models for atherosclerosis. The ODE models describe long time evolution of plaques in arteries. We show how the dynamics of the first atherosclerosis model (model A) can be understood using codimension-two bifurcation analysis. The Low-Density Lipoprotein (LDL) intake parameter (d) is the first control parameter and the second control parameter is either taken to be the conversion rate of macrophages (b) or the wall shear stress (\(\sigma\)). Our analysis reveals that in both cases a Bogdanov–Takens (BT) point acts as an organizing center. The bifurcation diagrams are calculated partly analytically and to a large extent numerically. The bifurcation curves show that the concentration of LDL in the plaque as well as the monocyte and the macrophage concentrations exhibit oscillations for a certain range of values of the control parameters. Moreover, we find that there are threshold values for both the cholesterol intake rate \(d_{\text{crit}}\) and the conversion rate of the macrophages \(b_{\text{crit}}\), which depend on the values of other parameters, above which the plaque volume increases with time. It is found that larger conversion rates of macrophages lower the threshold value of cholesterol intake and vice versa.

The very slow evolution of the radius of the artery compared to the other processes makes it possible to use a slow manifold approximation to study the dynamics in this case. We find that in this case the model predicts that the concentrations of the plaque constituents may go through a period of oscillations before the radius of the artery will start to decrease. These oscillations hence act as a precursor for the reduction of the artery radius by plaque

Conclusions

We demonstrate how bifurcation theory can be used to determine parameter values for the models used in atherosclerosis modeling and show that the analysis can be used to make qualitative predictions that may be tested in clinical environment.

References

Effect of altered blood flow on arterial permeability in immature and mature rabbits

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Introduction
The development of atherosclerosis has been attributed to elevated permeability of the arterial wall to circulating macromolecules such as low density lipoproteins, and also to different types of hemodynamic stresses acting on the endothelium. We have recently established a high-throughput method for mapping arterial wall permeability to fluorescently labelled albumin over large areas. Our results demonstrate that the uptake of the tracer is elevated in specific regions of the rabbit aortic wall around intercostal branch ostia, and that these regions change with age; they correlate with patterns of atherosclerotic plaque prevalence in rabbits, which also change with age. To test the relation of permeability to haemodynamic stresses, we have developed methods for acutely or chronically altering blood flow around branch ostia in the abdominal aorta of the rabbit and have investigated their effect on permeability patterns at different ages. We used Doppler ultrasound to determine blood flow waveforms in different regions and used them as boundary conditions for computational fluid dynamics modelling.

Methods
Patterns of arterial permeability to fluorescently labelled albumin were assessed around the mouth of the renal artery ostia in immature (13 weeks) and mature (1.5-2 years) male New Zealand White rabbits, using a confocal tracer detection method based on that of Clarke et al., 2012. For chronic studies, the left renal artery, vein and ureter were ligated and the left kidney was removed without disturbing the adrenal gland or its connection to circulation. Pulse wave velocity was measured prior to the operation and for 4 following weeks to assess any arterial stiffening. The velocity of the blood in the abdominal aorta was monitored before and following the nephrectomy using spectral Doppler ultrasound for use in computational simulations of steady flow, which were conducted using STAR-CCM+ software; geometries were obtained from microcomputed tomography of resin corrosion casts. Permeability was measured 5 weeks after nephrectomy. Acute studies were performed under anesthesia; tracer was administered 1 h after the left renal artery and vein were ligated. Maps of permeability were produced from en face confocal microscopy tile scans, summing fluorescence intensities over a depth of 10µm from the lumen into the arterial wall. Gels containing plasma were used to scale mean intensity values by plasma concentrations of tracer, and hence to calculate the mass transfer coefficients.

Results
We confirmed normal postoperative recovery and kidney function by analysis of protein and electrolyte concentration in plasma and urine 5 weeks post-operation and by measuring pulse wave velocity, which showed only a transient increase 2 weeks following the operation. Higher uptake was observed downstream than upstream of control left renal ostia in the young age group (n=4). The pattern slightly shifted towards a more equal distribution between upstream and downstream regions in the control mature age group (n=5), but the effect was not significant (p = 0.33, t-test). In our chronic studies we found a significant effect (p=0.03, t-test; naive n=4, L-nephrectomy n=6) of nephrectomy around the left renal ostium in the young age group: uptake was higher downstream of the ostium in control rabbits but higher upstream in nephrectomised rabbits. There was no significant effect of nephrectomy in the mature age group. Conversely, our acute ligation studies showed a significant effect (p= 0.05, t-test; sham n=2, ligation n=3) in the mature age group only, with greater tracer uptake upstream of the ligated renal ostium. Preliminary CFD results showed regions of greater wall shear stress downstream of control renal ostia at both ages, with some shift towards a more equal distribution between upstream and downstream regions in the mature group, due to increased filtration rates by the mature kidneys. Nephrectomy did not seem to change this pattern dramatically, even though extensive remodeling was obvious in both renal arteries.

Conclusions
Our data show a subtle change in the permeability pattern around renal branch ostia with increasing age of the rabbits, similarly to what has been reported on the prevalence of atherosclerotic plaques at different ages. We also demonstrated an effect of nephrectomy in young rabbits, while mature animals appeared to adapt better to such long-term effects of decreased outflow in the left renal artery. Interestingly, our assessment of macromolecular permeability only one hour following renal artery ligation showed that the mature aortas responded more than immature ones. This change could be due to different kidney filtration rates at different ages but also due to differences in adaptability of the arterial wall with age. Our results causally link blood flow to wall permeability. (Funded by the BHF).

The influence of asymmetry and mobility of a stenosis.

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Introduction
A stenosis gives rise to a different flow at the narrowed flow region. It can also influence the flow and stress distribution at some distance from the stenosis, and it can lead to additional in-stationarity of the flow. The flow can be further influenced if the stenosis is able to move in a prescribed way or in response to the flow.

Methods
Two numerical codes (in-house and fluent) are used to represent a plane channel with an obstruction at one wall. The obstruction can be kept stationary or allowed to be in-stationary. The motion of the obstruction can be prescribed or can be made to respond to the pressure over the obstacle by moving normal to the vessel wall. The obstruction then moves like a solid body, according to the equation for a mass attached to a spring. We assume laminar, Newtonian flow at a Reynolds number based on average velocity, channel height and viscosity of several hundred.

Results
The stationary obstacle already gives an interesting flow pattern with stationary inflow, where we see a set of regions with altered flow and lowered shear stress which are alternately at the lower and at the upper side of the channel, as also noted by Pedley [1] and as visualized by the resulting meandering flow pattern shown in figure 1. The positions of lowered stress are determined by flow velocity, height of the obstruction and movement.

Conclusions
An obstruction like a non-symmetric stenosis leads to interesting stress patterns at intuitively non-expected positions in a 2D channel. These positions are changed when the obstruction is able to move. Similar effects are expected for a round vessel, this is subject of further research. At the conference, a number of interesting, resulting flow patterns for a moving and a non-moving obstruction, and stationary and pulsatile inflow can be shown.

References

Figure 1. Axial velocity component in a 2D channel with an obstruction. A meandering flow profile results due to an obstruction at the bottom wall.
Coronary bifurcation stenting: Insights on plaque shift from numerical simulations

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Introduction
Although stenting of the main branch (MB), in particular the so called provisional side branch (PSB) technique, is a routine procedure to treat atherosclerotic lesions in a coronary bifurcation, this endovascular technique is still associated with high event rates. A major procedural complication is the side branch (SB) compromise, which is induced by the plaque/carina shift towards the SB after stent implantation in the MB. In this study we investigated the influence of plaque composition and the bifurcation distal angle on plaque/carina shift after PSB stenting using finite element simulations.

Methods
An accurate parametric model of a diseased left anterior descending (LAD) coronary bifurcation was created with a stenosis of 60% in each branch. Realistic diameters, plaque burden, and curvature values were included. Material properties of the tissues were retrieved from ex vivo data. We studied three different distal bifurcation angles (45°, 57.5°, 70°) with two extreme plaque lesion scenarios (fully fibrous and fully lipid plaques). A Multi-Link 8 stent (Abbott Lab., USA) was virtually placed following the PSB technique. The finite element solver ABACUS (Dassault Systèmes Simulia Corp., USA) was used. The plaque/carina shift was investigated by mimicking the clinically-used volumetric analysis on 5 mm long SB region as performed by Xu et al. [1], to compute SB ostial compromise, plaque, and carina shift.

Results
Our results showed that the plaque/carina shift is influenced by both plaque composition and bifurcation angle. A higher distal angle leads to lower SB ostial compromise and lower plaque shift (Fig.1A). Carina shift occurs only in the case with distal angle of 70°. The effects observed with fibrous plaques are magnified in the case of lipid rich plaques. The SB section ovalizes after stenting (Fig.1C) as the device pushes the carina towards the SB, still keeping a similar pre-stenting area. This might explain good FFR values even when the SB lumen appears narrowed on the top angiographic-like view (Fig.1B), in agreement with previous clinical studies.

Figure 1 - Representative results for lipid plaque geometries with angles 45° and 70°: A) Volume changes of vessel, lumen, and plaque in the SB ostium after PSB; B) Pre- and post-stenting models; C) Pre- and post-stenting SB cross-sections indicated in B for the 45° model.

Conclusions
The presented numerical strategy can help understand the mechanisms of coronary stenting in the vicinity of a SB and provide information that can be misinterpreted based on conventional angiography. Results highlighted that patho-anatomical differences of the LAD bifurcation might be good predictors of plaque/carina shift.

References
MicroRNA-374B induces Endothelial-Mesenchymal Transition by targeting MAPK7 signaling.

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Background & Objective
The endothelium plays a major role in fibroproliferative diseases, such as cardiac- or kidney fibrosis and neointima formation. Induced by inflammatory and pro-fibrotic growth factors and cytokines, endothelial-mesenchymal transition (EndMT) is induced, wherein the endothelium loses its endothelial characteristics and adopts a mesenchymal phenotype.

TGFβ is a major inducer of EndMT, and highly expressed inside neointimal lesions, which may cause EndMT. In contrast, MAPK7 is highly expressed by the endothelium outside the neointimal lesions and blocks EndMT induction.

Laminar shear stress (transmitted through the flow of blood), activates MAPK7 and inhibits the induction of EndMT by TGFβ1. Knockdown of MAPK7 in endothelial cells results in EndMT, even in the absence of exogenous TGFβ1, suggesting a pivotal balance between TGFβ and MAPK7 in the induction of EndMT and the formation of neointimal lesions. Indeed, TGFβ1 actively represses endothelial MAPK7 expression through unidentified mechanisms.

MicroRNAs (microRNAs) are small non-coding RNAs that cause post-translational repression of their target genes. TGFβ1 induces a shift in microRNA expression levels in endothelial cells that may affect MAPK7 expression and EndMT induction.

Here, we hypothesized that TGFβ1 induces the expression of microRNAs that affect MAPK7 signalling and induces EndMT.

Methods & Results
We used in silico analysis to identify microRNAs that putatively target MAPK7 signalling and identified miR-374b as a major regulator. Luciferase reporters revealed that miR-374b targets not only MAPK7, but also its upstream kinase MEKK3 and downstream transcription factors MEF2D and KLF4.

MicroRNA-374b is expressed in porcine neointima, and its expression is induced by TGFβ1. Blocking of TGFβ1 signalling using a small molecule inhibitor (SB431542) of TGFβR1 abrogated miR-374b expression.

Ectopic expression of miR-374b caused a drastic reduction in MEKK3, MAPK7, MEF2D and KLF4 expression. Endothelial cells underwent EndMT as indicated by repressed expression of endothelial cell markers VE-Cadherin and eNOS, as well as decreased angiogenic sprouting activity. Vice versa, cells expressing miR-374b increased expression of mesenchymal cell markers SM22α and Calponin and gained contractile behavior, suggesting miR-374b induces EndMT.

Conclusion & Future Perspective
We show that TGFβ1 induces EndMT by inducing the expression of miR-374b, which results in silencing of MAPK7 signalling in endothelial cells. As EndMT is associated with pathologies such as cardiac fibrosis and neointima formation, targeting miR-374b expression in these pathologies, may reduce disease progression.
The Polycomb methyltransferase EZH2 regulates endothelial proliferation and is inhibited under fluid shear stress.

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Background & Objective
High uniform fluid shear stress (FSS) is atheroprotective and preserves the endothelial phenotype and function through activation of downstream mediators such as MAPK7. Endothelial cells sense FSS through mechanotransduction. However, how the resulting signalling is integrated and resolved at the epigenetic level, remains elusive. We hypothesized that Polycomb methyltransferase EZH2 is involved in the effects of FSS in human endothelial cells.

Methods & Results
We show that FSS alters the endothelial transcriptome by the abrogation of the Polycomb methyltransferase EZH2 and the simultaneous activation of MAPK7 signaling. Notably, knock down of EZH2 activates MAPK7 signaling in endothelial cells in the absence of FSS. We acquired the transcriptome of endothelial cells with EZH2-deficiency, under FSS and under control conditions through RNA-sequencing analysis. This, allows for the identification of gene clusters affected by EZH2 and/or FSS. Gene Ontology cluster analysis identified enrichment of the cell cycle-related genes. Indeed, depletion of EZH2 in endothelial cells strongly inhibits proliferation.

We analyzed the aorta’s of mice that underwent aortic constriction for the expression of EZH2. Aortic constriction triggers endothelial proliferation and endothelial-mesenchymal transition. In line with our in vitro data, where depletion of EZH2 results in endothelial quiescence, EZH2 expression is markedly increased after aortic constriction which may attribute the fibroproliferative phenotype of the endothelium following injury.

