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A Collagen-Glycosaminoglycan-Fibrin Scaffold For Heart Valve Tissue Engineering Applications

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6th Biennial Meeting on Heart Valve Biology & Tissue Engineering

10th -12th of September 2014

Royal Society, London

Imperial College
London



Georgia Institute
of Technology

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Welcome

Welcome to the 6th Biennial Heart Valve Biology and Tissue Engineering Meeting, which promises to be a stimulating and informative meeting with a truly international faculty and audience.

The field of heart valve biology and tissue engineering a heart valve continue to expand. The presentations at this meeting reflect the advances made in both areas due to the multi-disciplinary approach taken by many laboratories.

This meeting provides a forum that fosters an environment in which these advances can be reported and discussed. There are also a number of keynote lectures at the start of each session, providing a state-of-the-art perspective for each session. Lastly, Wednesday evening entails a fully moderated poster session and a prize for the best poster.

On Thursday evening you are invited to join us on the boat cruise along the river Thames, through the heart of London, where you will be able to enjoy excellent views of many of the London landmarks as well as some good food and excellent company.

We hope you enjoy the meeting and the wonderful facilities offered by the Royal Society in this historic and inspiring environment!

Magdi Yacoub

Ajit Yoganathan

Adrian Chester

Programme at a Glance

Wednesday 10th September

- 8.15-8.30 **Welcome Address – Magdi Yacoub**
- 8.30-10.30 **Session 1 – Heart Valve Development and Function**
Chairs: Giovanni Ferrari & Jonathan Butcher
Keynote Lecture: Katherine Yutzy (Cincinnati Children’s Hospital)
- 11.00-13.00 **Session 2 – Major players in valve function in health and disease I**
Chairs: Adrian Chester & Murali Padala
Keynote Lecture: Jane Grande-Allen (Rice University)
- 14.00-16.00 **Session 3 – Major players in valve function in health and disease II**
Chairs: Magdi Yacoub & Colin Caro
Keynote Lecture: Peter Davies (University of Pennsylvania)
- 16.30-18.00 **Session 4 - Mechanobiology**
Chairs: Ajit Yoganathan & Craig Simmons
Keynote Lecture: Remi Peyronnet (Imperial College London)
- 18.00-20.00 **Session 5 – Posters and Welcome Reception**
Assessors: Michael Sacks, Frank Baaijens, Ulrich Stock & Dan Simionescu

Thursday 11th September

- 08.30-10.30 **Session 6 – Intelligent Scaffolds**
Chairs: Jerome Sohler & Ibrahim
Keynote Lecture: Karen Wooley (Texas AM University)
- 14.00-16.00 **Session 8 – Immuntolerance for tissue engineering applications**
Chairs: Jane Grande-Allen & Herman Waldman
Keynote Lecture: Kathy Liu (Harvard University)
- 16.30-18.00 **Session 9 – Gene Expression & Biomarkers in Valve Disease**
Chairs: Elena Aikawa & Najma Latif
Keynote Lecture: Dennis Cokkinos (Academy of Athens)

Friday 12th September

- 8.30-10.30 **Session 10 – The Ross Procedure**
Chairs: Jane Sommerville & Giovanni Battista Luciani
Keynote Lecture: Ismail El-Hamamsy (Montreal Heart Institute)
- 11.00-13.00 **Session 11 – Valve Conserving Operations - From Structure to Function**
11.00-11.30 **Chairs: Axel Haverich & Magdi Yacoub**
Keynote Lecture: Martin Misfeld (Liepzig)
- 13.30-13.30 **Session 12 – Taking Cardiac Surgery to the People**
Chair Jane Sommerville
Keynote Speaker: Magdi Yacoub (Imperial College London)

Acknowledgments

The Course Directors would like to thank the following companies and individuals:

Cryolife Inc. for their continued support, without which we would not be able to hold this meeting. We have been able to enjoy their generous support for this and the previous 5 meetings in this series.

Medtronic Inc. for their kind donation of the Educational Grant.

The staff of the Royal Society for the excellent facilities made available to us and the high quality service that they have provided.

The staff of the SHVD management company for handling the registration system as well as endless support and advice.

Dr. John Smith of the Harefield Heart Science Centre for building and managing the Website.

Dr. Najma Latif for her invaluable help and support with the final arrangements.

The Royal Society

The Royal Society, is a learned society for science, and is possibly the oldest such society still in existence. Founded in November 1660, it was granted a Royal Charter by King Charles II as the "Royal Society of London". The Society today acts as a scientific advisor to the British government, receiving a parliamentary grant-in-aid. The Society acts as the UK's Academy of Sciences, and funds research fellowships and scientific start-up companies.

The Royal Society started from groups of physicians and natural philosophers, meeting at variety of locations, including Gresham College in London. They were influenced by the "new science", as promoted by Francis Bacon in his *New Atlantis*, from approximately 1645 onwards. A group known as *The Philosophical Society of Oxford* was run under a set of rules still retained by the Bodleian Library.

After the English Restoration, there were regular meetings at Gresham College. It is widely held that these groups were the inspiration for the foundation of the Royal Society.

The Society has a variety of functions and activities. It supports modern science; it finances approximately 700 research fellowships for both early and late career scientists, along with innovation, mobility and research capacity grants. Its Awards, prize lectures, and medals all come with prize money intended to finance research, and it provides subsidised communications and media skills courses for research scientists. Through its Science Policy Centre, the Society acts as an advisor to the European Commission and the United Nations on matters of science. It publishes several reports a year, and serves as the Academy of Sciences of the United Kingdom. Since the middle of the 18th century, government problems involving science were irregularly referred to the Society, by 1800 it was performed accordingly.

The premises in which we are now in are a Grade I listed building and the current headquarters of the Royal Society. The first Carlton House was named after Baron Carleton, and was sold to Lord Chesterfield in 1732, who held it on trust for Frederick, Prince of Wales. Frederick held his court there until his death in 1751, after which it was occupied by his widow until her death in 1772. In 1783, the then-Prince of Wales, George, bought the house, instructing his architect Henry Holland to completely remodel it. When George became King, he authorised the demolition of Carlton House, with the request that the replacement be a residential area. John Nash eventually completed a design that saw Carlton House turned into two blocks of houses, with a space in between them. The building is still owned by the Crown Estates and leased by the Society; it underwent a major renovation from 2001 to 2004 at the cost of £9.8 million, and was re-opened by the Prince of Wales on 7th July 2004.

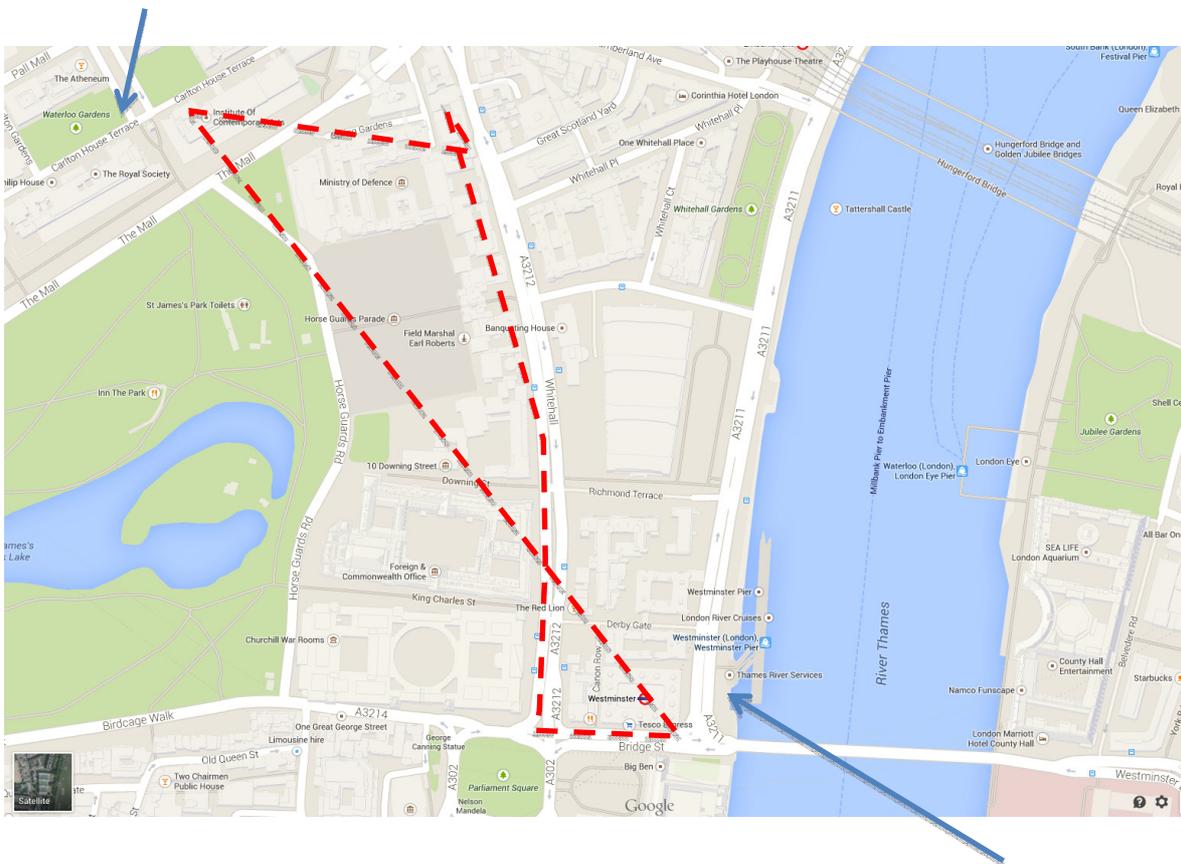
The Society is governed by its Council, which is chaired by the Society's President, according to a set of Statutes and Standing Orders. The members of Council and the President are elected from, and by, its Fellows, the basic members of the Society, who are themselves elected by existing Fellows. There are currently about 1,450 Fellows, allowed to use the post nominal title FRS (Fellow of the Royal Society), with up to 52 new Fellows appointed each year. There are also Royal Fellows, Honorary Fellows, and Foreign Members.

Conference Dinner

The conference dinner will be aboard the Pride on London riverboat. The boat will depart from Westminster Pier (see map) within a 10 minute walk from the Royal Society. The boat will depart at 19.45 and will return to Westminster at approximately 23.45. There will be a buffet style BBQ on the boat along with wine, beer and soft drinks. The boat is covered, with an upper viewing deck.