Conclusion & Future Perspective
Here, we link FSS-induced signaling to epigenetic alterations in endothelial cells. We show that decreased EZH2 levels enhance the activation of the atheroprotective MAPK7 signaling and is associated with endothelial quiescence. Contrasting, at sites of endothelial damage, where the endothelium adopts a fibroproliferative phenotype, expression of EZH2 is markedly increased. These data implicate that the methyltransferase EZH2 may be an interesting drugable target to confer an atheroprotective phenotype onto the endothelium.
Sprout Initiation Site and Sprout Elongation Are Controlled by Flow Dynamics During Angiogenesis

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Introduction
Angiogenesis is tightly controlled by a number of signaling pathways. Though our understanding of the molecular mechanisms involved in angiogenesis has rapidly increased, the role that biomechanical signals play in this process is understudied. One of the greatest difficulties in understanding the interplay of flow dynamics and angiogenesis is that few tools exist to study flow dynamics in real time in vivo.

Methods
We developed a technique to simultaneously time-lapse flow dynamics and vascular remodeling in the capillary plexus of avian embryos (Figure 1). We injected green fluorescently labeled acetylated low-density lipoprotein (acLDL), which specifically labels endothelial cells and macrophages. We then injected red fluorescent microspheres to follow blood flow dynamics. We imaged the vessel wall every 15 minutes and the flow dynamics every 2 hours for a period of 12 to 16 hours. Flow dynamics were imaged at 250 frames per second for 2 cardiac cycles. The green images for network morphology were processed using a MATLAB program to identify vessel walls and centerlines. The blood flow velocity at the inlets and outlets to the vascular network were analyzed using micro-particle image velocimetry (µPIV). The geometry and the inlet/outlet flow rates were then imported into COMSOL. We calculated viscosity based on embryonic stage and vessel diameter using a non-Newtonian model ¹. The domain was then solved every 15 to 30 minutes for an entire cardiac cycle, beginning 30 minutes before a sprout appeared until 30 minutes after lumenization of the new vessel.

Results
We find that sprouts always form from a vessel at lower pressure towards a vessel at higher pressure. Furthermore, sprout elongation rate is proportional to the pressure difference between these two vessels. We find that sprouts form at the location of a shear stress minimum, but avoid locations where two blood streams merge even if this point is at a lower level of shear stress than the sprouting location. Our results show that flow dynamics are predictive of the location of sprout formation in perfused vascular network and that pressure differences across the interstitium can guide sprout elongation and elongation rate strongly correlates to the magnitude of the pressure differential between the vessels.

Conclusions
Our results show that flow patterns play a significant role in sprouting angiogenesis. The branching pattern in the capillary network affects the hemodynamic efficiencies of the vascular bed. It therefore makes sense that flow dynamics have an important role in determining sprouting location.

References
Cigarette smoke extract abrogates atheroprotective effects of high laminar flow on endothelial function

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Introduction
Tobacco smoking and local hemodynamic forces are key stimuli in the development of endothelial dysfunction and atherosclerosis. This proinflammatory endothelial phenotype is accompanied by changes in gene expression and decreased bioavailability of nitric oxide (NO). High laminar flow has an atheroprotective effect on the endothelium. This leads to a reduced response of endothelial cells to cardiovascular risk factors compared to regions with disturbed or low laminar flow. The molecular mechanisms controlling the atheroprotective effect of high laminar flow and its effect on the cardiovascular risk factor of smoking is not well understood. We hypothesize that the atheroprotective molecular mechanisms of high laminar flow could be used to prevent the development of endothelial dysfunction by tobacco smoking. Therefore, we exposed human endothelial cells to cigarette smoke extract (CSEaq) under different flow conditions and studied gene expression, monocyte adhesion and wound healing.

Methods & Results
Primary human endothelial cells were stimulated with increasing dosages of CSEaq for 24-48h. CSEaq reduced cell viability in a dose-dependent manner. The main mediator of cellular adaption to oxidative stress is the NRF2 system. NRF2 and its target genes heme oxygenase 1 (HMOX1) and NAD(P)H dehydrogenase (quinone 1) (NQO1) were strongly increased by CSEaq in a dose-dependent manner. High laminar flow induced elongation of endothelial cells in the direction of flow, activated the PKB/AKT pathway, followed by increased eNOS expression and subsequent NO release. This increase was inhibited by CSEaq in a time-dependent manner. Induction of the NRF2 system by CSEaq was not further regulated by high laminar flow. In contrast, proatherosclerotic low laminar flow had no effect on eNOS expression and NO release compared to high laminar flow. Proinflammatory adhesion molecules ICAM1, VCAM1, SELE, and CCL2 were increased by CSEaq. Low laminar flow induced VCAM1 and SELE compared to high laminar flow. On a functional level, high laminar flow improved endothelial wound healing. This protective effect was inhibited by CSEaq in a dose-dependent manner. Low laminar flow did not affect wound healing compared to static conditions. Low laminar flow as well as high laminar flow decreased adhesion of primary monocytes to endothelial cells. Interestingly, monocyte adhesion was increased by CSEaq under low laminar flow, which was not evident under high laminar flow.

Conclusions
In conclusion, our data suggest novel molecular mechanisms that underlie the association between tobacco smoking and the development of endothelial dysfunction. In contrast to low laminar flow, high laminar flow mediates protective effects on tobacco smoke-induced endothelial inflammation and wound healing.
Disturbed flow induces glycolysis enzymes at atheroprone sites

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Introduction
Atherosclerosis is a chronic disease of arteries that leads to angina, stroke or heart attack. It is characterized by the accumulation of lipids and inflammatory cells at the inner wall of arteries and is initiated by endothelial cell (EC) dysfunction. Atherosclerotic plaques develop predominantly at regions of branches and bends of the arterial tree that are exposed to low or disturbed blood flow, which generates low wall shear stress (WSS) and also influences the transport of biomolecules and dissolved gases from blood to the vessel wall. Using microarray technology we demonstrated that hypoxia inducible factor 1a (HIF1α) and several of its downstream targets (including glycolysis enzymes HK2 and ENO2) were enriched at an atherosusceptible region of the porcine aorta exposed to disturbed flow (unpublished). Thus we hypothesized that hypoxia and/or low WSS may influence EC dysfunction by inducing HIF1α-dependent altered glucose metabolism in EC.

Methods
Endothelial cells were collected from inner (atheroprone site exposed to disturbed flow) and outer (protected site exposed to unidirectional flow) curvatures of the porcine aortic arch, and quantitative RT-PCR (qRT-PCR) was performed to assess the expression level of target genes. Cultured human umbilical vein EC (HUVEC) were exposed to WSS for 3 days using an orbital flow system that generates unidirectional high WSS at the periphery and oscillatory low WSS at the centre. HUVEC were treated with 0.5mM DMOG for 24 hours to induce HIF1a. Analysis of gene and protein expression in cultured cells was performed using qPCR and western blotting. En face immunofluorescence staining and confocal microscopy was performed to assess the expression of specific proteins in EC inner and outer curvatures of the murine aortic arch (C57BL/6 strain). The exposure of EC to hypoxia was measured at inner and outer regions of the murine aorta using pimonidazole.

Results
qPCR demonstrated that the expression of HIF1α and downstream glycolysis enzymes HK2 and ENO2 was enhanced at the inner curvature compared to the outer curvature in the porcine aorta (HIF1α p<0.015; HK2 p<0.008; ENO2 p<0.03). Similarly, en face staining of the mouse aortic arch demonstrated that expression of HK2 and ENO2 was higher at inner curvature compared to the outer curvature (p<0.04; p<0.001). Staining with pimonidazole revealed that EC at the inner curvature of the murine aortic arch were exposed to hypoxia, whereas cells at the outer curvature were not (p<0.02). Thus EC at the inner curvature were exposed to hypoxia and low WSS and they expressed elevated levels of glycolysis enzymes. To determine whether hypoxia and/or WSS influence glycolysis enzyme expression we exposed cultured HUVEC to WSS for 72h in the presence or absence of DMOG. Western blotting revealed that the expression of ENO2 and HK2 was higher in cells exposed to low WSS compared to high WSS. Both enzymes were induced by 0.5mM DMOG (2-3 fold change), and the combination of low WSS and DMOG had a synergistic effect on ENO2 and HK2 expression (4-6 fold change).

Conclusions
We conclude that EC at atheroprone sites of disturbed flow express HIF1α and the downstream glycolysis enzymes HK2 and ENO2. It is likely that disturbed flow regulates HK2 and ENO2 by altering both molecular transport and mechanical conditions since hypoxia and low WSS synergised to induce these molecules. Further studies are required to elucidate the mechanosensitive pathway responsible for the regulation of glycolysis enzymes by WSS, and to determine the relevance of this pathway to arterial injury and atherosclerosis.
Evaluating the influence of simulated hypergravity on endothelial cell behavior

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Introduction

The ability to regulate angiogenesis is of great value in the fields of tissue engineering and oncology. Endothelial cells (ECs) are influenced by mechanical forces, with their phenotype and function being continuously conditioned by local hemodynamics (fluid shear stress, pressure and associated stretch). Thus, we set out to determine the effect of gravitational force on angiogenesis. Hypo- and hypergravity conditions have been reported to affect ECs, in terms of migration and proliferation, as well as leading to reorganization of cytoskeletal proteins, affecting their ability to organize into capillary-like structures in vitro. The present work aims at investigating the influence of hypergravity on EC behaviour with special focus on angiogenesis, particularly on cytoskeleton organization and capillary-like structure formation under different exposure times (4 and 16 h) and g levels (3 and 10g).

Methods

Hypergravity conditions were generated using the Large Diameter Centrifuge (LDC) from the European Space Research and Technology Centre (ESTEC, ESA, The Netherlands). Two different populations of human umbilical vein ECs (HUVECs) were studied. To evaluate the effects of hypergravity on cytoskeleton reorganization, cells were cultured in 2D, whereas the formation of capillary-like structures was evaluated in a 3D matrigel assay. Additionally, the influence of pre-stimulating cells with hypergravity was also investigated by (i) culturing cells in 2D under hypergravity and then transferring them to a 3D matrigel assay; or by (ii) culturing cells in 3D matrigel first under hypergravity and then under normal gravity conditions (1g). HUVECs in 3D matrigel assay were observed and photographed under an inverted optical microscope. The number of capillary-like structures was counted. F-actin filaments of the cytoskeleton were stained using phalloidin and nuclei were counterstained with DAPI. Samples were visualized by fluorescence microscopy.

Results

Cell viability was determined after each exposure to hypergravity and was unaffected compared to control (1g) conditions. No major alterations were observed in the organization of F-actin filaments. Further analysis of other cytoskeletal components, including vinculin and tubulin, is currently being performed.

The number of capillary-like structures was diminished after culturing HUVECs under hypergravity conditions in the 3D environment. This was irrespective of the level and duration of hypergravity exposure. Strikingly, prestimulation (from 2D hyper- to 3D normal gravity) of HUVECs under 3g or 10g for 16h resulted in an increase in capillary-like structures compared to 4h under both g levels. By contrast, prestimulation in 3D showed opposite results, with a lower number of capillary-like structures being formed.

Conclusions

Culturing HUVECs under hypergravity conditions alters their ability to form capillary-like structures, dependent on 2D or 3D culturing. More research is required to determine the mechanisms associated to HUVEC response.

Acknowledgements

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Disturbed flow-induces the formation of multi-nucleated endothelial cells

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Introduction
Atherosclerosis is a chronic inflammatory disease of arteries with dysfunctional endothelium failing to maintain vascular homeostasis, leading to the formation of atherosclerotic plaques. Wall shear stress (WSS; the drag force exerted upon endothelial cells (EC) lining the blood vessels by flowing blood) is recognised as a major contributor of atherogenesis, with lesions often forming at bends and branches of the arterial tree that are exposed to disturbed blood flow patterns. While there are likely to be multiple mechanisms that induce a dysfunctional EC phenotype, Tokunaga and colleagues provided evidence to the existence of giant multi-nucleated EC (MNEC) in the human aorta [1]. Although senescence through excessive cellular replication is commonly associated with MNEC formation, the presence of odd-numbers of nuclei suggests mechanisms other than this canonical replication pathway. It was therefore hypothesised that disturbed WSS may promote MNEC formation via mechanisms other than replicative senescence and contribute to EC dysfunction.

Methods
An in vitro live-cell imaging platform was used to observe the formation of MNEC under disturbed WSS. Confluent monolayers of human umbilical cord vein EC (HUVEC) were exposed to oscillatory flow (±4dyn/cm² at 0.5Hz) using the Ibidi® pump system and monitored by microscopy (Nikon® TE300) with temperature- (37°C) and CO₂- (5%) control for up to 72 hours. For high-throughput analysis, immunofluorescence cytochemistry was performed on HUVEC exposed to disturbed flow using an orbiting plate system (rotation at 210rpm) to generate low oscillatory WSS (disturbed flow) in the centre of the well.

Results
Time-lapse imaging of HUVEC exposed to oscillatory WSS revealed the formation of MNEC via replicative senescence as well as by cell fusion. HUVEC exposed to disturbed flow using the orbital plate system also produced MNEC with two or more nuclei (2.68±0.61%), with a smaller proportion of MNEC contained three or more nuclei (0.45±0.28%). To determine the relative contribution of disturbed flow-induced fusion to endothelial multinucleation, HUVEC labeled with 5-ethynyl-2'-deoxyuridine (EdU) were combined with unlabelled HUVEC and the population was then subjected to disturbed flow. While MNEC formed via replicative senescence will have either EdU⁺VE⁺/⁻VE or EdU⁻VE⁻/⁺VE nuclei, about half of MNEC formed via cell fusion will have EdU⁻VE⁻/⁺VE nuclei. It was revealed that 11.33±0.67% MNEC were formed via fusion (representing 0.33±0.07% of total HUVEC). Immunocytochemistry analysis further demonstrated that MNEC formed via cell fusion expressed typical senescence markers of γH2AX, p53 and p21.

Conclusions
This study revealed that MNEC form under conditions of disturbed flow. In addition to the canonical pathway of replication-induced MNEC formation, we identified a novel mechanism of cell fusion-induced multinucleation under disturbed WSS. MNEC were dysfunctional as evidenced by their expression of several markers of senescence including γH2AX, p53 and p21. Further studies are required to identify the responsible mechanosensing pathway involved in cell fusion and its potential role in atherogenesis.

References
Activation and nuclear translocation of NF-κB in vascular endothelial cells exposed to shear stress – Live cell imaging and mathematical modelling

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Introduction: We investigate the impact of hemodynamics on the activation and nuclear translocation of NF-κB in vascular endothelial cells (ECs). NF-κB is a key promoter of inflammatory responses in vascular ECs and plays a pivotal role in cell growth, survival and apoptosis. Mis-regulation of NF-κB is related with inflammation, autoimmune and metabolic diseases and cancer. It has been shown that shear stress has a large effect on temporal and spatial concentration of NF-κB in ECs. However, the exact dynamics of the nuclear translocation of NF-κB in response to shear stress remain unknown. We aim to explore the real time translocation of NF-κB in ECs exposed to laminar and disturbed flows.

Experimental methods: We transfect Human Umbilical Cord Endothelial Cells (HUVECs) with plasmids sequenced with NF-κB, IκK and IκBo containing a protein tag of enhanced green fluorescent protein (eGFP). The transfected HUVECs are seeded in a parallel flow and in a backward facing step channel. Laminar flow of a physiological range is excited within the channels. The activation and nuclear translocation of NF-κB, IκK and IκBo is measured with live-cell imaging by the emission of the fluorescent protein tag.

Mathematical methods: We have developed an intracellular signalling model that predicts the temporal concentration of NF-κB in ECs. We first model single cell dynamics that include a temporal control of IκK, which cause the unbinding of NF-κB from IκBo. Secondly, with Monte Carlo methods we simulate the intracellular dynamics of a cell population to account for different excitation levels of the cell receptors, such as receptor tyramine kinase, integrins and PECAM-1 that activate IκK. Hence, we predict the NF-κB dynamics in a cell population in different flow environments (straight channel and backward facing step channel).

Results: We have observed a clear difference in the temporal activation and nuclear translocation of NF-κB between low and high shear stress. Low shear stress (1-3 dyne/cm²) causes slow activation of NF-κB and peaks after 2 hours and remains high. In comparison, when exposed to high shear stress (10-15 dyne/cm²) the nuclear concentration of NF-κB concentration peaks at around 30 minutes and then steadily declines until it remains at a low concentration after 80 minutes. Our model predictions of the activation and nuclear translocation of NF-κB of an endothelial cell population are in agreement with the experimental observations. In Figure 1 the nuclear NF-κB concentration for 137 cells exposed to 10 dyne/cm² shear stress are shown.

Conclusions: We demonstrate that low and high shear stress causes different temporal activation and nuclear translocation of NF-κB in vascular endothelial cells. Using a novel intracellular signaling model of NF-κB that incorporates dynamic activation of IκK we are able to predict the nuclear concentration of NF-κB in a cell population of endothelial cells exposed to shear stress. This model will be applied to image-derived vessel geometries to predict the nuclear NF-κB concentration and consequently point out areas that are high in risk of inflammatory responses.

References:
Shear Stress Dependent Regulation of P2X4 and P2X7 Receptors in the Endothelium
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Introduction
Regions of the arterial tree exposed to disturbed blood flow with low wall shear stress (WSS) are prone to atherosclerotic plaque development, whereas areas under undisturbed flow with high WSS are considered protected. In response to shear stress, endothelial cells release ATP extracellularly which activates nearby cell surface receptors. The endothelium expresses the ATP-gated cation channels P2X4 and P2X7, which previous studies have shown to be activated by shear stress-induced ATP release in the endothelium[1] and osteoblasts[2] respectively. As shear stress influences atherosclerotic plaque development, and the release of ATP, we hypothesised that P2X4 and P2X7 receptors are differentially regulated by shear stress and thereby contribute to the focal nature of atherosclerosis.

Methods
Human umbilical vein endothelial cells (HUVECs) were exposed to different levels of shear stress for 72 hours using either an orbital shaker or an ibidi flow pump system. The orbital shaker model (210 rpm) produces high shear undisturbed flow at the periphery and low shear disturbed flow in the centre of a 6 well plate. The ibidi pump modelled atheroprotective flow by exposing cultured HUVECs to +13 dynes/cm², as this falls within the range of physiological arterial shear stress, whereas +/-4 dynes/cm² was used to mimic atheroprone flow. Transcripts of P2X4 and P2X7 splice variants were identified using reverse transcriptase-PCR, quantitative real time-PCR or western blotting. Immunocytochemistry was used to identify subcellular localisation of P2X7 receptors and calcium imaging was used as a functional readout of P2X receptor activation. P2X7 receptor activation was modulated using BzATP, a P2X7 agonist, and A438079 hydrochloride, a potent antagonist of P2X7 receptors.

Results
We found that the endothelium expresses several P2X4 and P2X7 splice variants. Expression of P2X7 splice variants was significantly increased under high shear undisturbed flow relative to low shear disturbed flow. Immunocytochemistry confirmed that P2X7 was expressed on the surface of HUVECs, as well as a population in the ER/golgi. mRNA expression of P2X4 splice variants significantly increased under disturbed flow, but protein levels remained unchanged. Calcium imaging on static HUVEC showed the presence of functional P2X4 and P2X7 channels, which could be modulated pharmacologically. The P2X7 agonist BzATP induced calcium responses in endothelial cells that were modulated according to pre-conditioning with either high WSS (+13 dynes/cm²), low WSS (+4 dynes/cm²) or oscillating low WSS (+/-4 dynes/cm²), indicating that P2X7 responses may be altered after exposure to different WSS.

Conclusions
These studies show that P2X7 expression is augmented under high shear undisturbed flow. Our results also indicate that P2X7 activity may be differentially regulated in endothelial cells conditioned to different WSS conditions. Thus P2X7 may contribute to atheroprotection at sites of high shear by controlling downstream calcium signalling pathways. Our future studies will test this concept and determine how shear stress conditions alter P2X receptor trafficking and regulatory interacting proteins.

References
The Rho-GEF Trio regulates long-term flow-induced alignment of endothelial cells

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Endothelial cells line the lumen of the vessel wall and are constantly exposed to laminar flow. As a result, these cells align in the direction of the flow and protect the vessel wall from damage and leakage. However, disturbed flow patterns can induce an inflammatory phenotype of the vessel wall, ultimately resulting in vascular diseases such as atherosclerosis. Therefore, it is important to understand how endothelial cells translate changes in flow conditions to align in the direction of the flow.

In this study, we show that endothelial cells align within 12 h of laminar flow, as is illustrated by linear VE-cadherin-based stable cell-cell junctions. With the use of a novel FRET-based Rac1 biosensor with increased FRET efficiency, we were able to image over longer periods of time and showed for the first time that Rac1 remained activated and polarized during flow-induced endothelial cell alignment. We additionally showed that polarized Rac1 activation as well as cell alignment was dependent on the presence of the Rho-GEF Trio. Surprisingly, we found that Trio did not regulate flow-induced Rac1 activation, but rather controlled the localization of active Rac1 within the cell. To further assess the role of Trio in flow-induced alignment, we used fluorescent recovery after photo-bleaching (FRAP) techniques. These experiments revealed that flow reduced the mobility of Trio at endothelial cell-cell junction regions. In addition, loss of Trio resulted in perturbed monolayer integrity under flow conditions, assessed by Electric Cell Substrate Impedance Sensing (ECIS) and reduced linear junction morphology.

In conclusion, our data show that flow-induced alignment requires the Rho-GEF Trio as a scaffold protein to control flow-induced polarized Rac1 activation and endothelial permeability.
Using magnetic tweezers to probe mechanically-induced signalling in endothelial cells

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Introduction
Atherosclerosis is an inflammatory arterial disease that can cause heart attack and stroke. It develops in regions exposed to disturbed flow, such as at bends, while regions of steady flow are protected. Blood flow affects endothelial cells through drag forces (shear stress) and by modifying biomolecular motion around the cell (mass transport). Microfluidic flow systems are commonly used in vitro to examine the effect of flow on cellular signalling. However, the relative contributions of shear stress and mass transport to the overall response cannot be distinguished in a flow-based system. Furthermore, flow-based methods offer limited functional understanding of shear stress sensing mechanisms, since flow triggers multiple stress-sensitive receptors (mechanoreceptors) simultaneously. An alternative approach is to use magnetic tweezers, which overcome these difficulties by applying force directly to specific mechanoreceptors. Here, we describe the development of a magnetic tweezers system that enables two-dimensional forces to be applied and demonstrate its potential for studying live-cell signalling processes.

Methods and Results
A four-poled electromagnet was designed and built as part of a magnetic tweezers platform. The electromagnet had a mild steel core and 360 turns of coil per pole piece. Magnetic fields were generated by passing electrical current around the coils. Each pole was independently powered, enabling the two-dimensional magnetic field profile between the poles to be controlled. ANSYS software was used to solve the Biot-Savart equation to computationally model the magnetic field profile produced in the region between the pole pieces. Forces were calculated from the magnetic energy gradient of a superparamagnetic bead in the field, showing that the maximum force was 16 pN per bead. The electromagnet was embedded within a fluorescence microscope fitted with an incubation chamber heated to 37 ºC, enabling live-cell imaging during force application. Computer control of the magnetic field profile enabled the generation of forces of arbitrary magnitude, direction and oscillation frequency.

Human umbilical vein endothelial cells (HUVEC) were cultured in fibronectin-coated dishes until they reached confluency and were serum-starved prior to experimentation. Cells were loaded with a calcium-sensitive fluorescent dye, Cal-520 (Stratech), prior to the application of superparamagnetic beads coated with antibodies that recognise integrin-β1 (12G10, AbD Serotec). Cells were imaged for 3 min without force to define a signalling baseline, followed by 3 min of applied force. Force applied to the mechanoreceptors initiated calcium influx around the beads (fig. 1a-c). Using single-cell analysis, the amplitudes of the calcium influx peaks were quantified. Initial results indicated that peak amplitude significantly increased under constant 16 pN force and that the response under force was similar to that induced by 15 dyne/cm² laminar flow (fig. 1d and e).

Conclusions
Magnetic tweezers were built around a fluorescence microscope to enable live-cell imaging of cell signalling during force application. Using calcium signalling to validate the approach, the feasibility of using the magnetic tweezers to induce a mechanoresponse was demonstrated. The flexible design of the magnetic tweezers means it has the potential to uncover the mechanisms through which shear stress is converted into a biological signal, since it can be adapted to study a variety of mechanoreceptors, signalling pathways and force profiles.
Ubiquitin Proteasome System Does Not Play a Major Role in the Degradation of IκBα in Endothelial Cells Exposed to Steady Laminar Flow

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Introduction

Nuclear factor-κB (NF-κB) which is crucial in regulating inflammatory pathways of atherosclerosis is inhibited by IκBα protein. Phosphorylation of IκBα (p-IκBα) causes activation of NF-κB by degrading IκB via ubiquitin proteasome system (UPS). Shear stress is known to cause activation of NF-κB but the precise details of this phenomenon are not well defined. The aim of this study was to investigate the mechanism of the degradation of IκBα in endothelial cells under continuous laminar flow (CLF).

Methods

Bovine aortic endothelial cells (BAECs) seeded on fibronectin coated glass slides were exposed to CLF in a parallel plate flow chamber system for 4 hours with or without 7.5µM MG132, a UPS inhibitor. BAECs kept under static conditions served as control. Cell lysates were immunoblotted with anti-IκBα, anti-phospho-IκBα and anti-Ubiquitin antibodies. Fold change compared to static control at 0 hour was calculated and compared by t-test. P value of <0.05 was considered statistically significant.

Results

Exposure of BAECs to CLF for 4 hours significantly decreased the levels of IκBα compared to static control (56.4±24.7%; p < 0.05) but caused no significant change in the level of p-IκBα (204.2±83.1%; p=NS). Addition of MG132 significantly increased the level of p-IκBα (1578.0±442.7%; p=0.02) but failed to inhibit the decrease in the level of IκBα caused by CLF (58.4±29.0%; p=NS).

Conclusions

The degradation of IκBα under CLF occurs via an alternative, UPS-independent pathway. Further studies are to be done to elucidate this novel mechanism of degradation.
Wall Shear Stress, Permeability and Lesion Frequency in the Mouse: Role of PECAM-1

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Introduction
Putative local risk factors responsible for the patchy development of atherosclerosis include wall shear stress (WSS) and transport properties of the arterial wall. Platelet/endothelial cell adhesion molecule 1 (PECAM-1) expressed by endothelium is involved in mechanosensing WSS. In addition, studies of hyperlipidaemic mice crossed with PECAM-1-/- mice have revealed that knocking out PECAM-1 alters disease patterns. Focusing on the aortic arch, we investigated patterns of permeability in wild-type and PECAM-1-/- mice, and WSS and lesion prevalence in apoE-/- mice, as part of a programme to determine spatial correlations between these properties and the role of PECAM-1.

Methods
Excised aortas of apoE-/- mice fed a Western diet were stained with oil red O and imaged by fluorescence microscopy so that lesion prevalence could be mapped. To assess wall permeability, rhodamine-labelled albumin (3 mg, IV) was administered to wild-type or PECAM-1-/- mice. After 10 minutes, aortic arches were fixed in situ and excised; uptake of the fluorescent tracer was imaged by confocal microscopy. WSS was computed from numerical simulations of steady flow (Re = 140) using an in-house spectral/hp element framework and vessel geometries obtained by microCT of corrosion casts.

Results
Fig 1A shows lesion distribution around the brachiocephalic (BCA), left carotid (LCA) and left subclavian (LSCA) arteries of apoE-/- mice fed a Western Diet for 16 or 25 weeks. Permeability maps in wild-type and PECAM-1-/- mice are shown in Fig 1B, while Fig 1C shows WSS in an apoE-/- aortic arch. Lesions occurred mainly within the branch mouths, spreading upstream of them with increased time on the Western diet. Permeability was elevated upstream of the LCA and LSCA; mean permeability was higher in PECAM-1-/- than in wild-type mice. The wall upstream of branch mouths experience lower shear than the downstream region.