Please dress appropriately for the weather conditions on the day of the cruise.

Royal Society



Westminster Pier

Programme in Full

Wednesday 10th September

- 8.15-8.30 **Welcome Address – Magdi Yacoub**
- 8.30-10.30 **Session 1 – Heart Valve Development and Function**
Chairs: Giovanni Ferrari & Jonathan Butcher
- 8.30-9.00 **Keynote Lecture: Katherine Yutzy (Cincinnati Children’s Hospital)**
- 9.00-9.15 C1 **Extracellular Matrix Composition Regulates Endothelial to Mesenchymal Transformation**
Sudip Dahal¹, Jonathan Bramsen¹, Peter Huang², Gretchen Mahler¹
¹*Binghamton University, Department of Bioengineering, Binghamton, NY, USA,*
²*Binghamton University, Department of Mechanical Engineering, Binghamton, NY, USA*
- 9.15-9.30 C2 **Cyclic stretch orchestration of RhoA-Rac1 signaling axis is essential for embryonic valve remodeling**
Russell Gould¹, Huseyin Cagatay², Joanna MacKay³, Sanjay Kumar³, Jonathan Butcher¹
¹*Cornell University, Ithaca, NY, USA,* ²*Dogus University, Istanbul, Turkey,* ³*University of California, Berkeley, Berkeley, CA, USA*
- 9.30-9.45 C3 **Insights Into Regional Adaptations In The Growing Pulmonary Artery Using A Meso-scale Structural Model: Effects Of Ascending Aorta Impingement**
Michael Sacks¹, ¹*Institute of Computational Engineering and Sciences Department of Biomedical Engineering University of Texas at Austin, Austin, USA*
- 9.45-10.00 C4 **Modulation Of Human Valve Interstitial Cell Phenotype and Function Using A FGF2 Formulation**
Najma Latif^{1,2}, Alfred Quillon¹, Ann McCormack¹, Alec Lozanoski¹, Magdi Yacoub^{1,2}, Adrian Chester^{1,2}
¹*Imperial College, London, UK,* ²*Qatar Cardiovascular Research Centre, Doha, Qatar*
- 10.00-10.15 C5 **Deletion of Krox20 results to bicuspid aortic valve**
Gaelle Odelin^{1,2}, Emilie Faure^{1,2}, Corinne Maurel-Zaffran^{1,3}, Fanny Couplier^{4,5}, Piotr Topilko^{4,5}, Patrick Charnay^{4,5}, Stéphane Zaffran^{1,2}
¹*Aix Marseille Universite, Marseille, France,* ²*INSERM GMGF U910, Marseille, France,* ³*CNRS, IBDMML UMR7288, Marseille, France,* ⁴*INSERM U1024, IBENS, ENS, Paris, France,* ⁵*CNRS, UMR8197, IBENS, ENS, Paris, France*
- 10.15-10.30 C6 **Pharmacological Treatment in Marfan Mitral Valve: A Pilot Study**
Alexander Black¹, Vincent Griffiths¹, Dalibor Soukup², Jonathan McGuinness³, Darren McLoughlin³, John Byrne³, Mark Redmond³, Harry C. Dietz⁴, Peter Dockery¹
¹*Anatomy, School of Medicine, National University of Ireland, Galway, Galway, Ireland,* ²*Institute for Science and Technology in Medicine, Keele University, Stoke-on-Trent, UK,* ³*Department of Surgical Research, The Royal College of Surgeons in Ireland, Dublin, Ireland,* ⁴*Department of Medical Genetics, Johns Hopkins University Hospital, Baltimore, USA*
- 10.30-11.00 Coffee break

- 11.00-13.00 **Session 2 – Major players in valve function in health and disease I**
Chairs: Adrian Chester & Murali Padala
- 11.00-11.30 **Keynote Lecture: Jane Grande-Allen (Rice University)**
- 11.30-11.45 C7 **COX2 Inhibition Reduces Aortic Valve Calcification In Vivo**
Elaine Wirrig, Robert Hinton, Katherine Yutzey
The Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA
- 11.45-12.00 C8 **Extracellular Adenine Nucleotide Catabolism on the Calcified Aortic Valve Surface and its Clinical Correlates**
Barbara Kutryb-Zajac¹, Patrycja Sommerfeld¹, Marta Toczek¹, Paulina Zukowska¹, Romuald Lango², Jan Rogowski³, Ewa M. Slominska¹, Ryszard T. Smolenski¹
¹*Department of Biochemistry, Medical University of Gdansk, Gdansk, Poland,*
²*Department of Cardiac Anesthesiology, Medical University of Gdansk, Gdansk, Poland,*
³*Chair and Clinic of Cardiac and Vascular Surgery, Medical University of Gdansk, Gdansk, Poland*
- 12.00-12.15 C9 **Characterisation of human cardiovascular tissue affected by calcific disease using Raman and scanning electron microscopy**
Amanda You^{1,2}, Sergio Bertazzo¹, Jean-Philippe St-Pierre^{1,2}, Joseph Steele^{1,2}, Adrian Chester⁴, Magdi Yacoub⁴, Molly Stevens^{1,2}
¹*Department of Materials, Imperial College London, London, UK,*
²*Department of Bioengineering, Imperial College London, London, UK,*
³*Institute of Biomedical Engineering, Imperial College London, London, UK,*
⁴*National Heart and Lung Institute, Imperial College London, London, UK*
- 12.15-12.30 C10 **Analysis of The Retinoblastoma Protein In pAVIC Calcification**
Marina Freytsis¹, Jenna Calvino³, Lauren Baugh⁵, Lauren Black^{5,1}, Philip Hinds^{1,4}, Gordon Huggins^{2,3}
¹*Tufts University, Sackler School of Graduate Biomedical Research, Boston, MA, USA,*
²*Tufts Medical Center, Boston, MA, USA,*
³*Molecular Cardiology Research Institute, Boston, MA, USA,*
⁴*Molecular Oncology Research Institute, Boston, MA, USA,*
⁵*Tufts University, Department of Biomedical Engineering, Medford, MA, USA*
- 12.30-12.45 C11 **Investigating The Role Of Matrix Vesicles In Valve Interstitial Cell Calcification**
Lin Cui¹, Dongxing Zhu¹, Francesco Rao², Colin Farquharson¹, Vicky MacRae¹
¹*The Roslin Institute, Edinburgh, Midlothian, UK,*
²*Dundee Cell Products Ltd, Dundee, UK*
- 12.45-13.00 C12 **eNOS Uncoupling Drives Calcification Via Disruption of Endothelial Protection In Early And Late Stages Of Aortic Valve Disease**
Emily Farrar¹, Geoffrey Huntley², Jonathan Butcher¹
¹*Cornell University, Ithaca, NY, USA,*
²*Brown University, Providence, RI, USA*
- 13.00-14.00 Lunch
- 14.00-16.00 **Session 3 – Major players in valve function in health and disease II**
Chairs: Magdi Yacoub & Colin Caro
- 14.00-14.30 **Keynote Lecture: Peter Davies (University of Pennsylvania)**
- 14.30-14.45 C13 **Characterization of Sex-Related Differences in Valvular Interstitial Cell Response to Transforming Growth Factor-beta1**
Chloe McCoy, Ashley Quinn, Kelsey Duxstad, Kristyn Masters
University of Wisconsin, Madison, WI, USA

- 14.45-15.00 C14 **Insights into CAVD Pathobiology and Relationship to Atherosclerosis Provided by Familial Hypercholesterolemic Swine**
Ana Porras, Dhanansayan Shanmuganayagam, Jennifer Meudt, Christian Kreuger, Jess Reed, Kristyn Masters
University of Wisconsin-Madison, Madison, WI, USA
- 15.00-15.15 C15 **Deficiency Of C-Type Natriuretic Peptide Signaling Promotes Aortic Stenosis, Left Ventricular Dysfunction, And Bicuspid Aortic Valves In Mice**
Mark Blaser¹, Yu-Qing Zhou², Hangjun Zhang³, Scott Heximer³, Mark Henkelman², Craig Simmons^{1,4}
¹*Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada*, ²*Mouse Imaging Centre, Toronto Centre for Phenogenomics, Toronto, Ontario, Canada*, ³*Department of Physiology, University of Toronto, Toronto, Ontario, Canada*, ⁴*Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, Ontario, Canada*
- 15.15-15.30 C16 **Pharmacological Antagonist Of Serotonin Receptor 2A Reduces The Pathological Activation of Human Mitral Valve Interstitial Cells: Implication For Myxomatous Mitral Valve Disease**
Kathryn Driesbaugh¹, Erik Lai¹, Emanuela Branchetti¹, Juan B Grau^{1,2}, Robert C Gorman¹, Joseph H Gorman¹, Michael S Sacks⁴, Joseph E Bavaria¹, Robert J Levy^{1,3}, Giovanni Ferrari¹
¹*Perelman School of Medicine at the University of Pennsylvania, Philadelphia, USA*, ²*The Valley Hospital, Ridgewood, NJ, USA*, ³*The Children's Hospital of Philadelphia, Philadelphia, PA, USA*, ⁴*The University of Texas at Austin, Austin, TX, USA*
- 15.30-15.45 C17 **Effect Of Mitral Regurgitation Severity And Extent Of Leaflet Tethering On Leaflet Remodeling In Ischemic Mitral Regurgitation**
Kanika Kalra, Robert Guyton, Vinod Thourani, Muralidhar Padala
Emory University, Atlanta, USA
- 15.45-16.00 C18 **Filmain-A Mutations Causing Mitral Prolapse Alter Integrin Expression and Cellular Mechanotransduction**
Muralidhar Padala^{1,2}, Marzieh Zamani^{2,4}, Padmini Sarathchandra², Jean Merot³, Julia Gorelik⁴, Magdi Yacoub^{2,4}, Adrian Chester^{2,4}
¹*Emory University, Atlanta, GA, USA*, ²*Harefield Heart Science Centre, Harefield, Middlesex, UK*, ³*University of Nantes, Nantes, France*, ⁴*Imperial College London, London, UK*
- 16.00-16.30 Coffee Break
- 16.30-18.00 **Session 4 - Mechanobiology**
Chairs: Ajit Yoganathan & Craig Simmons
- 16.30-17.00 **Keynote Lecture: Remi Peyronnet (Imperial College London)**
- 17.00-17.15 C19 **Paracrine TGF- β Signaling Amplifies Mechanical Force-Regulated Pulmonary Valve Leaflet Gene Expression**
Xiaoyuan Ma, Katsuhide Maeda, Seta Stanbouly, Yasuhiro Fujii, Frank Hanley, R. Kirk Riemer
Stanford University, Stanford, CA, USA
- 17.15-17.30 C20 **Role of Shear- and Side-dependent MicroRNAs in Aortic Valve Biology**
Swetha Rathan¹, Anita Rajamani¹, Shan Lee¹, Sandeep Kumar², Ann Aphivantrakul¹, Hanjoong Jo^{1,2}, Ajit Yoganathan^{1,2}
¹*Georgia Institute of Technology, Atlanta, GA, USA*, ²*Emory University, Atlanta, GA, USA*