Conclusions
Our preliminary data provide evidence for a correlation between permeability and lesion frequency and an inverse correlation with WSS. Knocking out PECAM-1 increased permeability in some regions, suggesting a mechanism by which it alters lesion patterns. We are currently mapping disease in apoE/PECAM-1 double knockouts and simulating pulsatile WSS patterns. (Funded by the BHF).
Disturbed flow induces expression of the negative NF-kappaB regulator Cezanne

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Introduction
Atherosclerosis is a chronic inflammatory disease occurring at arterial sites exposed to disturbed flow, which generates low oscillatory wall shear stress (WSS). Endothelial cells (ECs) at these sites transduce haemodynamic forces into inflammatory signals through the NF-kappaB transcription factor family, leading to endothelial activation and lesion development. The deubiquitinase Cezanne negatively regulates NF-kappaB activity through disruption of upstream signalling\[1\][2]. In order to understand the potential role of Cezanne in the regulation of focal vascular inflammation, we assessed the influence of different flow patterns on the expression of Cezanne using in vitro and in vivo models.

Methods
Human umbilical vein ECs (HUVECs) were cultured in vitro under high (20 dyn/cm\(^2\)) or low oscillating (±5 dyn/cm\(^2\)) wall shear stress (WSS) using a commercial parallel plate flow system (ibidi); or in a six-well plate orbital shaker system which generates unidirectional high WSS in the periphery and multidirectional low WSS in the centre of each well. Gene silencing of Cezanne by RNA interference was carried out using electroporation. Quantitative real-time PCR (qPCR) and Western blotting were employed to detect changes in Cezanne mRNA and protein expression. Additionally, regions of high and low WSS within the murine vasculature were immunostained en face for Cezanne using fluorescent antibodies and analysed by laser scanning confocal microscopy.

Results
qPCR revealed that Cezanne mRNA was expressed at significantly higher levels in ECs exposed to low oscillating WSS compared to high WSS using either the orbital (p<0.001) or parallel plate (p<0.05) system. Silencing of Cezanne and subsequent Western blotting revealed the presence of multiple isoforms of Cezanne, and demonstrated that Cezanne protein expression was enhanced in EC exposed to low oscillating WSS compared to high WSS (p<0.0005). En face immunostaining analysis showed that Cezanne protein expression was elevated at a disturbed flow region of the inner curvature of the murine aortic arch in comparison to a uniform flow region in the outer curvature.

Conclusions
Cezanne expression in EC was enriched at an atheroprone site in the murine aorta where cells are exposed to complex flow pattern with low WSS. The upregulation of Cezanne in two in vitro models of arterial WSS is consistent with the mechanism of induction being related to local haemodynamics. It is plausible that Cezanne functions to limit inflammation at low WSS disease-prone sites by inhibiting NF-kappaB. This hypothesis will be tested by silencing of Cezanne expression in cultured ECs and by using Cezanne knockout mice.

References
The evaluation and characterization of mammalian thrombi models for the investigation for vascular occlusion in acute ischaemic stroke

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Introduction
A lack of blood supply to any part of the brain for an unknown period of time can lead to stroke. Stroke is the third most common cause of death and the leading cause of acquired long-term disability worldwide. Over 80% of all strokes are ischaemic and are caused by a thrombus occlusion of a major cerebral artery. Often the thrombus may have originated elsewhere in the cardiovascular system, but embolised and lodged in the cerebral vasculature. The retrieval of thrombi from humans for the investigation of stroke is expensive and highly regulated. Therefore, embolus analogues manufactured from mammalian blood¹ are necessary in understanding the pathophysiology, morphology and mechanical characteristics of thrombi. This project concerns the histological and mechanical examination and characterisation of thrombin induced mammalian thrombi.

Methods
Mammalian blood, of ovine and bovine origin, was harvested from a local abattoir and induced immediately with bovine thrombin. To investigate the effect of thrombin on thrombus composition, the thrombin concentration was varied among the samples (0-10 NIH units/ml blood). Haemotoxylin and Eosin (H&E) and Martius, Scarlet and Blue (MSB) stains were completed for compositional analysis. Tensile (n=13) and compression tests (n=14) were conducted on the corresponding thrombi models and results were compared to literature values². 9 patient specific scans of the aortic arch in DICOM format were imported into Mimics Version 16.0 to generate 3D models using segmentation techniques. Models were geometrically characterised and differentiated for flexible model production. These models were incorporated into a cerebral test facility for releasing the animal thrombi and tracking the trajectory within the cerebral vasculature³.

Results
The presence of nuclei and erythrocytes correlated significantly with stained human thrombi samples (Figure 1A). The presence of fibrin and collagen was determined in high concentrations of thrombin induced mammalian thrombi (Figure 1B). Tensile and compression tests were conducted at varying strain displacements. Initial results indicate a change in stiffness with increase in thrombin concentration.

Figure 1 H&E stain ovine thrombus (6NIH Units/ml blood) (A), MSB stain bovine thrombus (9 NIH Units/ml blood) (B)

Conclusions
The successful development of such thrombus models and future clot flow studies may provide a basis for the characterisation of post-operative thrombi removed from humans. Knowledge about the histological characteristics and lodgement morphologies of thrombi may provide a means for improving current endovascular therapies and the development of new treatment strategies for revascularisation in patients with acute ischemic stroke. These findings could indicate that the composition of thrombi is a potential key variable regarding the selection of the appropriate treatment options for ischemic stroke patients and in predicting the performance of mechanical thrombectomy devices and thrombolytics.

References
Bundling of fibrils governs the viscoelastic response of fibrin clots to small and large shear stresses

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Introduction
Blood clots are mechanically supported by a three-dimensional mesh consisting of branched fibrin fibers. Recent works have started to explore the extensibility of fibrin fibers and the links between the mechanical properties of fibrin clot and its underlying network structure. Such efforts have proven to be a challenging multiscale enterprise, however, as it has been increasingly realized that macroscale properties of whole clots are strongly affected by nanoscale properties and variabilities of the molecules making up the clots.

Methods
In this work, we aim to better understand how the remarkable mechanical properties of the fibrin clots derive from the self-assembled structures at the fiber and molecular levels. To this end, we combined rheological measurement of the viscoelastic response of fibrin clots to varying degrees of shear stresses with structural quantification at different length scales using electron and fluorescence microscopy as well as light scattering approaches.

Results
We show that fibrin clots can dramatically stiffen—up to more than 100-fold—as they are increasingly stressed. The response at small stresses can be quantitatively predicted by accounting for the bundle size, i.e., the number of protofibrils within each fibrin fibers, assuming strong lateral association between the protofibrils. At large stresses, the clot response is governed by the stiffening of single fibers, indicating that the stress–strain relationship of single fibers dominates at large clot deformations. To corroborate the role of lateral association between protofibrils, we examined the effect of Factor XIII-mediated clot ligation, which has been previously reported to enhance clot stiffness. Ligation is found to (1) physically compact the fibers and (2) enhance lateral coupling between protofibrils, thus effectively stiffening the fibers and thereby the clot.

Conclusions
We find that structural hierarchy plays a major role in determining the integrity and behavior of clots. The structural architecture of fibrin fibers can thus account for the enormous elastic resilience characteristic of blood clots. A better understanding of the structure–mechanics interconnection can not only shed important light on the molecular origins of fibrin behavior and regulation in thrombosis, but also provide guidance in designing haemostatic materials, particularly in surgical settings and tissue engineering.
Numerical modeling of the deposition of activated platelets in a stagnation point flow

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Introduction
Thrombus formation depends on local properties of blood, wall and blood flow also known as Virchow Trias. The role of blood flow mostly is studied in parallel flow cells with the focus on wall shear stress (WSS). Only a few experimental studies examined the deposition in non-parallel flows, so that current platelet deposition models may be limited in their application. Based on the depositions of activated platelets in a stagnation point flow, we propose a two-parameter numerical model.

Methods
Fresh human blood was drawn from volunteers using an S-Monovette\(^\circ\) with Trisodium citrate solution (Sarstedt, Germany). The blood platelets were labeled with Calcein Red Orange for fluorescent microscopy. This prepared blood is drawn into a flow chamber with continuous adding of Adenosine-Diphosphate for platelet activation. It enters the chamber through a canula with an inner diameter of 650 µm. The pipe ends 480 µm above a cover glass and the blood impinges perpendicular on it. There a stagnation point flow is formed. The cover glass is coated with von-Willebrand-factor. The depositions were observed and analyzed to obtain the surface coverage for different distances from the stagnation point. The flow rate was varied in a range from 20 to 80 ml/h to result in WSS maxima of up to 480s\(^{-1}\). The WSS was assessed with flow simulations (CFD). Further details on these parts are given in [1]. Based on this data, a two-parameter deposition model was developed. For that platelet quantity conservation in a volume above the coated surface of certain height was examined. This volume was subdivided radially into rings with a certain width. Numerical results taken from (CFD) the platelet rates inflow from the bulk flow into the volume rings are known. From the experimental data the deposition rates are known, so that a ratio of depositing to incoming platelets for each ring volume can be calculated starting at the stagnation point. Assuming further a hemodynamic independent value for this ratio, for each run a parameter pair – height and ratio – was determined. The ratio was reinterpreted to a width independent deposition probability parameter using decay functions.

Results
An example of a deposition pattern is shown in the image with a deposition maximum near the stagnation point. In the box plots the fitting quality \(R^2\), the values of the deposition probability and the height \(H\) of volume layer are shown as median, the 25th and 75th percentiles and extreme values for each flow rate.

Conclusions
The model shows a promising agreement with experimental data. This model shows the importance of the velocity component towards the wall, but neglect the aggregate detachment by the flow.

References
In vivo determination of wall strain in the young, old and aneurysmal aorta with real time 3D speckle tracking ultrasound

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Introduction
Rupture of the infrarenal aortic aneurysm, with its inherent mortality and morbidity, remains a major clinical problem. Therefore, determining biomechanical properties of the aorta is an important diagnostic step to identify those patients with high aneurysm rupture risk. Although ultrasound measurement of aortic diameter may be sufficient for aneurysm screening and allows control of aneurysm progression, analysing biomechanical properties of the aneurysm enables better rupture prediction. So far, prospective risk stratification has been encumbered with offline analysis, significant radiation exposure and or contrast medium administration. The aim of this study was to analyze the biomechanical properties of the infrarenal aortic aneurysm with real time 3D speckle tracking ultrasound to localize wall areas with higher rupture risk.

Methods
In a prospective study, biomechanical properties of the aortic wall in 46 patients with a normal aorta (younger than 60 y n = 21, older than 60 y n = 25) and in 19 patients with infrarenal aortic aneurysm were examined employing real time 3D speckle tracking ultrasound. The heterogeneity of the circumferential strain in the infrarenal aorta was defined by global (circumferential) strain amplitude, spatial heterogeneity index, (circumferential) local peak strain and the ratio of local peak to global mean strain 1.

The dyssynchrony of the infrarenal aorta was described by systolic delay und the systolic dyssynchrony index. Subsequently, high resolution 4D models of the aorta were examined from 15 patients with infrarenal aortic aneurysm to determine regions exhibiting pathological strain.

Results
Younger patients had a higher mean circumferential strain amplitude than older patients (p <0.05). Aortic aneurysms displayed a significantly higher spatial heterogeneity index (p <0.05) than non-aneurysmal aorta. The ratio of local maximum circumferential strain amplitude to global average circumferential strain amplitude was lower in younger patients (p <0.05), but non-aneurysmal and aneurysmal aorta could not be differentiated independently from aortic diameter using this parameter (p> 0.05). The mean global strain amplitude was significantly higher in the aneurysm neck, compared to the maximal aneurysm diameter (p <0.05). The areas with maximum local circumferential strain were predominantly localized in posterolateral regions of the aneurysm wall. For the first time it has become possible to identify pathological regions with 4D ultrasound, which correspond in situ to an area prone to infrarenal aortic aneurysm rupture.

Conclusions
Real time 3D speckle tracking ultrasound reflects age and pathology of the aortic wall. It allows the description of a patient-specific strain and displacement pattern throughout the cardiac cycle and identifies rupture prone regions of the aortic aneurysm. The infrarenal aortic aneurysm displays highly heterogeneous circumferential strain with the highest amplitude in posterolateral regions. In a first clinical case it was possible to associate pathologic strain distribution with the rupture site in an infrarenal aortic aneurysm.

References
Patient specific stress and rupture analysis of ascending thoracic aneurysms

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Introduction
Thoracic aortic aneurysm is defined as an abnormal widening or ballooning of a portion of the aorta due to a pathological weakness in the wall. The most common cause of this pathology is hardening of the arteries (atherosclerosis), which is common in people with high cholesterol, long-term high blood pressure, or who smoke [1]. For patients with aneurysms of the ascending aorta, surgery to replace the aorta by plastic or fabric graft is recommended if the aneurysm is larger than 5.5 cm [2]. These aneurysms have a much higher risk of rupture if they are not replaced.

This manuscript presents a method for obtaining the wall stress and retrospective rupture risk for ascending thoracic aortic aneurysms (ATAAs) on a patient specific basis.

Methods
In this study, the ATAAs were obtained during elective surgeries performed in the CHU hospital of Saint Etienne, where each patient's aneurysm was replaced with a synthetic graft. In addition, the preoperative ECG-gated dynamic CT scans for each patient were provided by the same hospital. The material properties and rupture stress were determined by performing bulge inflation tests on the collected aneurysm samples. The dynamic CT scans were used to generate patient specific geometries for a finite element model of each patient's aneurysm. The material properties from the bulge inflation tests were implemented in the finite element model and the wall stress distribution at 4 different pressures was estimated.

Results
Three different rupture risk assessments were compared: The maximum diameter, the peak wall stress, and the overpressure index. The peak wall stress for patients varied from 412 to 783 kPa and was between 28% and 94% of the ATAA's failure stress. The peak wall stresses identified for each of the patients was loosely correlated with the maximum diameter of the aneurysm. The largest aneurysm did not have the largest peak wall stress, and the local geometry of the aneurysm seems to have an important impact on the wall stress. Moreover, the hypertensive patient, who has the smallest aneurysm, had the highest rupture risk.

Conclusions
Using the results of the finite element analysis, we proposed a method to estimate the risk of rupture for ATAA on a patient-specific basis using dynamic CT scans. In the future, we plan to perform a larger study population to confirm that the patient specific material properties can directly be identified from CT images.

References
A noninvasive in vivo approach for the identification of elastic AAA wall properties using 3D ultrasound data

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Introduction
Computational biomechanical models of abdominal aortic aneurysms (AAA) aim at obtaining patient-individual rupture risk predictors in addition to the statistical diameter criterion. Despite the great inter-individual variance of the parameters determining the mechanical behavior of the AAA wall, most of the approaches are patient-specific only with regard to geometry and blood pressure. In contrast, population averaged constitutive equations are used in most cases [1]. In the present study, we have determined the individual nonlinear and orthotropic elastic properties of the AAA wall noninvasively in vivo. We have investigated the impact of the use of patient-individual vs. population averaged constitutive behaviour on the peak wall stress predicted by a finite element analysis of the AAA wall.

Methods
Three-dimensional volume data sets of a saccular AAA with a maximum diameter of 32 mm were acquired by use of a real time 3D-echocardiography system (Artida™, Toshiba Medical Systems, Japan). A discrete field of 1080 material points on the aneurysm wall were obtained for 19 geometrical configurations throughout the pulse cycle by use of a customized commercial speckle tracking algorithm (Advanced Cardiology Package, Toshiba Medical Systems, Japan). Diastolic and systolic blood pressure was measured at the brachial artery. In case of the model with the patient-individual material properties (patient-individual model) the constitutive parameters of a nonlinear orthotropic strain-energy function (SEF) [2] were identified by use of an inverse finite element model updating method that accounts for prestresses in the imaged reference configuration [3]. In case of the model with a population averaged SEF (population averaged model) a 2nd order Yeoh constitutive equation with the parameters $c_{10} = 177$ kPa and $c_{20} = 1881$ kPa was used [4]. Finally, systolic wall stresses were calculated for both models by applying hydrostatic transmural pressure to the prestressed diastolic geometries.

Results
The identified individual constitutive parameters are: $\mu = 0.228$ MPa, $k_1 = 4.736$ MPa, $k_2 = 1960.65$ MPa, $\kappa = 0.207$, $\varphi = 4.6^\circ$. The patient-individual model shows a peak wall stress of 2.11 MPa, which is close to the upper limit of the range of AAA wall failure stresses (0.34 to 2.35 MPa) that is reported in [5]. It exceeds the peak wall stress of the population averaged model (0.91 MPa) by more than 2 times.

Conclusions
Both models predict increased peak wall stress for an AAA that is not prone to rupture according to the diameter criterion. The patient-individual model indicates a much higher rupture risk according to the peak wall stress criterion compared to the population averaged model. Thus, the presented in vivo constitutive parameter identification method is a promising step towards more patient-specific computational models of AAA.

References
A new device to assess the visco-elastic and active isotonic properties of periodically stretched isolated mouse aortic segments

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Introduction
Besides shear stress also cyclic stretch is known to substantially alter the biomechanical properties of arteries. While most studies on isolated mouse aortas are done at low stretch frequency or even under static conditions and subphysiological stretch amplitude, pacing experiments done in rodents and humans show that arterial compliance is strongly stretch frequency- and amplitude-dependent. Here, we present a new technique that exposes isolated mouse aortic segments to cyclic stretch at physiological frequencies and amplitudes.

Methods
The Rodent Oscillatory Tension Set-up to study Arterial Compliance (ROTSAC) is an in-house developed device that clamps small rodent aortic segments (width 2 mm, diameter 0.5 to 3 mm) to imposed preloads at physiological rates of ≤600bpm in an organ bath setting. Force and displacement are measured simultaneously and can be clamped at different amplitudes and rates. The technique enables us to perform force-displacements clamps and to assess the (visco)-elastic and active isotonic properties of periodically stretched isolated mouse aorta of mice with different expression of eNOS (eNOS+/−, WT and eNOS-Tg mice with reduced, normal and over-expression of eNOS, respectively).

Results
Using our set-up, we successfully acquired pressure diameter (PD) loops at physiological pressure (extrapolated from imposed preloads) and frequency (Fig A) with high reproducibility in WT mouse aorta (Fig B). Well-standardized wire myograph experiments showed altered biomechanics in aortic segments from mice with different eNOS expression (Fig C). These results were confirmed by the ROTSAC set-up, i.e. the slope of the PD loop is increased with lower eNOS expression (Fig D). Further, we were able to confirm the strong frequency-dependency of arterial compliance, indicating the added value of this device to study vascular biomechanics (data not shown).

Figure Calculating the slope of the linear upstroke of 5 consecutive PD loops of an isolated aorta segment of a WT mouse in (A) yields a highly reproducible measure of aortic stiffness (B). (C) Myograph experiment showing that, when gradually stretched, wall stress increases steeper in the isolated aorta of eNOS/− mice (n=4) and flatter in eNOS-tg mice (n=6) as compared with WT (n=5). The grey area depicts the physiological pressure range (80-120 mmHg). (D) ROTSAC experiment showing that the slope of the upstroke of the pressure-diameter loop (80-120 mmHg pressure range and cyclic stretch frequency of 10 Hz) is increased in eNOS−/− mice and decreased in eNOS-tg mice as compared with WT mice, confirming the results of the well standardized wire myograph experiments. **p<0.01, ***p<0.001, 1-way ANOVA with Bonferroni's post-hoc test.

Conclusions
The Rodent Oscillatory Tension Set-up to study Arterial Compliance (ROTSAC) allows to study biomechanical properties of isolated arterial segments at physiological stretch amplitude and frequency.
Comparison of the cyclic longitudinal and circumferential deformation of the ascending and abdominal aorta measured by 3D ultrasound combined with speckle tracking

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Introduction
Knowledge and understanding of the cyclic deformation of the healthy human aorta is important for the development of physiologically optimal biomaterials, grafts for the replacement of diseased aortic segments, and endografts. Measuring the three-dimensional and dynamic cyclic deformation of the aorta in vivo is still a challenge [1]. Most available imaging techniques are either too slow to capture the dynamics of aortic wall motion (CT, MRI) or they do not provide 3D geometry data. Time resolved 3D-ultrasound combined with speckle tracking algorithms is a new imaging technique that has the potential to overcome these shortcomings.

Methods
3D volume data sets of 8 ascending aortic segments close to the aortic root and of 18 abdominal aortas were acquired with a temporal resolution of 15 - 23 Hz by use of a commercial real-time 3D-echocardiography system (ArtidaTM, Toshiba Medical Systems, Japan). Only volunteers without cardiovascular risk factors (27±8.2 yrs.) were included in the study. Discrete displacement fields of about 964 nodal points on the aortic wall (Fig. 1) were obtained for 15 to 23 time steps throughout the pulse cycle for each sample by use of a customized commercial speckle tracking algorithm (Advanced Cardiac Package, Toshiba Medical Systems, Japan). 3D geometries and centerlines of the aortic lumen were constructed, average lengths and diameters were calculated and longitudinal and circumferential strains were derived.

Results
Ascending and abdominal aorta show comparable circumferential strain (A. asc.: 11.4±1.1%, A. abd. 12.3±1.2%). In contrast, the ascending aorta is subjected to significantly larger longitudinal strain (15.1±3.0%) than the abdominal aorta (2.2±0.5%, \(p = 0.00005\)). However, cyclic changes of length and diameter are not synchronous. Maximum length and longitudinal strain are reached in the ascending aorta 14.2±5.4% of the pulse cycle later [Fig. 1] and in the abdominal aorta 8.1±6.2% earlier than maximum diameter and circumferential strain.

Conclusions
Quantification of the three-dimensional cyclic deformation based on time resolved 3D-ultrasound speckle tracking is able to provide new insight into the specific loading conditions of different aortic segments. While the cyclic deformation of the abdominal aorta is characterized by diameter change and almost constant length, the ascending aorta undergoes a more complex biaxial deformation with length and diameter changes of the same order of magnitude and a phase shift between both cyclic deformations.

References
Finite element modeling of force transmission in a monolayer of endothelial cells

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Introduction

Responses of endothelial cells (ECs) to hemodynamic forces play a significant role in vascular health and disease. It is well known that ECs transduce the fluid shear stress (FSS) resulting from blood flow into intracellular signals that affect gene expression and cellular functions. Although potential mechanosensors have been identified, the precise biomechanical mechanisms by which the apical shear stress leads to localized inter-/intracellular signaling at the mechanosensors are not well understood. In the present study, a confluent vascular EC monolayer is modeled by finite element method (FEM) to investigate the redistribution and amplification of hemodynamic forces applied at the glyocalyx surface to inter-/intracellular organelles where forces are transduced to biochemical signals. Stress transmission throughout the EC monolayer is analyzed, for the first time, where all major cellular elements are incorporated in the model (the glyocalyx layer, actin cortical layer, nucleus, focal adhesions (FAs), cytoskeleton network, and adherens junctions (ADJs)). Results may help to identify the role of each individual mechanosensor in early mechanotransmission stages.

Methods

In the present study, the endothelial cell monolayer consists of seven ECs. Each EC is modeled as a hexagonal cell at its base. The surface topology of each EC is modeled as a sinusoid. The surface function is given as:

\[ y = \eta \cos(\alpha x) \cos(\beta z) \]

where \( \eta \) is the amplitude of the surface contour.

Results

Figure 1A demonstrates the schematic view of EC, its connection to neighboring cells, and subcellular structures and 1B shows the EC monolayer from the side. Figures 2A and 2B demonstrate the von Mises stress distributions over the FAs and ADJs oriented perpendicular to FSS, respectively.

Conclusions

The model predicts that the stresses are amplified 250-600 fold over apical values at ADJs and 175-200 fold at FAs for ECs exposed to a mean shear stress of 10 dyn/cm². Estimates of forces per molecule in the cell attachment points to the external cellular matrix and cell-cell adhesion points are on the order of 8 pN at FAs and as high as 3 pN at ADJs, suggesting that direct force-induced mechanotransmission by single molecules is possible in both.

References

Age-related changes of aortic hemodynamics from 4D flow MRI in 56 healthy volunteers

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Introduction: It is well known that adult cardiovascular structure and function changes with age. Given this knowledge, it is important to establish normative values over a range of ages for a number of functional parameters. We performed 4D flow MRI in a large cohort of healthy volunteers (n=56) to measure the age dependency of aortic diameter and a number of hemodynamic factors in the entire thoracic aorta. We hypothesize that age-related significant differences in aortic hemodynamics exist that should be taken into account when comparing patient cohorts to healthy controls.

Methods: ECG and navigator gated 4D flow MRI of the thoracic aorta was performed in 56 volunteers (age: 19-78 year old, 37 men) on MAGNETOM Skyra systems (Siemens Healthcare, Erlangen, Germany, spatial resolution = 2.5-3.2x1.8-2.3x2.4-2.6mm³; temporal resolution = 38-42ms, TE/TR/FA = 2.4-2.8ms/4.8-5.3ms/7-15°; Venc = 150-250cm/s). All 4D flow MRI data was corrected for Maxwell terms and eddy currents. 3D PC-MRA images were created by multiplying the phase contrast magnitude images with absolute velocity images averaged over all time frames. The 3D PC-MRA data were used to semi-automatically segment the thoracic aorta using a commercial software package (MIMICS, Materialise, Leuven, Belgium). A volume centerline was calculated based on the aortic 3D segmentation to automatically characterize the mid-ascending aortic diameter (MAA). The time frame with the maximum average absolute velocity within the segmentation was defined as peak systole. Systolic 3D WSS along the entire aorta lumen surface was calculated using the algorithm developed by Potters et al.¹ Mean velocity and mean WSS were calculated in four segments: 1) the total aorta, 2) the ascending aorta (AAo), 3) the aortic arch and 4) the descending aorta (DAo), see figure 1. The fifty-six included controls were subdivided in five age categories: 19-30 years, 31-40 years, 41-50 years, 51-60 years and 61-78 years. Age-categorized spatially-resolved average velocity and WSS maps were generated by a previously described methodology². Differences between regional and age-categorized velocity and WSS were tested with a Kruskal-Wallis test. Linear regression was performed to investigate relations between age, MAA diameter and hemodynamics in the entire aorta. P<0.05 was considered significant.

Results: In figure 1 the age-categorized cohort-averaged velocity and WSS maps are shown. There is a significant reduction in mean velocity and WSS in the aortic regions with age (table 1 and figure 1). Velocity and WSS averaged over the total aorta decreased significantly with age (R²=0.32 and R²=0.39, P<0.001, respectively). MAA diameter increased significantly with age (R²=0.35, P<0.001).

Conclusions: The decrease in mean velocity and WSS may be related to the significant increase in aortic diameter with age (diameter-velocity: R²=0.21, P<0.001 diameter-WSS: R²=0.27, P<0.001). The significant hemodynamic differences over age are important to consider when comparing velocity and WSS between patients and controls. The results presented here show the importance of matching the patient age with an appropriate control group.

Bicuspid Valve Morphology Determines the Position of Elevated Velocity and WSS

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Introduction: Bicuspid aortic valve (BAV) disease occurs in 1-2% of all newborns and is associated with two dominant patterns of aortic dilation, that is: a ‘type 1’ pattern in which the dilation involves the root and proximal portion of the tubular ascending aorta (AAo); or a ‘type 2’ in which the distal AAo and arch are dilated. There is recent evidence that aortopathy may be related to hemodynamic changes (i.e. outflow patterns and wall shear stress [WSS]) due to the orientation of the valve leaflets1. This study uses a volumetric technique to investigate the valve morphology and hemodynamic hypothesis in a cohort of 202 4D flow MRI exams.