- 17.30-17.45 C21 **Influence of Aortic and Ventricular Shear Stresses on the stiffness of Valve Endothelial Cells**
 Napachanok Mongkoldhumrongkul¹, Jose Sanchez-Alonso¹, Padmini Sarathchandra¹, Julia Gorelik¹, Magdi Yacoub^{1,2}, Adrian Chester^{1,2}
¹Imperial College London, London, UK, ²Qatar Cardiovascular Research Centre, Doha, Qatar
- 17.45-18.00 C22 **Valve Interstitial Cell Remodeling Under Abnormal Mechanical Stress is Mediated by 5HT and FGF2 Signaling**
 Ngoc Thien Lam¹, John Carradini¹, Atefeh Razavi¹, Shree Sharma², Kartik Balachandran¹
¹University of Arkansas, Fayetteville, AR, USA, ²University of Arkansas for Medical Sciences, Little Rock, AR, USA
- 18.00-20.00 **Session 5 – Posters and Welcome Reception**
Assessors: Michael Sacks, Frank Baaijens, Ulrich Stock & Dan Simonescu
- P1 **Chondrogenic Pathways Involved In The Development of Myxomatous Valve Disease**
 Alexia Hulin¹, Jonathan Cheek¹, Christina Alfieri¹, Robert Hinton¹, Alain Colige², Katherine Yutzey¹
¹The Heart Institute, Cincinnati Children's Hospital, Cincinnati, Ohio, USA, ²GIGA, Laboratory of Connective Tissues Biology, Sart-Tilman, Belgium
- P2 **Versican Is Markedly Elevated During The Early Pathogenesis Of Murine Aortic Valve Disease**
 Mark Blaser¹, Jung-Woo Kwon¹, Kuiru Wei¹, Yu-Qing Zhou², Mark Henkelman², Craig Simmons^{1,3}
¹Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada, ²Mouse Imaging Centre, Toronto Centre for Phenogenomics, Toronto, Ontario, Canada, ³Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, Ontario, Canada
- P3 **Non-Linear Optical Visualisation Technologies For Characterisation Of Aortic Valve Sclerosis In A Murine Model**
 Anett Jannasch¹, Saskia Faak¹, Christian Schnabel², Roberta Galli², Edmund Koch², Klaus Matschke¹, Thomas Waldow¹
¹Department of Cardiac Surgery, Faculty of Medicine, TU Dresden, Herzzentrum Dresden, Dresden, Germany, ²Institute of clinical Sensoring and Monitoring, Faculty of Medicine, TU Dresden, Dresden, Germany
- P4 **Patient-Specific Fluid Structure Interaction Simulation of Ascending Thoracic Aortic Aneurysm Using In Vivo Magnetic Resonance Imaging**
 Liang Ge^{1,2}, Henrik Haraldsson¹, David Saloner^{1,2}, Michael Hope^{1,2}, Elaine Tseng^{1,2}
¹University of California San Francisco, CA, USA, ²San Francisco VA Medical Center, San Francisco, CA, USA
- P5 **Tissue-Engineered Textile-Reinforced Mitral Valve - Second Generation TexMi**
 Ricardo Moreira¹, Valentine N. Gesche², Luis G. Hurtado-Aguilar¹, Nuno Alves¹, Thomas Schmitz-Rode¹, Julia Frese¹, Stefan Jockenhoevel^{1,2}, Petra Mela¹, ¹Department of Tissue Engineering & Textile Implants, AME-Hemholtz Institute for Biomedical Engineering, RWTH Aachen, Aachen, Germany, ²Institut für Textiltechnik, RWTH Aachen, Aachen, Germany

- P6 **Novel CALP Pericardium Shows Superior Hydrodynamics and Fluid Structure Interaction Compared to Current Bioprosthetic Valve Tissues: A Computer Simulation**
Yaghouab Dabiri¹, Todd J Anderson¹, John V Tyberg¹, Janet Ronsky², Imtiaz Ali¹, Michael Wong¹, Kishan Narine¹
¹University of Calgary Libin Cardiovascular Institute of Alberta, Calgary, Canada, ²University of Calgary Schulich School of Engineering, Calgary, Canada
- P7 **Characterization and Validation of a 3D PEG-NB Screening Platform for Improved MSC-Based Heart Valve Tissue Engineering**
Jenna Usprech¹, Craig Simmons^{1,2}
¹Institute of Biomaterials and Biomedical Engineering, Toronto, Ontario, Canada, ²Mechanical and Industrial Engineering, Toronto, Ontario, Canada
- P8 **Assessment of Parylene C Thin Films for Heart Valve Tissue Engineering**
Isra Marei^{1,2}, Adrian Chester¹, Themistoklis Prodromakis³, Tatiana Trantidou³, Magdi H. Yacoub¹
¹Heart Science Centre, Harefield Hospital, National Heart and Lung Institute, Imperial College London, London, UK, ²Qatar Science Leadership Program, Qatar Foundation, Doha, Qatar, ³Centre for Bio-Inspired Technology, Institute of Biomedical Engineering, Imperial College London, London, UK
- P9 **Surface Functionalization Of Anisotropic Nanofibers For Driving Human Mesenchymal Stem Cell Behaviour**
Ivan Carubelli¹, Jerome Sohler², Ann McCormack¹, Padmini Sarathchandra¹, Adrian Chester¹, Magdi Yacoub¹
¹Magdi Yacoub Institute, Harefield Heart Science Centre, Imperial College London, Harefield, UK, ²Laboratory of Tissue Biology and Therapeutic Engineering (LBTI), UMR 5305, CNRS, Lyon, France
- P10 **PNGase F removes glycocalyx structures of decellularized porcine pulmonary heart valve matrices**
Katja Findeisen, Axel Haverich, Andres Hilfiker
MHH; LEBAO, Hannover, Germany
- P11 **BMP-pSmad1/5/8 Pathway Activation in a Novel Mouse Model of Calcific Aortic Valve Disease**
M. Vicky Gomez^{1,2}, Jonathan D. Cheek¹, Elaine E. Wirrig¹, Katherine E. Yutzey^{1,2}
¹Cincinnati Children's Hospital, Cincinnati, OH, USA, ²University of Cincinnati, Cincinnati, OH, USA
- P12 **Mild Hyperlipidemia Induces Aortic Valve Disease And An Osteoporotic Phenotype In Aged Mice**
Rachel Adams¹, Mark Blaser¹, Yu-Qing Zhou², Celeste Owen², R. Mark Henkelman², Craig Simmons¹
¹Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada, ²Mouse Imagine Centre at the Toronto Centre for Phenogenomics, Toronto, Ontario, Canada
- P13 **Relationship Between Free Aldehyde Groups In Bioprosthetic Heart Valves And Calcification**
Tara Tod, Jeff Dove
Edwards Lifesciences, Irvine, Ca, USA
- P14 **Investigating the Potential of Valve Interstitial Cells to Act in a Pericyte Manner by Promoting Angiogenesis and Invasion by Valve Endothelial Cells**
Christopher Arevalos, Kathryn Grande-Allen
Rice University, Houston, TX, USA

- P15 **MG53-Mediated Protection in Heart Valve Biology**
T.M. Ayodele Adesanya¹, Hua Zhu¹, Tao Tan¹, Ki Ho Park¹, Joy Lincoln^{2,1}, Jianjie Ma¹
¹The Ohio State University, Columbus, Ohio, USA, ²Nationwide Children's Hospital, Columbus, Ohio, USA
- P16 **Relevance of YAP/TAZ Transcriptional Co-factors on Mechanotransduction of Aortic Valvular Interstitial Cells**
Claudia Dittfeld, Wolfgang Witt, Anett Jannasch, Janin Andres, Maria Feilmeier, Klaus Matschke, Thomas Waldow
 Medical Faculty, TU Dresden, Dresden, Germany
- P17 **An In Vitro Model for the Investigation of Potential Mechanical Interactions Between Surgical Sealants and Aortic Tissue**
 Stacy Arnold¹, David Gale¹, David Hollinworth¹, Davide Pacini²
¹CryoLife, Kennesaw, GA, USA, ²University of Bologna, Bologna, Italy
- P18 **Optimization Of Cyclic Stretching For Acceleration Of Collagen Production In Fibrin-Based Engineered Tissue Equivalents**
Robert Tranquillo, Jill Schmidt
 Univ. of Minnesota, Minneapolis, MN, USA
- P19 **Parameter Estimation of Heart Valve Leaflet Hyperelastic Mechanical Behavior Using An Inverse Modeling Approach**
Ankush Aggarwal¹, Michael S. Sacks¹, ¹Center for Cardiovascular Simulation Institute of Computational Engineering and Sciences Department of Biomedical Engineering, Austin, USA
- P20 **A Computational Framework for Multiscale Modeling of the Mitral Valve**
Chung-Hao Lee¹, Michael S. Sacks¹, ¹Center for Cardiovascular Simulation Institute of Computational Engineering and Sciences Department of Biomedical Engineering University of Texas at Austin, Austin, USA
- P21 **A novel experimental/numerical method to assess BHV biomaterial fatigue response in-vivo**
Kristen R. Feaver¹, Will Zhang¹, Hobey Tam², Jeremy R. McGarvey³, Norihiro Kondo³, Robert C. Gorman³, Joseph H. Gorman³, Narendra Vyavahare², Michael S. Sacks¹
¹University of Texas at Austin, Austin, USA, ²University of Clemson, Clemson, USA, ³University of Pennsylvania, Philadelphia, USA
- P22 **Adipose-Derived Stem Cells as a Potential Source of Valve Endothelial Cells**
Napachanok Mongkoldhumrongkul¹, Magdi Yacoub^{1,2}, Adrian Chester^{1,2}
¹Imperial College London, London, UK, ²Qatar Cardiovascular Research Centre, Doha, Qatar
- P23 **A New Strategy for Heart Valve Decellularization for Tissue Engineering**
Jessica Boldt, Georg Lutter, Beke Sarrahs, Jan Schoettler, Jochen Cremer, Anja Metzner
 University hospital Schleswig-Holstein, Campus Kiel, Department of Cardiovascular Surgery, Kiel, Germany
- P24 **WITHDRAWN**
- P25 **Adipose Tissue-Derived Stem Cell Sourcing for Heart Valve Tissue Regeneration; Selection of the Optimal Anatomical Site and Harvesting Procedure in Sheep**
 Marius Harpa¹, Terezia Preda¹, Ionela Movileanu¹, Ovidiu Cotoi^{1,2}, Dan Simionescu^{1,2}
¹University of Medicine and Pharmacy, Tirgu Mures, Romania, ²Clemson University, Clemson SC, USA

- P26 **Decellularisation of Allogeneic Aortic and Pulmonary Valves using Low Concentration SDS**
 Tayyebeh Vafaee¹, Daniel Thomas¹, Paul Rooney², John N Kearney², Helen Berry³, Louise Jennings¹, John Fisher¹, Eileen Ingham¹
¹University of Leeds, Leeds, UK, ²NHS Blood and Transplant Tissue Services, Liverpool, UK, ³Tissue Regenix Group plc, York, UK
- P27 **Comparative Analysis of the Chemoattractive Properties of Human Mesenchymal Stem Cells**
 Debora Kehl¹, Benedikt Weber¹, Marten Schneider², Simon P. Hoerstrup¹
¹Swiss Center for Regenerative Medicine, University of Zürich, Zürich, Switzerland, ²Laboratory of Vascular Immunology, University Hospital Zürich, Zürich, Switzerland
- P28 **In Vivo Investigation of Stented Monoleaflet PGA-P4HB Based Valves in The Arterial Circulation of a Rat Model**
 Agnieszka Ksiazek¹, Benedikt Weber¹, Katharyn Mitchell², Colin Schwarzwald², Simon Peter Hoerstrup¹
¹Swiss Center for Regenerative Medicine, University Hospital of Zurich, Zurich, Switzerland, ²Equine Department, Clinic for Equine Internal Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
- P29 **Acellular Mitral Valve Scaffold with Intact Annulus and Chordae Tendinae for Tissue Engineering**
 Cristopher deBorde¹, Lee Sierad¹, Sourav Patnaik², Jun Liao², Agneta Simionescu¹
¹Clemson University, Clemson, SC, USA, ²Mississippi State University, Starkville, MS, USA
- P30 **Whole Aortic Root Decellularization and Recellularization: An Elusive Task in Heart Valve Tissue Engineering**
 Leslie Sierad^{1,2}, Eliza Shaw¹, Rebekah Odum¹, Allison Kennamer¹, Marius Harpa², Ovidiu Cotoi², Terezia Preda², Lucian Harceaga², Victor Raicea², Imre Egyed², Zoltan Pavai², Annamaria Szanto², Horatiu Suci², Klara Branzaniuc², Radu Deac², Agneta Simionescu^{1,2}, Dan Simionescu^{1,2}
¹Clemson University, Clemson, SC, USA, ²University of Medicine and Pharmacy, Targu Mures, Romania
- P31 **Reasons Of Reoperation After The Ross Procedure In Different Age Groups - A Report From The German Ross Registry**
 Doreen Richardt¹, Wolfgang Hemmer², Armin Gorski³, Ulrich Franke⁴, Sigmar Sachweh⁵, Arlindo Riso⁵, Jürgen Hörer⁶, Rüdiger Lange⁶, Anton Moritz⁷, Roland Hetzer⁸, Michael Huebler⁹, Ulrich Stierle¹, Hans-Hinrich Sievers¹
¹UKSH, Campus Luebeck, Department of Cardiac and Thoracic Vascular Surgery, Luebeck, Germany, ²Sana Clinic Stuttgart, Department of Cardiac Surgery, Stuttgart, Germany, ³University Clinic of Wuerzburg, Department of Cardiac and Thoracic Aortic Surgery, Wuerzburg, Germany, ⁴Robert-Bosch-Hospital Stuttgart, Department of Cardiac Surgery, Stuttgart, Germany, ⁵Universitäres Herzzentrum Hamburg, Hamburg, Germany, ⁶Deutsches Herzzentrum Muenchen, Muenchen, Germany, ⁷University Clinic Frankfurt, Frankfurt, Germany, ⁸Deutsches Herzzentrum Berlin, Berlin, Germany, ⁹Universitätshospital Zuerich, Zuerich, Switzerland

Thursday 11th September

- 8.30-10.30 **Session 6 – Intelligent Scaffolds**
Chairs: Jerome Sohier & Ibrahim El Sherbiny
- 8.30-9.00 **Keynote Lecture: Karen Wooley (Texas AM University)**
- 9.00-9.15 C23 **Peptide Amphiphiles Induced Collagen Production In Adipose Derived Stem Cells In Relation To Tissue Engineering Application**
Yuan-Tsan Tseng^{1,2}, Navaneethakrishnan Krishnamoorthy^{1,2}, Ivan Carubelli¹, Jerome Sohier³, Ann McCormack², Padmini Sarathchandra², Adrian Chester², Magdi Yacoub^{1,2}
¹Qatar Cardiovascular Research Center (QCRC), Qatar Foundation, Doha, Qatar, ²The Magdi Yacoub Institute, Imperial College London, London, UK, ³Centre National de la Recherche Scientifique, Lyon, France
- 9.15-9.30 C24 **A Collagen-Glycosaminoglycan-Fibrin Scaffold For Heart Valve Tissue Engineering Applications**
Claire Brougham^{1,2}, Stefan Jockenhoevel^{3,4}, Thomas Flanagan⁵, Fergal O'Brien^{1,6}
¹Tissue Engineering Research Group, Department of Anatomy, Royal College of Surgeons in Ireland, Dublin, Ireland, ²School of Mechanical & Design Engineering, Dublin Institute of Technology, Dublin, Ireland, ³Department of Tissue Engineering & Textile Implants, AME-Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany, ⁴Institut für Textiltechnik, RWTH Aachen University, Aachen, Germany, ⁵School of Medicine & Medical Science, University College Dublin, Dublin, Ireland, ⁶Advanced Materials & Bioengineering Research (AMBER) Centre, RCSI & TCD, Dublin, Ireland
- 9.30-9.45 C25 **Towards Designing Multifunctional Biomimetic Scaffolds for Tissue Engineering Heart Valves**
Navaneethakrishnan Krishnamoorthy^{1,2}, Yuan Tsan Tseng^{1,2}, Poornima Gajendrarao¹, Adrian Chester², Magdi Yacoub^{1,2}
¹Qatar Cardiovascular Research Center, Doha, Qatar, ²Imperial College London, National Heart and Lung Institute, Heart Science Centre, London, UK
- 9.45-10.00 C26 **Surface-Immobilized LL37 Exhibits Mitogenic Effect On Endothelial Cells**
Matthias Gabriel¹, Robert Szulcek², Geerten van Nieuw-Amerongen²
¹Qatar Cardiovascular Research Center, Doha, Qatar, ²VU University Medical Center, Amsterdam, The Netherlands
- 10.00-10.15 C27 **In-Vitro Evaluation Of A Newly Developed Bio-Hybrid Tissue Engineered Aortic Valve Scaffold For TAVI-Application**
Jangsun Lee¹, Nikolaus Thierfelder¹, Fabian Koenig^{1,3}, Ulrike Haas¹, Cornelia Fano², Trixi Hollweck¹, Christoph Schmitz¹, Christian Hagl¹, Bassil Akra¹.
Medical Center Munich University, Munich, Germany, ²Institute of Textile Technology and Process Engineering, Denkendorf, Germany, ³Institute of Medical Engineering, Technical University of Munich, Munich, Germany, Christoph Schmitz¹, Christian Hagl¹, Bassil Akra¹
¹Medical
- 10.15-10.30 C28 **A New Single-Body Silicone-Polycarbonate-Urethane/PDMS Heart Valves: Material Characterization And Prototype Realization**
Marianna Asaro¹, Tamer Al Kayal¹, Silvia Volpi¹, Paola Losi¹, Simona Celi², Mattia Glauber¹, Giorgio Soldani¹
¹National Council of Research, Institute of Clinical Physiology, Massa, Italy, ²Scuola Superiore S'Anna, Massa, Italy