Methods: 4D flow MRI exams were performed in 140 BAV patients and 62 healthy controls on 1.5 and 3T MAGNETOM Avanto, Espree, Aera and Skyra MRI systems (Siemens Healthcare, Erlangen, Germany). The BAV morphology was assessed on balanced steady state free precession images at the level of the valve and classified according to the Sievers scheme, with Sievers type 0 AP and LAT indicating BAV with no raphe, and opening in anterior-posterior and lateral direction, respectively. Sievers type 1 RL and RN indicate BAV with a raphe, with fusion of the right-left coronary valve (and opening in AP direction) and fusion of the right-non-coronary valve (and opening in LAT direction), respectively. All subjects had no stenosis (peak velocity <2m/s). Sinus of Valsalva (SOV) and mid-ascending aortic (MAA) diameters were measured from contrast-enhanced MRA data. Two methods were used to determine the difference in peak systolic aortic velocity and WSS between different BAV morphology configurations: 1) cohort-averaged velocity and WSS maps using a previously published methodology2, and 2) P-value maps2 delineating significantly higher or lower velocity and WSS (Wilcoxon rank sum test, P<0.05 was considered significant) for all patient cohorts, as compared to the healthy age-matched control groups.

Results: Subject demographics and aortic diameters are given in table 1. In Fig 1 it can be seen that results were very similar for valves opening in AP direction (Sievers 0 AP and Sievers 1 RL) and for valves opening in LAT direction (Sievers 0 LAT and Sievers 1 RN). Fig 1b shows that the average outflow jet for Sievers 0 AP and Sievers 1 RL subjects is directed more towards the anterior part of the proximal aorta, whereas for Sievers 0 LAT and Sievers 1 RN, the outflow jet is directed more towards the posterior part of the proximal aorta and impinges on the outer curvature of the aorta at a more distal location. Accordingly, the velocity P-value maps in Fig 1c for Sievers 0 AP and Sievers 1 RL show a larger volume of significantly higher velocity in the proximal and anterior aorta than Sievers 0 LAT and Sievers 1 RN. The highest WSS for Sievers 0 AP and Sievers 1 RL is found at the level of the right pulmonary artery, whereas for Sievers 0 LAT and Sievers 1 RN, the highest WSS is found more distally (Fig 1d). For the P-value WSS maps in Fig 1e, significantly lower WSS than controls on the aortic root can be distinguished on the Sievers 0 LAT map and the Sievers 1 RN map, whereas the proximal aorta of the Sievers 0 AP map and the Sievers 1 RL map shows significantly elevated WSS compared to controls.

Conclusions: The cohort-averaged and P-value maps revealed important differences in aortic velocity and WSS for AP- and LAT-opening BAV patients. It is intriguing that these WSS differences match with the reported differences in aortic dilation expression for the different BAV-fusion patterns. Larger studies with longer patient follow-up (for detection of aortic growth) are necessary to evaluate the influence of outflow patterns and WSS expression on the pattern of aortic dilation.

The effect of resolution on viscous dissipation measured with 4D flow MRI in patients with Fontan circulation: Evaluation using computational fluid dynamics

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Introduction
There is growing evidence that viscous dissipation inside Fontan circulation is associated with the limited exercise performance of Fontan patients, suggesting that determining viscous dissipation in vivo might contribute to evaluation of the patients with impaired outcome [1]. Viscous dissipation is generally obtained with computational fluid dynamics (CFD) but it can also be derived from 4D-flow MRI velocities. In the latter approach, the results might depend on the spatial resolution and noise of the measurements since viscous dissipation calculation involves spatial derivatives of velocities. In this study, we aim to evaluate the influence of resolution and noise on viscous dissipation calculation in Fontan patients.

Methods
6 Fontan patients underwent 4D-flow MRI scans with coverage of heart and great arteries. Using MRI measurements, Fontan geometries were reconstructed and time-resolved subject-specific CFD simulations were performed. Viscous dissipation was calculated for 1) high resolution CFD velocities, 2) down-sampled CFD velocities, 3) down-sampled CFD velocities with added noise level obtained from MR images, and 4) in-vivo 4D-flow MRI velocities. Relative viscous dissipation was calculated as viscous dissipation divided by the mean viscous dissipation for each velocity type. Mean velocity and viscous dissipation were reported.

Results
Maximum intensity projection of velocities and viscous dissipation obtained by CFD, down-sampled CFD with added noise and 4D flow MRI are shown in Figure 1 for a representative case. The velocity patterns based on CFD and MRI were generally in good agreement. 4D-flow MRI velocities (15.9±3.8 cm/s) were higher, but not significantly different than CFD velocities (14.0±4.6 cm/s, p=0.09), down-sampled CFD velocities (13.4±4.5 cm/s, p=0.06) and down-sampled CFD velocities with added noise (13.8±4.2 cm/s, p=0.06). CFD-based viscous dissipation (0.75±0.52 mW) was significantly higher than those based on down-sampled CFD (0.75±0.52 mW, p=0.03), down-sampled CFD with noise (0.25±0.15 mW, p=0.03) and 4D-flow MRI (0.30±0.16 mW, p=0.03). However, there was no significant difference between relative viscous dissipations (p=0.56).

Conclusions
Viscous dissipation was underestimated using velocities at low resolution. Nevertheless, relative viscous dissipation between different subjects was maintained irrespective of resolution and noise, which may imply that comparison of viscous dissipation between patients is still possible and cases with elevated viscous dissipation can be detected using low resolution 4D-flow MRI velocities.

Optimizing Spatio-temporal resolution for hemodynamic calculations

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Introduction

Previous studies suggest that low and oscillating wall shear stress (WSS) is associated with atherosclerotic plaque initiation and progression within the arteries in the presence of risk factors. Oscillatory Shear Index (OSI) is the suggested WSS parameter to determine oscillations by quantifying the change in the vector direction of WSS over the cardiac cycle. Although both WSS and OSI are important parameters to be determined concerning cardiovascular diseases progression, most of the flow MRI based studies focus only on the mean or maximum WSS values. This is due to the fact that it is intrinsically challenging to obtain velocities in both high spatial and temporal resolution, which is necessary in estimation of WSS and OSI, at reasonable measurement duration. In this study, our aim was to assess the influence of spatio-temporal resolution of MRI measurements on WSS and OSI estimations using an in vitro setting.

Methods

A silicon phantom of a carotid bifurcation based on the anatomical MRI images of a healthy volunteer was 3D printed and connected to a flow setup supplying a pulsating water flow within a closed system. The flow in common carotid artery (CCA) was measured with echo probe outside of the MR room before scans. Time-resolved 2D phase contrast MRI (PC-MRI) scans were performed with 36 different spatio-temporal resolutions varying between 0.2 to 1 mm and 6 to 140 ms at CCA using a 3T MR system (Ingenia R4, Philips Healthcare, Best, NL) with a solenoid rat coil. WSS was calculated by fitting a spline to each point on the vessel wall to calculate shear rate as suggested by Potters [1] and OSI was calculated with the method by He [2]. The viscosity was set as 1 mPas. We present the flow curve and the cycle averaged mean WSS and OSI at CCA only.

Results

The flow curves determined at high temporal resolutions from the PC-MRI measurements match well with echo probe measurements (figure 1). At low temporal resolution, the estimated peak is shifted and the mean velocity at peak is underestimated. Figure 2 shows the mean WSS and OSI for all measurements. The average WSS was 0.63±0.14 Pa and OSI was 0.22±0.16 (a.u.) at 0.2 mm and 22 ms spatio-temporal resolution. Both WSS and OSI were underestimated for lower spatio-temporal resolutions.

Conclusions

WSS and OSI are both influenced greatly by the spatial and temporal resolution. OSI is especially affected at low temporal resolution while WSS magnitude is underestimated specifically by spatial resolution.

References

Quantitative Coronary Angiography Based Reconstructions for Wall Shear Stress Calculations in Bifurcations

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Introduction
For this study we investigated the effect of angiography-based segmentation and reconstructions on wall shear stress (WSS) computations in coronary bifurcations. For this we computed the WSS in phantoms of which the geometry was known. These WSS results were compared with the WSS results obtained from computations in angiography-based reconstructions of those phantoms.

Methods
Four phantoms having the geometrical characteristics of diseased coronary bifurcations were previously created [1]. The images were segmented by two clinically experienced readers blind to the original geometry [2]. Based on the segmentations, 3D surface meshes were generated using a newly developed module within the CAAS software package. The computer-aided designs of the phantoms were used to generate surfaces meshes as well. In total three meshes per phantom were constructed: one gold-standard from the phantom model itself, and one mesh from each reader. Steady-state CFD computations were performed using standard numerical techniques.

Results
The confidence intervals of the WSS difference in the areas proximal to the stenosis were within 0.25 Pa, and were therefore clinically indifferent (α=0.05). Qualitatively the WSS in the stenosis areas agreed nicely. Distal of the bifurcation all maps show the same crescent-shaped low WSS area. This is the result of recirculation zones, caused by jet formation in the stenosis. Small off-sets in the reconstruction of the side branches resulted in skewed zones of low WSS. Deviations in the segmentation of the stenosis caused changes in size and location of the low WSS zone.

Conclusions
Representative WSS results were computed with angiography-based reconstructions for coronary bifurcation. Subtle deviations in the segmentation procedure near the stenosis and the side branch, influenced size and shape of the low WSS zone. Therefore intravascular imaging techniques might be required to provide the resolution needed to reconstruct the stenosis within the necessary accuracy when jets are present.

References
Grid Resolution and Shear Stress Scaling Issues from the Aortic Mouse Model to the Human Aorta

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Introduction
The mouse model is commonly used in studies of artheriosclerotic processes. This allows for long-term results being obtained in a relatively short time span compared to e.g. pig models. The underlying assumption is that the shear stress data can be scaled up to human size following the principles of allometric scaling[1], [2]. However, hydrodynamic scaling laws contradict this assumption and predict a different dynamical flow behaviour for the large scale and recent studies looking into unsteady WSS indicate that the secondary flow dynamics are distinctly different between mouse and human scale.

Methods
A numerical model that was created based on mouse data [3] is scaled to human size. Flow velocity was measured on the mouse using echocardiography and the velocity pulse was scaled from mouse heart rate \( HR_{\text{mouse}} = 419 \text{ bpm} \) to \( HR_{\text{human}} = 60 \text{ bpm} \). Blood is modelled as a Newtonian fluid. A grid sensitivity study was performed for both mouse and human scale and the time dependant spatially averaged WSS was compared for the entire aortic wall and for defined ROI on the inner and outer curvature.

Results
Dimensional analysis shows that the characteristic Reynolds, Womersley and Dean Numbers are one order of magnitude higher in the human than in the mouse, bringing the flow from purely laminar in the mouse to transitional/turbulent flow in the human. The effect of the higher dynamic energy in the flow is clearly seen in the fluctuations of WSS in the separation bubble on the inner curvature, making this the dominant WSS contribution in the deceleration phase of the flow.

Conclusions
While the mean and average WSS for the whole aortic wall ROI seem to follow allometric scaling the dynamic behaviour on the inner curvature is highly influenced by the higher energy in the flow on the larger scale. This only becomes evident in higher resolution simulations and does have an impact on time dependent WSS derived wall damage indicators like OSI and temporal and spatial WSS gradients.

References
An account of curvature in a 1D model of hemodynamics

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Introduction
Modeling of arterial wave propagation in the arterial tree is important for understanding of cardiovascular physiology. Due to progress in computational methods and resources, complex 3D fluid-structure interaction models are now available. However, the importance of simpler 1D models remains because of their power to supply realistic boundary conditions or in the study of total body hemodynamics. We propose an improvement of the now classical 1D hemodynamic model by taking into account the curvature of a vessel. An influence of curvature on hydrodynamic resistance and shear stresses are estimated.

Methods
While the classical derivation of the 1D hemodynamic equations is based on averaging in a straight cylindrical segment, we have considered a segment of a torus, which represents the vessel with some fixed curvature. During the averaging, the problem is separated into a longitudinal 1D master equation and a planar equation for the velocity profile for the curved tube. The planar part can be solved assuming a negligible convection term (linear approximation). This approach was introduced by Womersley for a velocity profile of nonstationary flow in straight tube [1].

Results
The longitudinal and planar parts of the problem of blood flow were considered separately. The first contains an explicit account of curvature to second order and reduces to the solution for the straight tube for negligible curvature. The second gives implicit influence of the curvature, but to first order and results in the estimation of the shear stress value as a function of curvature, see Figure 1. The solution of the planar problem (that is velocity profile in a section of the vessel) for ratio of the vessel radius and radius of curvature equal to 2, is illustrated in Figure 2. We also compared our 1D model with a full fluid-structure interaction 3D model of blood flow. This demonstrates that taking into account the curvature of a vessel reduces the relative error in pulse wave calculations.

Conclusions
We presented a 1D hemodynamic model including curvature of vessels. It was shown that influence of curvature is considerable for a ratio of radius of curvature and radius of vessel value < 3. A comparison with full 3D simulations demonstrated added value of our model. We will now apply this to 1D modeling of strongly curved arteries.

Acknowledgements
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References
Hemodynamic Changes in Treated Cerebral Aneurysms and Correlations with Long-Term Outcomes

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Introduction
Endovascular cerebral aneurysm treatment methods, e.g. flow-diverting stents or coil embolization, are believed to reduce flow, circulation and stresses in the aneurysm sac, promoting flow stasis and thrombosis. However, 15-30% of aneurysms treated with a flow diverting stent or coil remain patent at midterm angiographic follow-up. The hemodynamic environment in patients undergoing endovascular treatment is considered an important factor in long-term treatment outcome.

Methods
Changes in pre- and post-treatment aneurysm hemodynamics are investigated using computational flow simulations. Patient-specific 3D models of the diseased vasculature are reconstructed from rotational angiography. Treatment methods are computationally imposed via inspection of post-treatment contrast enhanced flat-pannel CT. Patient-specific flow and pressure boundary conditions are prescribed using intravascular pressure and velocity measurements. Simulations of the unsteady hemodynamics are performed for pre- and post- treatment configuration in 7 patients. Changes in hemodynamic variables are compared in patients with successful and unsuccessful treatment outcomes which are evaluated at six month angiographic follow-up.