- 10.30-10.45 Coffee break
- 10.45-12.45 Session 7 – New Approaches to Tissue Engineering**
Chairs: Simon Hostrup & Ulrich Stock
- 10.45-11.15 **Keynote Lecture: Frank Baaijens (Eindhoven University of Technology)**
- 11.15-11.30 C29 **Off-the-shelf Decellularized Tissue-Engineered Heart Valves Repopulate and Remodel Rapidly in Pre-clinical Animal Models**
Petra E. Dijkman¹, Laura Frese¹, Benedikt Weber¹, Anita Driessen-Mol², Maximilian Y. Emmert¹, Bart Sanders², Jacques Sherman³, Juerg Gruenenfelder², Nikola Cesarovic¹, Volkmar Falk¹, Peter Zilla³, Frank P.T. Baaijens², Simon P. Hoerstrup^{1,2}
¹University and University Hospital Zurich, Division of Surgical Research, Zurich, Switzerland, ²Eindhoven University of Technology, Department of Biomedical Engineering, Eindhoven, The Netherlands, ³Cardiovascular Research Unit, Cape Heart Centre, University of Cape Town, Cape Town, South Africa
- 11.30-11.45 C30 **Improved geometry of decellularized tissue engineered heart valves for future enhanced long term in vivo performance**
B. Sanders¹, S. Loerakker¹, A. Driessen-Mol¹, E.F. Fioretta¹, P.E. Dijkman², L. Frese², S.P. Hoerstrup^{2,1}, F.P.T. Baaijens¹
¹Eindhoven University of Technology, Eindhoven, The Netherlands, ²University and University Hospital Zurich, Zurich, Switzerland
- 11.45-12.00 C31 **Tubular Heart Valves Fabricated From Decellularized Engineered Tissue Tubes**
Robert Tranquillo, Zeeshan Syedain, Jay Reimer, Jill Schmidt, Lee Meier
 Univ. of Minnesota, Minneapolis, MN, USA
- 12.00-12.15 C32 **Influence Of Small Intestinal Submucosa (SIS) Configuration On Tricuspid Valve Remodeling After Three Months In An Ovine Model**
Anna Fallon¹, James Cox², Robert Matheny¹
¹CorMatrix Cardiovascular, Roswell, GA, USA, ²Washington University, St. Louis, MO, USA
- 12.15-12.30 C33 **Tissue-Engineered Heart Valves with a Tubular Leaflet Design for conventional and minimally invasive implantation**
Nuno Alves¹, Ricardo Moreira¹, Miriam Weber¹, Valentine Gesché², Thomas Schmitz-Rode¹, Julia Frese¹, Stefan Jockenhövel^{1,2}, Petra Mela¹
¹AME-Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany, ²Institut für Textiltechnik, RWTH Aachen University, Aachen, Germany
- 12.30-12.45 C34 **Mechanical Conditioning Of Cell Seeded PCL Fibers Under Pulsatile Pressure**
Ivan Carubelli¹, Jerome Sohler², Ann McCormack¹, Padmini Sarathchandra¹, Adrian Chester¹, Magdi Yacoub¹
¹Magdi Yacoub Institute, Harefield Heart Science Centre, Imperial College London, Harefield, UK, ²Laboratory of Tissue Biology and Therapeutic Engineering (LBTI), UMR 5305, CNRS, Lyon, France
- 12.45-13.30 Lunch

- 13.30-15.30 Session 8 – Immuntolerance for tissue engineering applications**
Chairs: Jane Grande-Allen & Herman Waldman
- 13.30-14.00 **Keynote: Kathy Liu (Harvard University)**
- 14.00-14.15 C35 **Minimization of Human Allograft Immunogenicity by Ice-free Cryopreservation**
Kelvin G.M. Brockbank^{1,2}, Lia H. Campbell¹, Zhenzhen Chen¹, Elizabeth D. Greene¹, Ulrich A. Stock³, Martina Seifert⁴
¹Cell & Tissue Systems, Inc., North Charleston, South Carolina, USA, ²Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, South Carolina, USA, ³Department of Thoracic, Cardiac and Vascular Surgery University Hospital Frankfurt, Frankfurt am Main, Germany, ⁴Institute of Medical Immunology and Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Charité Universitätsmedizin, Berlin, Germany
- 14.15-14.30 C36 **Histological Change of A Novel Autologous Tissue Engineered Heart Valve after Implantation**
Yoshiaki Takewa¹, Yasuhide Nakayama¹, Hirohito Sumikura¹, Satoru Kishimoto¹, Kazuma Date¹, Keiichi Kanda², Tsutomu Tajikawa³, Takaharu Tanaka⁴, Eisuke Tatsumi¹
¹National Cerebral and Cardiovascular Center, Osaka, Japan, ²Kyoto Prefectural University of Medicine, Kyoto, Japan, ³Kansai University, Osaka, Japan, ⁴GOODMAN Co.,Ltd., Aichi, Japan
- 14.30-14.45 C37 **Dynamic Relationship between the Mitral Annulus and the Basal Left Ventricular Myocardium in the Beating Ovine Heart**
Matts Karlsson¹, Neil Ingels²
¹Linköping University, Linköping, Sweden, ²Stanford University, Palo Alto, CA, USA
- 14.45-15.00 C38 **Canonical and Non-canonical TGF β Inhibition Differentially Regulate Matrix Homeostasis in Porcine Aortic Valve Interstitial Cells**
Varun Krishnamurthy, Andrew Stout, Kristi Lim, David Allison, K. Jane Grande-Allen
Rice University, Houston, TX, USA
- 15.00-15.15 C39 **A computational approach for in situ estimation of aortic valve interstitial cell mechanical state from tissue level measurements**
Rachel M. Buchanan¹, Michael S. Sacks¹, ¹Center for Cardiovascular Simulation Institute of Computational Engineering and Sciences Department of Biomedical Engineering University of Texas at Austin, Austin, USA
- 15.15-15.30 C40 **Mimicking Heart Valve Disease In An Ex Vivo Flow Model**
Boudewijn Kruithof^{1,2}, Samuel Lieber³, Marianna Kruithof-de Julio¹, Vinciane Gaussin², Marie-José Goumans¹
¹Leiden University Medical Center, Leiden, The Netherlands, ²University of Medicine and Dentistry of New Jersey, Newark, USA, ³New Jersey Institute of Technology, Newark, USA
- 15.30-15.45 Coffee break

- 15.45-17.15** **Session 9 – Gene Expression & Biomarkers in Valve Disease**
Chairs: Elena Aikawa & Najma Latif
- 15.45-16.15 **Keynote Lecture: Dennis Cokkinos (Academy of Athens)**
- 16.15-16.30 C41 **Bicuspid Aortic Valve: Determining Susceptibility for Dilation of the Ascending Aorta by Histopathology**
Nimrat Grewal¹, Adriana C. Gittenberger-de Groot¹, Robert J.M. Klautz¹, Robert E. Poelmann¹, Marie-José Goumans¹, Sjoerd N. Duim¹, Johannes H.N. Lindeman¹, Monique R.M. Jongbloed¹, Salah A. Mohamed², Hans-Hinrich Sievers², Ad J.J.C. Bogers³, Marco C. DeRuiter¹
¹*Leiden University Medical Center, Leiden, The Netherlands,* ²*University of Lübeck, Lübeck, Germany,* ³*Erasmus University Medical Center, Rotterdam, The Netherlands*
- 16.30-16.45 C42 **Second Harmonic Generation Microscopy For Analysis Of Collagen Fiber Alterations In Calcific Aortic Valve Disease**
Heather Hutson, Kristyn Masters
University of Wisconsin, Madison, Wisconsin, USA
- 16.45-17.00 C43 **Comparative in vivo analysis of ovine tissue-engineered heart valves re-endothelialized with ECs derived either from umbilical cord blood or peripheral blood**
Karolina Theodoridis¹, Anna-Lena Wendland², Igor Tudorache¹, Serghei Cebotari¹, Alexandru Mogaldea¹, Tobias Goecke¹, Samir Sarikouch¹, Tanja Meyer¹, Doreen Unger¹, Karl-Heinz Waldmann², Axel Haverich¹, Andres Hilfiker¹
¹*Hannover Medical School, Division of Thoracic and Cardiovascular Surgery, Hannover, Germany,* ²*University of Veterinary Medicine, Clinic for Swine, Small Ruminants, Forensic Medicine and Ambulatory Service, Hannover, Germany*
- 17.00-17.15 **Expression and activity of extracellular ectoenzymes (CD39 and CD73) in aortic valves cells and in endothelial cells under the shear stress conditions**
Ewa Kaniewska¹, Alicja Sielicka¹, Padmini Sarathchandra², Iwona Pelikant-Malecka¹, Adrian Chester², Magdi H. Yacoub², Ryszard T. Smolenski¹
¹*Medical University of Gdansk, Gdansk, Poland,* ²*Heart Science Centre, Imperial College London at Harefield Hospital, London, UK*
- 20.00-23.00 Conference Dinner –Aboard the Pride of London while cruising on the River Thames

Friday 12th September

8.30-10.30 Session 10 – The Ross Procedure

Chairs: Jane Sommerville & Giovanni Battista Luciani

8.30-9.00

Keynote Lecture: Ismail El-Hamamsy (Montreal Heart Institute)

9.00-9.15

C45 **Reoperation Rate After The Ross Procedure Compared To Bioprostheses In Different Age Groups - A Report From The German Ross Registry**

Doreen Richardt¹, Wolfgang Hemmer², Armin Gorski³, Ulrich Franke⁴, Sigmar Sachweh⁵, Arlindo Riso⁵, Jürgen Hörer⁶, Rüdiger Lange⁶, Anton Moritz⁷, Roland Hetzer⁸, Michael Huebler⁹, Ulrich Stierle¹, Hans-Hinrich Sievers¹

¹UKSH, Campus Luebeck, Department of Cardiac and Thoracic Vascular Surgery, Luebeck, Germany, ²Sana Clinic Stuttgart, Department of Cardiac Surgery, Stuttgart, Germany, ³University clinic of Wuerzburg, Department of Cardiac and Thoracic Aortic Surgery, Wuerzburg, Germany, ⁴Robert-Bosch-Hospital Stuttgart, Department of Cardiac Surgery, Stuttgart, Germany, ⁵Universitäres Herzzentrum Hamburg, Hamburg, Germany, ⁶Deutsches Herzzentrum München, München, Germany, ⁷University Clinic Frankfurt, Frankfurt, Germany, ⁸Deutsches Herzzentrum Berlin, Berlin, Germany, ⁹Universitätshospital Zuerich, Zuerich, Switzerland

9.15-9.30

C46 **Decellularized Valves For RVOT Reconstruction During The Ross Operation - Should Cryopreservation Be Abandoned?**

Francisco Costa^{1,2}, Eduardo Mendel², Ana Beatriz Costa¹, Marcia Olandoski¹, Daniele Fornazari², Sergio Lopes¹, Tiago Fernandes¹, Andrea Ferreira¹, Claudinei Colatusso¹

¹Santa Casa de Curitiba, Curitiba, Parana, Brazil, ²Instituto de Neurologia e Cardiologia de Curitiba, Curitiba, Parana, Brazil

9.30-9.45

C47 **A multicenter evaluation of the autograft procedure for young patients undergoing aortic valve replacement: Results from the German Ross Registry**

Hans-Hinrich Sievers¹, Ulrich Stierle¹, Marc Albert², Ulrich Franke², Armin Gorski³, Efstratios Charitos¹, Roland Hetzer⁴, Jürgen Hörer⁵, Rüdiger Lange⁵, Anton Moritz⁶, Arlindo Riso⁷, Jörg Sachweh⁷, Wolfgang Hemmer⁸

¹University of Lübeck, Lübeck, Germany, ²Robert-Bosch-Hospital, Stuttgart, Germany, ³University of Würzburg, Würzburg, Germany, ⁴German Heart Center, Berlin, Germany, ⁵German Heart Center, Munich, Germany, ⁶University Clinic of Frankfurt am Main, Frankfurt am Main, Germany, ⁷University of Hamburg, Hamburg, Germany, ⁸Sana Heart Surgery, Stuttgart, Germany