Results
Results indicate a reduction in time-averaged and peak intra-aneurysmal flow, wall shear stress (WSS), wall shear stress gradient (WSSG), and energy dissipation for all patients following treatment. The greatest average reduction is seen in time-averaged and systolic WSS, which were reduced by 34% and 54% respectively. The median reduction in WSS is 20% greater in patients with successful treatment than in those with incomplete sac occlusion, figure 2. However, the median reduction in inta-aneurysmal flow is consistent for both treatment outcome groups.

Conclusions
The results from this study confirm that flow-diverting stents and coils do reduce intra-aneurysmal flow, as well as WSS, WSSG, and energy dissipation within the aneurysm dome. Preliminary results suggest that changes in WSS are more indicative of treatment outcome than intra-aneurysm flow rate. The results and methods of this ongoing study suggest that patient-specific CFD can be used to quantify a treatment’s probability of success by computing the change in pre- and post- treatment hemodynamics in cerebral aneurysms.

References
Velocity and WSS in the Circle of Willis in Sickle Cell Disease using 4D flow MRI

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Introduction: Increased risk for stroke and vasculopathy¹ as a result of endothelial dysfunction in sickle cell disease (SCD) could be related to abnormal WSS but this hasn’t been studied in the circle of Willis (CoW). Viscosity needed for WSS calculations is dependent on hematocrit (Hct), which is abnormally low in sickle cell disease (SCD). In this pilot study, we explore the feasibility of 4D flow MRI in the CoW and WSS estimation in the Middle Cerebral Artery (MCA) in SCD. We hypothesize that mean velocity and MCA diameter will be higher in the sickle cell patients, while we expect WSS to be lower, according to previous allusions to atherogenic mechanisms attributed to low WSS with vasodilation¹. On the other hand one might expect WSS to also increase, since, when velocity increases, so too should WSS².

Methods: 9 patients diagnosed with SCD HbSS or HbSβ⁰-thalassemia genotype (aged 13±2 years, range 8-15 years), and 5 age matched controls (aged 16±4, P=0.07, Wilcoxon rank sum test, P<0.05 was considered significant), were recruited from two Dutch centers (Emma Children’s Hospital, Amsterdam, and Sophia Children’s Hospital, Rotterdam) and were scanned with a 3.0 Tesla Intera clinical scanner (Philips Healthcare, Best, The Netherlands). The MRI protocol comprised a 4D flow MRI sequence over 2-4 heart phases, imaged at the level of the CoW. Scan parameters were: TE/TR 3.2/6.5ms, flip angle 15-20°, pixel size 0.5 x 0.5 x 0.5mm², SENSE 2-3, venc 100 cm/sec, scan time 5 min. The CoW was segmented from phase contrast magnitude images using a commercial software package (Mimics, Materialise, Leuven). The velocity fields in the Circle of Willis were filtered with a 3x3x3 voxels median filter. The MCAs were manually segmented in Matlab (Mathworks, Natick, NC). WSS was calculated in the MCA using the algorithm based on Potters et al.³ with an added module for automatic MCA diameter extraction. The mean velocity and WSS were calculated for each measured cardiac time frame and were subsequently averaged over the frames. Hct was measured from blood drawn from an antecubital vein. Viscosity was calculated from Hct using:

\[ \eta = 1.24 \times 10^{-3} \frac{1}{\text{Pa.s}} \]

A standard Hct value of 38% was assumed for controls. A Wilcoxon rank sum test was used to test for significant differences in mean velocity, mean WSS and MCA diameter between patients and controls; P<0.05 was considered significant.

Results: Hct values were 24.3±3.9% (range: 18–30%) and mean viscosity was 0.0022 Pa.s for the patients. Table 1 shows that: 1) velocity in patients showed a trend towards increased velocity compared with controls, 2) in the MCA a significantly lower WSS was found in patients than in controls and 3) the diameter of the MCA was significantly larger in patients, indicating vasodilation compared with controls. In figure 1, velocity in the entire circle of Willis and WSS in the left and right MCA are shown for a healthy volunteer (a) and a patient (b), respectively. It can be seen that the WSS is lower for the SCD patient compared to the control.

Conclusions: In a small patient sample of 9 and only 5 controls, significant differences were apparent for velocity and WSS in the Circle of Willis in SCD patients compared to age-matched controls. Despite a slightly higher velocity, lower WSS was found in the MCA for sickle cell patients, which may induce vascular inflammation. In conclusion, we show that WSS measurements are feasible in the circle of Willis and that with careful consideration of confounding variables 4D flow MRI can be used to measure velocity and WSS in SCD in a short scan.


Table 1. Mean velocity, mean WSS and diameter in the left and right middle cerebral artery for SCD patients compared to controls.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean velocity (m/s)</td>
<td>LMCA</td>
<td>0.47±0.07</td>
<td>0.53±0.08</td>
</tr>
<tr>
<td></td>
<td>RMCA</td>
<td>0.46±0.06</td>
<td>0.52±0.05</td>
</tr>
<tr>
<td>Mean WSS (Pa)</td>
<td>LMCA</td>
<td>5.0±0.59</td>
<td>3.3±0.62</td>
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<tr>
<td></td>
<td>RMCA</td>
<td>3.1±0.65</td>
<td>3.1±0.44</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>LMCA</td>
<td>2.1±0.45</td>
<td>2.8±0.29</td>
</tr>
<tr>
<td></td>
<td>RMCA</td>
<td>2.0±0.10</td>
<td>2.9±0.19</td>
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</tbody>
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The 10th International Symposium on Biomechanics in Vascular Biology and Cardiovascular Disease
How is the Fractional Flow Reserve in a Coronary Bifurcation influenced by the Distal Angle and the Side Branch Stenosis? A Computational Fluid Dynamic Study

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Introduction
In interventional cardiology, fractional flow reserve (FFR) has become a feasible invasive measurement to assess potential myocardial ischemia under high work load by measuring the pressure drop across coronary artery stenosis under hyperemic conditions [1]. Despite the widespread acceptance of FFR, a deeper comprehension of the physiological basis and diagnostic features is needed, in particular when bifurcation lesions are treated [1]. In this context, we evaluated the influence of distal angle and side branch (SB) stenosis on the FFR by performing computational fluid dynamics (CFD) analyses in literature-based coronary bifurcation models.

Methods
A parametric model of the left anterior descending coronary artery (LAD) with its first diagonal branch was created with realistic diameters, stenosis in the proximal main branch (MB), distal MB, and SB, and curvature on the heart. A hyperemic state was replicated with steady-state CFD simulations in Fluent (ANSYS Inc., USA) by imposing a flow-rate of 120 mL/min (three times the physiological value at rest; coronary flow reserve of 3) at the inlet. 27 cases were investigated by combining 3 distal angles (40°, 55°, 70°), 3 degrees of SB stenosis (40%, 60%, 80%), and 3 flow splits as outlet boundary condition (55%-45%, 65%-35%, 75%-25% for the MB and SB outlets, respectively).

Results
Differences in FFR of the MB due to distal angle (Fig. A) and degree of SB stenosis were below 2% in all cases. FFR of the SB is strictly linked to both SB and proximal MB stenosis. For lower SB stenosis, the FFR in the SB is dominated by the proximal MB stenosis, thus it is insensitive to flow through SB and distal angle (data not shown). For higher SB stenosis (80%, Figs. B and C), FFR values of the SB are significantly affected by differences in flow rate and distal angle: a flow rate of 55 mL/min in the SB gives a 0.25 (0.55—0.30) difference in FFR value between the 40° and 70° (black circles in Fig. B).

Figures – A) FFR of the MB with respect to the distal MB flow-rate for all cases with 80% SB stenosis. B) FFR of the SB with respect to the SB flow-rate for all cases with 80% SB stenosis. C) Pressure contour maps for the cases indicated by circles in (B).

Conclusions
Our findings suggest that FFR of the MB is minimally influenced by SB lesion severity, distal bifurcation angle, or differences in flow ratio through SB and MB. However, we showed that the FFR of the SB is dependent, as expected, by SB stenosis and distal angle, even when the flow is kept constant. Despite the need of further in-vivo exploration with combined pressure-flow measurements of the SB and MB, our findings suggest that the interpretation of FFR values in SB with large bifurcation angles needs caution.

References
Recent Development in Modeling and Simulation of the WSS Dependent Multi-Solutal Mass Transfer within Multi-Layered Arterial Wall

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Introduction
To be able to a priori determine regions in the vascular system where initial development of atherosclerosis could take place is a very first requirement of the mathematical modeling of the atheroma plaque formation and growth. In present study, we focus on development, validation and application of the comprehensive multi-solutal transport model in simplified multiple-layered arterial wall configurations under steady and pulsating blood flow conditions.

Methods
The present form of the mathematical model includes the mass transfer of following solutal components: low-density lipoprotein, monocyte, oxidized low-density lipoprotein, macrophages, cytokines, foam cells, contractive smooth muscle cells, synthetic smooth muscle cells and collagen (as presented in Ref.[1]). In addition, the plaque growth is modeled through additional equation for dynamical evolution of the mass balance of foam cells, smooth muscle cells and collagen (main constitutes of the atheroma plaque). The multi-layered structure of the arterial wall is modeled to include endothelium, intima, internal elastic layer (IEL), media and adventitia, as previously reported in Refs.[2] and [3]. The direct coupling of multi-solutal mass transfer with the blood flow is done through the WSS dependency of mass transfer across the lumen/endothelium interface.

Results
The analysis starts with some simplified artery geometries with pre-specified stenotic extensions of 20% (mild) 40% (intermediate) and 60% (severe) of the characteristic radius of the artery. Different values of transmural pressure are considered: 70 mmHg, 120 mmHg and 160 mmHg. The fully developed laminar velocity profiles are imposed at the inlet, which correspond to typical Reynolds numbers between 100 and 500 (to mimic blood flow in the carotid artery). The comparative assessment of LDL distributions within arterial wall with experimental measurements of Ref.[4] (for identical transmural conditions) showed good agreement. Next, in addition to LDL, the multi-solutal components are introduced and their spatial and temporal evolutions are analyzed. Finally, dynamical evolution of the volume of the atheroma plaque is solved. The special attention is devoted to an efficient time integration (a second-order fully-implicit time-integration scheme) due to necessity to simulate large time scales (up to 10 years).

Conclusions
A comprehensive mathematical model is developed that combines the blood flow in lumen with the WSS dependent multi-solutal mass transfer within the simplified multi-layered arterial wall. This, together with a separate dynamical equation for growth of the atheroma plaque, makes it possible to model the atherosclerosis progression from its initial development stage till pre-defined large time intervals (up to 10 years).

References
A Novel Method to Study Permeability of Endothelium Chronically Exposed to Different Shear Stresses in Vitro

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Introduction
Endothelial permeability to circulating macromolecules and patterns of haemodynamic wall shear stress both vary from site to site within the arterial system. We aimed to develop new methods to investigate in vitro whether there was a causal link.

Methods
We imposed chronic shear stresses on cultured porcine aortic endothelial cells (PAECs) grown in multiwell culture plates by swirling the plates on an orbital shaker: the orbital motion produces a wave of culture medium that rotates around the well [1]. The resulting shear stresses experienced by the endothelial cells at each location on the bottom of the well and at each time during the orbit were obtained using numerical methods. Spatially-resolved measurements of permeability were made by using molecular recognition between the substrate underneath the cells (biotinylated gelatin) and the fluorescent tracer initially placed above them (FITC-neutravidin or phycoerythrin-neutravidin; relative molecular masses of 66 and >300 kDa, respectively); the tracer binds to the substrate once it is transported through the monolayer, and is detected by confocal microscopy [2].

Results
Permeability maps of static cultures exhibited a uniform permeability across the well. Chronically sheared monolayers showed an increase in permeability at the center compared to the edge of the well; there was a decrease in overall permeability compared to the static cultures. At the cellular scale, binding patterns of the tracers differed. FITC-neutravidin accumulated underneath bicellular and tricellular borders whereas phycoerythrin-neutravidin was mostly found underneath tricellular borders. Shear stress metrics varied radially in the well but there were no clear correlations between any of them and the pattern of permeability.

Conclusion
The variation in binding locations of the two different size tracers suggests two pathways exist for macromolecule transport. The shear stress studies suggest that more than one feature of shear stress may affect endothelial permeability.

References

Figure Left: Mean permeability of FITC-neutravidin for endothelial monolayers sheared and cultured under static conditions at different radii in the well (n=6 cultures). Right: Equivalent values for phycoerythrin-neutravidin (n=6 cultures).
Recreating the Physical Geometry of Stented Arteries In Silico and In Vitro

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Introduction
Angioplasty and stent placement modify arterial physiology, in part by altering local mechanical conditions. Stents induce complex haemodynamics that vary in magnitude and kinetics according to vascular geometry and pulsatility. To study these processes, we have created a device to observe flow patterns and cell behaviour within stented vessels in vitro, and have complemented this with in silico computational fluid dynamics modelling. While previous studies used simple or idealised vascular geometries, we aimed to improve the accuracy and translational potential of our observations by recreating the exact geometry of stented arteries in vitro and in silico.

Methods
Stents were placed in porcine coronary arteries or in polydimethylsiloxane (PDMS) tubes. Resulting geometries were determined by reconstructing high-resolution micro computed tomography (µCT) scan images using a SkyScan 1172 scanner and CTVol software. While stent structures were easily extracted, relatively radiotransparent vessel walls were more challenging. Further scans were performed to evaluate potential solutions. First, vessels were imaged following the injection of contrast agents to better define the wall/lumen interface. Secondly, vessels were filled with PDMS to create casts of the entire interior geometry, which were then scanned following the removal of the stent and surrounding material.

Results
In applying intensity thresholds to reduce image artefacts around metallic stent struts, both PDMS and porcine vessel walls were obscured. In addition, the apparent dimensions of struts varied as intensity thresholds were altered. Therefore, some effort was required to attain the true stent geometry.

Imaging with contrast agents did not define wall/lumen boundaries in PDMS phantoms, as the attenuation of the two materials was too similar, but did allow the boundary to be visualised in stented coronary arteries. However, strut dimensions remained difficult to define and, additionally, the lower intensity thresholds required by the agent resulted in interference local to the stent.

Casts of both stented coronary arteries and in vitro PDMS vessels were successfully created. Scan reconstruction was found to be less complex than the contrast agent approach, as intensity thresholds were optimised for the PDMS alone in the homogeneous casts. The material was also less susceptible to the effect of thresholding on apparent dimensions.

Conclusions
Through micro computed tomography scanning we created in silico representations of physical stented vessels. Correcting the issues inherent in the use of contrast agents was time intensive and greatly reduced accuracy. Casting proved to be the more successful method, with PDMS capable of capturing micron-scale features; though this detail can easily be lost should bubbles be present at the outer surface.

Analysing stents under various flow conditions could allow their efficacy to be assessed, compared and potentially predicted. A µCT protocol which is both accurate and time-efficient will enable high-throughput scanning and modelling of a wide range of commercial stent designs.
Understanding the Differential Performance of Autologous Coronary Artery Grafts

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Introduction
The mechanical response of an autologous vascular graft plays a critical role in determining patient outcomes following coronary artery bypass graft (CABG) surgery. Maladaptive vascular remodeling, particularly at the anastomotic junction, can compromise graft patency and is most likely elicited by mechanical, compositional and geometrical incongruities between the host and graft vessels. In this study, the passive mechanical properties of the porcine left descending coronary artery (LAD), internal thoracic artery (ITA), radial artery (RA), great saphenous vein (GSV) and lateral saphenous vein (LSV) were assessed via an integrated theoretical-experimental approach. Generated data and constitutive modeling of the passive vessel properties were used to develop a novel metric for understanding tissue source-dependent outcomes following autologous grafting in the coronary circulation.

Methods
All porcine tissues were harvested immediately following animal sacrifice, rinsed with phosphate buffered saline, and mounted in a chambered mechanical testing system configured for inflation-extension experiments. The in-vivo axial stretch ratio ($\lambda_{in-vivo}$) of each vessel type was approximated via a series of force-displacement tests [1]. Samples were mechanically preconditioned at the in-vivo axial stretch ratio with 5 inflation-deflation cycles, and then subjected to a quasi-static inflation (0 – 200 mmHg, 20 mmHg steps) at three levels of axial stretch that span $\lambda_{in-vivo}$. The vessel outer diameter and axial force were continuously recorded throughout mechanical testing using integrated software and an externally mounted camera. Following mechanical testing, ring samples (1 mm width) were obtained from the central region of each vessel and used to quantify the stress-free configuration of the vessel following established protocols [2]. Additional ring samples were used for histological studies, in which the mass fractions of structural constituents within the vessel wall were quantified via analytical microscopy.

Results
Mechanical and histological data were processed to parameterize a four-fiber structure-based constitutive model of each vascular tissue type [3]. Models were then used to predict the vessel mechanical response under conditions that simulate pre- and post-transplantation, i.e. with account of the local loading conditions. The differences between each vessel’s native homeostatic and initially grafted states in terms of circumferential stress, axial stress, compliance, and inner radius were used to define an “overall mechanical difference metric”, $\Omega$. Our findings show that the ITA has the lowest $\Omega$, indicating the least mechanical mismatch when transplanted into the coronary circulation. Overall, $\Omega$ values among examined vessel types correlate well with CABG clinical outcomes [4]. Moreover, by examining $\Omega$ as a function of axial stretch, we predict a vessel-specific optimal axial stretch ratio for implantation that will minimize the mechanical discrepancy between host and graft vessels.

Conclusions
Our results indicate that among the vessels studied, the ITA is the most mechanically compatible for grafting in the coronary circulation. Further analysis suggests that manipulating the degree of axial stretch during graft implantation in a source-specific manner may improve patency and outcomes. Our findings provide explanation for the differential performance of autologous vascular grafts and have implications for the design of engineered vascular tissue.

References
Functional and anatomical measures for outflow boundary conditions in atherosclerotic coronary bifurcations

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Introduction
When studying atherosclerosis in coronary arteries two hemodynamic parameters of interest can directly be obtained with computational fluid dynamics (CFD). The first is fractional flow reserve (FFR), which is defined as the pressure ratio over a coronary stenosis during hyperemia. The second parameter is wall shear stress (WSS). Sites with low or oscillatory WSS patterns have a higher propensity to develop atherosclerosis, yielding coronary bifurcations to be at high risk of developing lesions. The aim of this research was to study the influence of outflow boundary conditions for computational fluid dynamics on FFR and WSS in diseased human coronary bifurcations.

Methods
Ten patient-specific coronary bifurcations were reconstructed from computed tomography images. Three methodologies were followed to set the outflow ratio over the outlets: First, they were based on the diameter of the outlets. This followed from the hypothesis that the diameter size adapts to the flow demand [1]. Second, they were based on the volume of the myocardial masses corresponding to the outlets. The size of the myocardial volume can be regarded as representative for the flow demand. Third, they were based on flow measurements derived from computed tomography perfusion (CTP). CTP measures the myocardial blood flow based on the distribution of contrast medium, providing a functional measure of the myocardium. [2]

Results
The differences in the outflow ratios from the perfusion-based approach and the volume-based approach were small: -2% [-2%:1%]. The differences between the outflow ratio from the perfusion-based and diameter-based results were larger and showed more spread: -6% [-15%:7%]. A quantitative analysis of the WSS results showed very high correlations between the methods with $r^2$ ranging from 0.90 to 1.00. But despite the high correlations the diameter-based and volume-based approach generally underestimated the WSS compared to the perfusion-based approach. The computed FFRs of all three methodologies matched very well.

Conclusions
We demonstrated the potential of CTP for setting patient-specific boundary conditions for atherosclerotic coronary bifurcations. The FFR - a global parameter - was unaffected by the variations in outflow ratios. But for WSS, which is a local parameter, considerable differences were observed between outflow conditions based on either anatomical or functional measures. A functional measure of the outflow ratio might be a necessity to accurately determine the WSS.

Fig: Three measures to set the outflow ratio for CFD in coronary bifurcations. Left: The diameters of the outlet. Middle: Volume of the myocardium corresponding to one of the outlets. Right: CTP measurements. The mean flow in a volume was obtained from multiple regions of interest (ROI) within the CTP data.

A Fluid-Structure Interaction (FSI) Model of the Coupling Blood-Artery

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Introduction
Numerous studies indicate that cardiovascular diseases are the cause of a significant increase in annual deaths in the modern world [1,2]. According to the BHF annual statistics (2014), only in the UK, the annual cost related to the treatment of cardiovascular diseases as atherosclerosis peaked at £6.8bn in 2013 - an increase of about 30% in 10 years [2]. Plaque morphology driven by mechanical forces is an important factor [3]. Moreover, studies related to the enhancement of the transport of species in areas of low shear stress [4] and its relation with the abnormal renewal of endothelial cells contributed to the understanding of how plaque progresses. We have previously suggested that under disease condition blood particles flowing in plasma might affect the mechanical forces that modulate cellular activities in areas of vulnerable plaques [5]. Modelling the vessel wall as a rigid surface, we found that the velocity distribution changes with particles at predilection sites. This work presents a more complete study that accounts for the effects of wall movement in plaque build-up.

Methods
To address the problem, we are simulating blood-artery interactions using a three steps coupling (co-simulation) strategy [6]. The equations of motion of the solid (arterial wall) and the fluid (blood) domain are resolved separately. Forces resulting from fluid-wall interactions are considered to be elastic and based on Hooke's law. These forces are exchanged between the solid and the fluid phases through a surface of interaction, which is a computational entity used to map the fluid-wall interface. The fluid phase is modelled as a Newtonian incompressible fluid using the Navier-Stokes equations. At each time step, the solid phase is resolved and the forces resulting from the application of the non-slip condition at the surface of interaction are distributed over the fluid domain. The equations of motion of the fluid phase are resolved and the interface forces used to feedback the next solid integration time step.

Results
We found that the results for a control geometry are in agreement with the analytical solution. This test was carried out to assess the correctness of the FSI strategy and the appropriateness of the boundary conditions. There is no spurious reflections at the outlet in unsteady flow simulations. Our results are quantitatively comparable with the literature [7]. The velocity magnitude slightly differs due to differences in the numerical approach. The assessment of flow characteristics at arterial curvatures indicates that the wall movement enhance recirculation at predilection sites for plaque deposition.

Conclusions
Simulations of a fluid-structure interaction (FSI) model that mimic the coupling blood-artery have yielded results comparable with the literature. The effects of the arterial morphology in flow was assessed using an idealized model of mouse aorta. Currently, we are assessing differences in flow due to both geometrical and physiological variations in different species: mouse and pig.

References
Efficient Sensitivity Analysis of Models with Many Model Parameters to Guide Model Personalization

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Introduction
Advances in cardiovascular modeling have led to initiatives that aim for predicting the outcome of an intervention for individual patients using these models. This patient-specific approach requires that the parameters and boundary conditions are adjusted to the individual patient and that the uncertainty in the prediction is properly quantified (uncertainty analysis). Since it is impossible in practice to measure all model parameters, it is required to identify the parameters that have the largest influence on the model output and are therefore most rewarding to measure. A variance-based sensitivity analysis can be used to apportion the outcome variance (uncertainty) to the direct effects and the interaction effects of the model parameters. This information can guide the formulation of the measurement protocol so that the model outcome uncertainty will be minimized. Variance-based sensitivity indices that express the direct and interaction effects of the model parameters are defined by multi-dimensional integrals. Monte Carlo estimates of these integrals can be calculated efficiently using Saltelli’s method. However, for models with many model parameters, Saltelli’s method becomes impractical, as the number of required model runs is in the order of $O(k \cdot 10^3)$, with $k$ the number of parameters, which can be prohibitively large. Other methods for deriving the sensitivity indices have also been reported in literature. In these approaches, a metamodel of the output space is constructed using multivariate polynomial basis functions. The sensitivity indices can be derived analytically from the metamodel. Compared to Saltelli’s method, the metamodeling approach requires much fewer model runs if the number of model parameters $k$ is not too large (i.e. $k < 25$). For the sensitivity analysis of models with many parameters, it is therefore advised to reduce the number of parameters before the metamodeling approach is used. Therefore, the aim of this study is to introduce such a two-step approach.

Methods
The effective number of parameters is reduced by first applying a screening method. After fixing parameters that were identified as non-important within their uncertainty domain, a metamodel is constructed with a basis in the remaining parameters. This metamodel is used to derive the sensitivity indices. We demonstrate the efficiency of this approach by applying it to a pulse wave propagation model with 73 parameters.

Results
The relative $L_2$-error in the obtained sensitivity indices of both methods is shown as a function of the (total) number of model runs in Figure 1. The reference analysis, performed using the Saltelli’s method, requires more than $10^5$ runs to obtain $\varepsilon(L_2) < 10^{-2}$ (i.e. slow convergence). In contrast, the proposed two-step approach requires fewer than $10^4$ runs to obtain $\varepsilon(L_2) < 10^{-2}$ (i.e. very fast convergence), especially if a sufficient polynomial order $z$ (second- or third order) is selected.

Conclusions
We conclude that the proposed two-step approach can be used to obtain accurate estimates of the sensitivity indices at a relatively low computational cost. This means that at a fraction of the traditional computational cost, the same conclusions can be drawn with regards to which parameters should be measured with high accuracy and which parameters can be fixed within their uncertainty interval.
Optical Clearing of Arteries as a Tool For Mapping Macromolecular Transport From Lumen to Adventitia

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Introduction
The entry of macromolecules (e.g. LDL) into the arterial wall is implicated in the pathogenesis of atherosclerotic lesions. Regions of high macromolecule permeability occur at branch mouths and bifurcations where shear stress and the structural composition of vessels are highly variable. Uptake and distribution of macromolecules may be related to the shape of the intimal cushion, the composition of the basement membrane or differences in vessel structure between dorsal and ventral surfaces as well as to fluid mechanical factors. We aimed to answer these questions by optically clearing tissue exposed to a fluorescent macromolecule tracer and imaged using confocal microscopy.

Methods
Samples of rat and rabbit aorta exposed to rhodamine labelled albumin for either 2hrs ex vivo or 10mins in vivo were fixed in 15% formaldehyde, mounted and imaged in 3D using confocal laser scanning microscopy (using excitation 476nm, emission 500-530nm for tissue autofluorescence and excitation 561nm, emission 580-640nm for tracer fluorescence). Samples were subsequently unmounted, dehydrated in alcohol, optically cleared in a 2:1 benzyl alcohol: benzyl benzoate solution (BABB) and re-imaged. A subset of samples was stained with Draq5 before clearing to highlight cell nuclei (excitation 633nm, emission 640-730nm).

Results
Vessels immersed in BABB rapidly became transparent. Before clearing, tissue autofluorescence could be imaged with a 20x immersion objective to a depth of ~15-20µm before signal intensity rapidly diminished; by 20µm, intensity dropped >50%. After optical clearing, >100µm was achieved (Figures A&B), the main limitation being the working distance of the objective lens; with a 10x objective, intact vessels could be imaged from one wall to the other, albeit with reduced depthwise resolution. Autofluorescence and albumin uptake throughout the entire vessel thickness could be routinely imaged in cleared rat tissue (20x imm.objective). Albumin uptake was visible between elastic lamellae and in the adventitia. Optical clearing did not adversely affect mean albumin fluorescence intensity; in fact it increased signal by as much as 200% for a given PMT gain. Draq5 staining highlighted the 3D cellular arrangement and alignment throughout the wall and was unaffected by the use of 15% formaldehyde or BABB (Figure C: rabbit aorta). In rabbit tissue, the enhanced rhodamine fluorescence enabled visualisation of finer details of albumin transport around cell borders, especially in combination with Draq5.

Conclusions
Macromolecule uptake through the entire vessel wall can be imaged en face by optically clearing tissue. This enables improved assessment of the influence on transport of wall structure, mechanical stresses and their interaction. (This study was funded by the BHF).