9.45-10.00

C48 **An Assessment of Shape and Function of the Pulmonary Autograft 10 or more Years after the Ross Procedure**

Ryo Torii^{1,2}, Michael Ibrahim³, Tarun Mittal⁴, Mohamed Donya⁵, Ismail El-Hamamsy³, Su-Lin Lee³, Yun Xu³, Magdi Yacoub^{3,2}

¹University College London, London, UK, ²Qatar Cardiovascular Research Centre, Doha, Qatar, ³Imperial College London, London, UK, ⁴Harefield Hospital, Harefield, UK, ⁵Aswan Heart Centre, Aswan, Egypt

10.00-10.15

C49 **Decellularized valve allografts combined with vascularized autologous small intestine for the reconstruction of the right ventricular outflow tract (RVOT)**

Karolina Theodoridis¹, Serghei Cebotari¹, Igor Tudorache¹, Dagmar Hartung², Tanja Meyer¹, Anatol Ciobutaru¹, Alexandru Mogaldea¹, Axel Haverich¹, Andres Hilfiker¹

¹Hannover Medical School, Division of Thoracic and Cardiovascular Surgery, Hannover, Germany, ²Hannover Medical School, Institute for Diagnostic and Interventional Radiology, Hannover, Germany

- 10.15-10.30 C50 **Outcome Of Pregnancy After The Ross Operation - A Report From The German-Dutch Ross Registry**
Doreen Richardt¹, Wolfgang Hemmer², Armin Gorski³, Ulrich Franke⁴, Sigmar Sachweh⁵, Arlindo Riso⁵, Jürgen Hörer⁶, Rüdiger Lange⁶, Anton Moritz⁷, Roland Hetzer⁸, Michael Huebler⁹, Ad JJC Bogers¹⁰, Johanna JM Takkenberg¹⁰, Ulrich Stierle¹, Hans-Hinrich Sievers¹
¹UKSH, Campus Luebeck, Department of Cardiac and Thoracic Vascular Surgery, Luebeck, Germany, ²Sana Clinic Stuttgart, Department of Cardiac Surgery, Stuttgart, Germany, ³University Clinic of Wuerzburg, Department of Cardiac and Thoracic Aortic Surgery, Wuerzburg, Germany, ⁴Robert-Bosch-Hospital Stuttgart, Department of Cardiac Surgery, Stuttgart, Germany, ⁵Universitäres Herzzentrum Hamburg, Hamburg, Germany, ⁶Deutsches Herzzentrum Muenchen, Muenchen, Germany, ⁷University Clinic Frankfurt, Frankfurt, Germany, ⁸Deutsches Herzzentrum Berlin, Berlin, Germany, ⁹Universitätsspital Zuerich, Zuerich, Switzerland, ¹⁰Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands
- 10.30-11.00 Coffee Break
- 11.00-13.00 Session 11 – Valve Conserving Operations - From Structure to Function**
- 11.00-11.30 **Chairs: Axel Haverich & Magdi Yacoub**
- Keynote: Martin Misfeld (Lipzig)**
- 11.30-11.45 C51 **Haemodynamics in Anatomically Shaped Sinus Prostheses Analysed by 4D Flow MRI**
Thekla Oechtering¹, Michael Beldoch¹, Claudia Schmidtke^{2,3}, Michael Scharfschwerdt², Peter Hunold¹, Jörg Barkhausen¹, Alex Frydrychowicz¹, Hans-H. Sievers², ¹Clinic for Radiology and Nuclear Medicine, UKSH, Lübeck, Germany, ²Department of Cardiac and Cardiothoracic Surgery, UKSH, Lübeck, Germany, ³Department of Cardiac Surgery, Segeberger Clinic, Bad Segeberg, Germany
- 11.45-12.00 C52 **Aortic Valve Dynamics After Valve-sparing Procedure With The New ‘Sinus’ Prosthesis Used For Valve Sparing Aortic Root Replacement**
Claudia Schmidtke, Hans-H. Sievers
Klinik für Herz- und thorakale Gefässchirurgie, Universitätsklinikum SH, Campus Lübeck, Lübeck, Germany
- 12.00-12.15 C53 **In Vitro Analysis of The Alterations Produced by Transcatheter Aortic Valve in The Valsalva Sinuses**
 Andrea Ducci, Francesco Pirisi, Spyridon Tzamtzis, Gaetano Burriesci
University College London, London, UK
- 12.15-12.30 C54 **Aortic valve replacement with decellularized aortic allografts: First clinical results**
Igor Tudorache, Anatol Ciubotaru, Thomas Breyman, Samir Sarikouch, Dietmar Boethig, Alexander Horke, Luitgard Meschenmoser, Serhhei Cebotari, Axel Haverich
Medizinische Hochschule Hannover, Hannover, Germany
- 12.30-12.45 C55 **Patient-Specific Finite Element Modeling of Ascending Thoracic Aortic Aneurysm Using In Vivo Magnetic Resonance Imaging**
Liang Ge, Michael Hope, David Saloner, Andrew Wisneski, Kapil Krishnan, Julius Guccione, Elaine Tseng
University of California San Francisco Medical Center, San Francisco, CA, USA

- 12.45-13.30** **Session 12 – Taking Cardiac Surgery to the People**
Chair: Jane Sommerville
- 12.45-13.30 **Keynote Speaker: Magdi Yacoub (Imperial College London)**
- 13.30-13.40 Concluding remarks and adjourn for Lunch

Abstracts – Oral Presentations

C1

Extracellular Matrix Composition Regulates Endothelial to Mesenchymal Transformation

Sudip Dahal¹, Jonathan Bramsen¹, Peter Huang², Gretchen Mahler¹

¹*Binghamton University, Department of Bioengineering, Binghamton, NY, USA,*

²*Binghamton University, Department of Mechanical Engineering, Binghamton, NY, USA*

Endothelial to mesenchymal transformation (EndMT) is a valve morphogenic mechanism associated with aortic valve regeneration and disease initiation. The aortic valve fibrosa is the initial site of inflammatory and calcific degeneration, and EndMT occurs on the valve fibrosa local to calcified nodules. In healthy valves, the fibrosa matrix is primarily composed of circumferentially-aligned, collagen fibers. Valve disease is characterized by the deposition of chondroitin sulfate and dermatan sulfate-rich proteoglycans into the sub-endothelial fibrosa layer, and the presence of the glycosaminoglycan (GAG) hyaluronan near calcified nodules. The goal of our work is to identify if and how this altered extracellular matrix (ECM) composition promotes EndMT and eventual calcification. In this study we examined aortic valve endothelial cell response to ECM changes using *in vitro* cell cultures and hybrid continuum-stochastic modeling. Our *in vitro* results show that the addition of GAGs (chondroitin sulfate, dermatan sulfate, and hyaluronan) to a 3D collagen I-based matrix strongly induces EndMT. Our computational model captures EndMT and cell migration within the local ECM environment. The composition, structure, and mechanical behavior of the ECM are expressed in the adhesion stresses during stochastic EndMT and resistance to biased cell migration. The model has been developed to contain measurable quantities validated by our *in vitro* experimental data and to predict which cell-ECM interactions are critical to mesenchymal transformation and cell migration. Determining the matrix cues that induce and promote EndMT will advance tissue regeneration strategies, tissue engineering approaches, and understanding of *in vivo* endothelial cell behavior in valve disease progression.

C2

Cyclic stretch orchestration of RhoA-Rac1 signaling axis is essential for embryonic valve remodeling

Russell Gould¹, Huseyin Cagatay², Joanna MacKay³, Sanjay Kumar³, Jonathan Butcher¹

¹*Cornell University, Ithaca, NY, USA*, ²*Dogus University, Istanbul, Turkey*,

³*University of California, Berkeley, Berkeley, CA, USA*

Embryonic valves rapidly evolve from disorganized globular “cushions” to highly organized thin fibrous leaflets. These multi-scale interactions occur within a demanding hemodynamic environment and are essential to maintain unidirectional flow. The mechanisms by which mechanical forces direct cellular response to coordinate this cycle of morphogenesis and function are not known. In this study, we determined how mechanosensitive small GTPases RhoA and Rac1 coordinate atrioventricular valve (AV) differentiation and morphogenesis. RhoA activity is elevated during early cushion formation, but decreases considerably over development. In contrast, active Rac1 increases as cushions mature into valves. Using gain and loss of function assays, we determined that the RhoA-SRF pathway was essential for a myofibroblastic contractile phenotype in early cushion formation, but was surprisingly insufficient to drive matrix condensation during valve maturation. Instead, sustained cyclic stretch was a potent inhibitor of myofibroblastic activation by decreasing RhoA-GTP levels, and promoted a Rac1 signaling “switch” via nuclear membrane localization of FilGAP. The downstream Rac1-p38 pathway was necessary and sufficient to cause directed matrix condensation through cell polarization, leading to enhanced stress fiber alignment and adhesion necessary for valvular remodeling. Finally, we used partial atrial ligation experiments to show that altered cyclic mechanical loading *in vivo* augmented or restricted cushion growth directly through potentiation of RhoA and Rac1 activity. Together, these results demonstrate that mechanical signaling is essential to coordinate a transition from RhoA to Rac1 based signaling, to produce a thin organized leaflet with a quiescent phenotype.

C3

Insights Into Regional Adaptations In The Growing Pulmonary Artery Using A Meso-scale Structural Model: Effects Of Ascending Aorta Impingement

Michael Sacks

Institute of Computational Engineering and Sciences Department of Biomedical Engineering University of Texas at Austin, Austin, USA

Delineating the normal postnatal development of the pulmonary artery (PA) and valve can inform our understanding of congenital abnormalities. Utilizing the extensive experimental structural and mechanical measurements from our previous study, we developed a structural constitutive model for the growing PA at the juvenile and adult stages. Our key findings were that while there are regional variations with age, the major effects of the age related changes were exhibited in the medial aspect of the PA wall. Specifically, we observed that structurally, the elastin and collagen fibers' in-plane splay underwent opposite changes, and a trend towards more rapid collagen fiber recruitment with respect to strain. The most profound changes were observed with the fiber moduli, with the elastin modulus increasing by ~50% and the collagen modulus reducing to only ~25% of the juvenile value. As observed in our geometric studies the focal changes observed in the medial region are likely due to the impingement of relatively stiff ascending aorta on the growing PA. Clearly, during the postnatal somatic growth, local stresses can substantially modulate the development of regional tissue microstructure and mechanical behaviors in the PA. Finally, we underscore that in our previous studies suggest an increase in effective PA wall stress with postnatal maturation. This observation is contrary to the accepted theory of maintenance of homeostatic stress levels in the regulation of vascular function, and suggests alternative mechanisms regulate postnatal somatic growth.

ACKNOWLEDGMENTS

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C4

Modulation Of Human Valve Interstitial Cell Phenotype and Function Using A FGF2 Formulation

Najma Latif^{1,2}, Alfred Quillon¹, Ann McCormack¹, Alec Lozanoski¹, Magdi Yacoub^{1,2}, Adrian Chester^{1,2}

¹Imperial College, London, UK, ²Qatar Cardiovascular Research Centre, Doha, Qatar

Valve interstitial cells comprise the main fibroblastic component of valve leaflets however in culture it is accepted that they differentiate into a myofibroblastic phenotype. A fibroblast culture media (FIB) formulation was assessed for its ability to maintain the fibroblastic phenotype. Normal human VICs were cultured in standard DMEM and in FIB consisting of FGF2 (10ng/ml), insulin (50ng/ml) and 2% FCS for at least one week. VICs in DMEM exhibited large, spread cells whereas VICs in FIB were smaller, elongated and spindly. Aspect ratio and size were both significantly higher in DMEM ($p < 0.01$). The level of expression of α -SMA was significantly reduced in DMEM ($p < 0.01$). There was a significant decrease in the proliferative potential of VICs in FIB after 1 ($p < 0.05$) and 2 ($p < 0.01$) weeks in culture. Contraction of collagen gels was significantly reduced in FIB from 4 hours post dislodgment ($p < 0.05$). Focal adhesions were assessed in relation to this contraction and VICs were found to have significantly fewer supermature ($p < 0.001$) and smaller focal adhesion ($p < 0.01$) in FIB. VICs in FIB demonstrated a slower migratory ability after wounding at 72 hours ($p < 0.01$). Treatment of human VICs with this FIB formulation has the ability to dedifferentiate the VICs back to a fibroblastic phenotype with phenotypic and functional characteristics ascribed to quiescent VICs. The use of quiescent VICs in the study of normal valve biology, pathology and in the field of tissue engineering important to recapitulate the physiological properties of VICs in vitro.

C5

Deletion of Krox20 results to bicuspid aortic valve

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Aortic valve disease may be life threatening and is highly prevalent conditions in Western countries whose impact is continuously increasing in the aging population. Among the aortic valve disease, bicuspid aortic valve (BAV) occurs when the aortic valve has two cusps, rather than three, and represents the most common form of congenital cardiac malformation, affecting 1-2% of the population. Despite the clinical significance of this pathology, its etiology is poorly understood. To date, only few genes (as NOTCH1, HOXA1 or TGFBR2) have been associated with BAV. Our recent work showed that the transcription factor Krox20 is expressed in the mesenchymal cells of the aortic valves of embryo and adult mice. Krox20 mutant embryos develop abnormal aortic valves with hypercellularization of interstitial cells and disorganization of the extracellular matrix (ECM). Our data revealed that aortic valve defect is caused by increased contribution of cardiac neural crest-derived cells and direct downregulation of fibrillar collagen Col1a1 and Col3a2 genes. Here, we report that 25% of Krox20 mice have BAV. This defect is essentially due to a failure of normal development of the embryonic cushions in the outflow tract resulting in a fused right- and non-coronary cusp. Our analysis shows that Krox20 regulated transcriptional levels of eNos (endothelial nitric oxide synthase), which is required for endothelial differentiation and vascular remodeling. We are currently analyzing the genetic interaction between Krox20 and eNos. Thus, Krox20 is a candidate gene for human aortic valve disease such as BAV.

C6

Pharmacological Treatment in Marfan Mitral Valve: A Pilot Study

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Marfan syndrome is caused by mutations in the gene that encodes fibrillin-1. Mitral valve prolapse is a cardiovascular complication in Marfan patients. This study examined the mitral valve of normal controls, untreated Marfan mice (using the murine model of Marfan syndrome, C1039G) and Marfan mice treated with either: Pravastatin (100mg/kg); Losartan (60mg/kg) or Doxycycline (24mg/kg). Valves were analysed both ultrastructurally and stereologically.

The C1039G murine model of Marfan Syndrome exhibits ultrastructural changes in the mitral valve compared to control animals. These include aberrant collagen orientation and phenotypic change to Valvar Interstitial Cells (VIC). Pharmacological treatment of the Marfan mouse is seen to change the microanatomy of the mitral valve; using transmission electron microscopy and a variety of stereological techniques these changes are demonstrable and quantifiable. Of the three pharmacological treatments used, pravastatin is the only one to decrease mitral cusp thickness whilst both Doxycycline and Losartan increase valve thickness in μm (40.96 ± 13.26) above the level of Marfan Syndrome - itself thicker than the control. This may have implications in valve stiffness and compliance during the cardiac cycle. Marfan Syndrome and each treatment group exhibited an increase in the absolute volume of VIC per μm^3 (221.29 ± 59.49), VIC nucleus (101.82 ± 25.41) and VIC rER (27.24 ± 11.51) compared to control. VIC and VIC rER was greatest in the pravastatin treated group. This may suggest that pravastatin treatment of Marfan Syndrome mice may have a beneficial effect on protein production by VICs in the mitral valve, thereby supporting valvar function and tonicity.

C7

COX2 Inhibition Reduces Aortic Valve Calcification *In Vivo*

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Calcific aortic valve disease (CAVD) is characterized by calcific nodule formation, stenosis of the valve, and induction of an osteogenic gene program. *Klotho*-deficient mice were used to study the molecular mechanisms and cellular origins of CAVD as they develop robust aortic valve (AoV) calcification. Through microarray analysis of AoV tissues from *klotho*-deficient and wild type mice, increased expression of the gene encoding cyclooxygenase 2 (COX2) was found. Aside from a role in inflammation, COX2 activity contributes to bone differentiation and homeostasis. Thus, we hypothesize that COX2 has a critical role in AoV calcification. In *klotho*-deficient mice, COX2 expression is increased throughout regions of AoV calcification in the valve interstitial cells (VICs). Similarly, COX2 expression is increased in human CAVD. To determine if the developmental origin of VICs confers a unique potential to calcify, the contribution of endothelial versus neural-crest derived VICs to the calcified area was assessed in *klotho*-deficient mice. COX2-expressing VICs in calcified regions were primarily of neural crest origin and rarely of endothelial origin, suggesting that neural crest derived VICs activate COX2 gene expression and have the capacity to calcify. Treatment of cultured porcine VICs with osteogenic media induced bone marker gene expression, which was blocked by inhibition of COX2 activity. Furthermore, genetic loss of function or pharmacological inhibition of COX2 activity reduced AoV calcification and blocked osteogenic gene induction in *klotho*-deficient mice. Thus, COX2 expression is activated in calcifying VICs of neural crest developmental origin, and COX2 inhibition is sufficient to reduce AoV calcification *in vivo*.

C8

Extracellular Adenine Nucleotide Catabolism on the Calcified Aortic Valve Surface and its Clinical Correlates

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Enzymes of extracellular nucleotide metabolism participate in regulatory functions, such as immunomodulation, thromboregulation, bone remodeling or cell proliferation by controlling extracellular nucleotide metabolite concentrations. In valves, disturbances of these processes may lead to calcification. This study aimed to investigate the changes in extracellular nucleotide metabolism in pathology of human aortic valves. Stenotic aortic valves were obtained from patients after aortic valve replacement (n=100) and non-stenotic valves were obtained after heart transplantation or Bentall procedure (n=20). Valves were analyzed for ecto-nucleoside triphosphate diphosphohydrolase (eNTPD), ecto-5'-nucleotidase (e5NT) and ecto-adenosine deaminase (eADA) activities by exposing specific area of fibrosa or ventricularis side to medium with nucleotide substrates and tracing conversions by high performance liquid chromatography. These measurements were correlated with clinical data.

On the fibrosa of stenotic aortic valve, activities of eNTPD, e5NT and eADA were 1.80 ± 0.09 , 1.11 ± 0.06 , 0.95 ± 0.07 nmol/min/cm² respectively. Whereas, in the non-stenotic valves these activities were higher: 2.60 ± 0.32 (eNTPD) and 2.11 ± 0.21 (e5NT) or lower: 0.55 ± 0.09 nmol/min/cm² (eADA). No differences between stenotic and non-stenotic valves were observed on the ventricular side. Negative correlations were found between: eNTPD and e5NT activities and plasma low density lipoproteins as well as between eADA activity and ejection fraction. Positive correlation was demonstrated between eADA activity and left ventricular end-systolic diameter.

These results demonstrate that extracellular nucleotide breakdown in the valve is adversely modified in aortic stenosis. Diminished activities of eNTPD and e5NT with increase in eADA activity may affect extracellular nucleotide concentrations in the way that favors valve inflammation and calcification.

C9

Characterisation of human cardiovascular tissue affected by calcific disease using Raman and scanning electron microscopy

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Certain pathological processes give rise to ectopic soft tissue calcification. A better understanding of the structures and nano-scale architecture of ectopic calcification in tissues such as aortae affected by arteriosclerosis and other calcific diseases can lend insight into the biomineralisation process of the different diseases. Aortae, aortic valve, and coronary artery samples from patients and healthy human donors were analysed using Raman spectroscopy and scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS). A previous study in our group identified spherical calcium phosphate deposits within aortic tissues of diseased and even healthy donors. These spherical deposits were a highly crystalline form of calcium phosphate^{1, 2}. In this study, further characterisation with Raman mapping of the aorta wall tissue supports these results, based on intensity heat maps of the 960 cm⁻¹ calcium phosphate and the 1080 cm⁻¹ phosphate bands; indicating discreet regions of calcium phosphate deposits within the tunica media of the aortic wall tissue. Furthermore, the mineral composition of the calcified spheres was determined to be magnesium-substituted hydroxyapatite, as found by Raman, EDS, and ICP, in stark contrast to calcification of atherosclerotic plaques and bone mineral, which are poorly crystalline apatite. Interestingly, this is true for calcified spheres of the tunica media in both healthy and diseased tissue, suggesting the same origin. This study indicates that the calcification in vascular tissues is different from that of bone. The characterisation and study of these tissues can thus contribute to a better understanding of pathophysiological tissue calcification processes.

C10

Analysis of The Retinoblastoma Protein In pAVIC Calcification

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Calcific aortic valve disease (CAVD) shares several features with osteogenesis. This pathological process is similar to normal development of bone, phenotypically and transcriptionally. Because the retinoblastoma protein (Rb) pathway is critical to bone development, we hypothesized that the Rb pathway also regulates development and progression of CAVD. To test this hypothesis we cultured porcine aortic valve interstitial cells (pAVICs) infected with lenti-viruses expressing shRNA constructs targeting porcine Rb mRNA. Knockdown of the Rb transcript was confirmed by qRT-PCR (80% and 69% reduced expression by shRb1 and shRb3, respectively, $p < 0.005$) and we consistently observed reduced Rb protein by Western blot compared to a scrambled shRNA control. AVICs lacking Rb had similar ki-67 positivity compared to control cells (21-36%, $p = \text{NS}$), suggesting that cell-cycle regulation is not impaired in pAVICs with Rb knockdown. Calcification was induced by culturing the pAVICs in medium treated with ascorbic acid, B- glycerophosphate, and dexamethasone. After three weeks, expression of bone-specific genes (Runx2, alkaline phosphatase, osteocalcin and osteonectin) was increased compared to pAVICs cultured in normal medium, as determined by qRT-PCR analysis. Calcification, detected by alizarin red S staining, was significantly higher in cells treated with calcification medium ($p < 0.05$). We observed modestly increased calcification of pAVICs transformed with the shRb3 construct compared to scrambled control. The role of pRb in pAVIC calcification is currently under investigation both *in vitro* and *in vivo* by cre-mediated knockout. These studies will determine the potential role for Rb in pAVIC calcification and CAVD.

C11

Investigating The Role Of Matrix Vesicles In Valve Interstitial Cell

Calcification.

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Accelerated progression of calcific aortic valve disease (CAVD) is frequently observed in patients with end stage renal disease (ESRD) however the cellular mechanisms responsible remain unclear. Elevated serum calcium (Ca) and phosphate (Pi) levels in ESRD patients enhance vascular smooth muscle cell calcification and the subsequent release of matrix vesicles (MV), which are specialised structures that initiate mineral nucleation. In the present study, we determined whether valve interstitial cells (VICs) contribute to aortic valve calcification through the release of MVs.

Rat VICs were cultured with control medium or calcifying medium containing 2.7 mM Ca and 2.5mM Pi (Ca x Pi) for 3 days. A significant increase in calcium deposition was observed (10.8 fold, P<0.001) by quantitative calcium assay and alizarin red staining. In addition, elevated levels of Ca x Pi significantly increased the mRNA expression of calcification regulators *PiT-1* (1.6 fold, P<0.01), *Enpp1* (2.9 fold, P<0.01) and *Phospho1* (2.6 fold, P<0.001). However, the expression of the osteogenic markers *Runx2*, *Ocn*, and *Msx2* were unaltered. MVs released by rat VICs were harvested by ultracentrifugation. Western blotting revealed that MVs released through Ca x Pi stimulation showed increased expression of the MV markers Annexin 2 and Annexin 6. These studies are the first to establish the *in vitro* calcification of rat VICs as a model of CAVD. Our data has demonstrated that calcifying VICs release MVs, suggesting a potential novel mechanism underpinning the pathogenesis of CAVD.

C12

eNOS uncoupling drives calcification via disruption of endothelial protection in early and late stages of aortic valve disease

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The role of eNOS uncoupling in aortic valve disease progression is unknown, despite its known role in vascular pathology. Here we show that inflammation drives increased valve oxidative stress via eNOS uncoupling, leading to endothelial dysfunction and calcification. Inhibition of eNOS uncoupling and its effects with tetrahydrobiopterin or superoxide dismutase can mitigate valve degeneration by blocking the downstream effects of eNOS uncoupling.

Calcified human aortic valves expressed side-specific elevation of superoxide levels in the endothelium, co-localized with high VCAM-1 expression, linking oxidative stress, inflammation, and valve degeneration. TNF α treatment increased superoxide and decreased eNOS and VE-cadherin acutely over 48 hours in aortic valve endothelial cells (VEC) and chronically over 21 days in *ex vivo* AV leaflets. Co-treatment of VEC with tetrahydrobiopterin (BH₄) but not apocynin mitigated TNF α -driven VEC oxidative stress, indicating eNOS uncoupling as the primary mechanism of increased valve endothelial oxidative stress. Co-treatment of VEC or *ex vivo* AV leaflets with TNF α +BH₄ or TNF α +superoxide dismutase (SOD) rescued endothelial function and mitigated inflammatory responses. In *ex vivo* AV leaflets, TNF α additionally caused extracellular matrix disorganization, myofibroblastic activation, and calcium and mineral deposition, which were ameliorated by co-treatment with TNF α +BH₄ or TNF α +SOD.

These results present endothelial inflammatory oxidative stress as a new mechanism that connects early valve pathology with later stages of degeneration. Targeting these mechanisms via tailored antioxidant therapy could provide new avenues for treatment of CAVD.

C13

Characterization of Sex-Related Differences in Valvular Interstitial Cell Response to Transforming Growth Factor-beta1

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Male sex is a significant risk factor for developing calcific aortic valve disease (CAVD), but this phenomenon has not previously been investigated on the cellular scale. Cellular-scale sex-related differences have been noted in other cardiovascular conditions, leading us to investigate whether male and female valvular interstitial cells (VICs) exhibit differential behavior in the context of CAVD. VIC cultures have not previously been separated by sex, and in vitro evaluation revealed intrinsically higher apoptosis, proliferation, and α SMA expression in male porcine VICs compared to female VICs; all of these markers are associated with CAVD pathobiology. Additionally, male VICs were significantly more sensitive to treatment with TGF-beta1, an inflammatory cytokine that plays a key role in CAVD progression. For instance, in male VICs, α SMA was significantly increased by TGF-beta1 and decreased by a neutralizing antibody to TGF-beta1, while female VICs were unresponsive to both treatments; endogenous TGF-beta1 production was similar across sexes. Expression of alkaline phosphatase was significantly decreased upon TGF-beta1 treatment of male VICs, while female VICs were unresponsive. Male VICs expressed higher levels of TGF-beta receptor 2, which may be one mechanism by which males exhibit increased sensitivity to this stimulus. Moreover, equivalent levels of phosphorylated Smad2 but increased p38 MAPK phosphorylation in male VIC cultures indicate involvement of the non-canonical TGF-beta1 signaling pathway in these sex-related differences. The finding that male and female VICs exhibit significant cellular-scale differences, particularly in their responsiveness to pro-disease stimuli, has the potential to substantially impact our characterization of CAVD etiology and possible treatments.

C14

Insights into CAVD Pathobiology and Relationship to Atherosclerosis Provided by Familial Hypercholesterolemic Swine

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Familial hypercholesterolemia (FH) is a prevalent hereditary disease associated with coronary artery disease and increased risk of calcific aortic valve disease (CAVD). The Rapacz familial hypercholesterolemic (RFH) swine is a well-established model of human FH and atherosclerosis in general, but has not been studied in the context of CAVD. We hypothesized that these unique animals may provide insight into CAVD pathobiology and its relationship to atherosclerosis. Coronary arteries and aortic valves from wild-type and RFH swine (0.25, 1, 2, and 3 year-old) were characterized across multiple scales and found to possess complex atherosclerotic lesions and hallmarks of early-stage human CAVD, respectively. Adult RFH swine exhibited significant leaflet thickening, increased lipid oxidation, macrophage infiltration, and extensive extracellular matrix remodeling, including proteoglycan enrichment, collagen disorganization, and elastin fragmentation. Intracardiac echocardiography showed leaflet thickening in adult RFH aortic valves, and valve function ranged from unimpaired to mildly sclerotic. Microarray analysis of adult and juvenile RFH valves revealed significant upregulation of inflammation-related genes and several commonalities with atherosclerosis; pathway analysis also showed substantial overlap of the RFH model with previous microarray comparisons of healthy vs. diseased human valves. Overall, adult RFH swine exhibited several hallmarks of early human CAVD that have not previously been demonstrated in other animal models, and may help to elucidate CAVD etiology in both FH and non-FH individuals. The development of advanced atherosclerotic lesions but only early-stage CAVD in RFH swine also support the hypothesis of an initial shared disease process, with additional stimulation or co-morbidities necessary for further progression.

C15

Deficiency Of C-Type Natriuretic Peptide Signaling Promotes Aortic Stenosis, Left Ventricular Dysfunction, And Bicuspid Aortic Valves In Mice

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C-type natriuretic peptide (CNP), which signals through the particulate guanylate cyclase receptor NPR2, is elevated in disease-protected regions of the aortic valve and reduced in stenotic valves. In vitro, CNP inhibits valvular cell myofibro/osteoblastogenesis, yet its functional impact in vivo is unknown.

Male *Npr2*^{+/-} mice and WT littermates were aged for 4 or 8 months (n = 9-15/group). Valve/cardiac function was assessed by echocardiography, and valve matrix composition/morphology by Movat's.

4-month *Npr2*^{+/-} mice developed deficits in aortic valve function, with elevated transvalvular velocity (normalized to ejection fraction; V/EF) (+30.7% vs. WT, p < 0.01). V/EF was further increased in 8-month *Npr2*^{+/-} mice vs. WT (+44.4%, p < 0.001). This was accompanied by impaired systolic function in 8-month *Npr2*^{+/-} mice: EF, fractional shortening, and left ventricular outflow tract velocity were reduced by 22.0%, 26.9%, and 18.5%, respectively, vs. all others (p < 0.001). *Npr2*^{+/-} leaflets were thickened, rich in proteoglycans, and developed collagenous remodeling of the attachment region. 9.5% of *Npr2*^{+/-} mice have congenital bicuspid aortic valves (BAVs) (none in WT), with the worst valve function (V/EF increased by 70.0% vs. tricuspid *Npr2*^{+/-} mice, p < 0.01), flow disturbances, most severe leaflet proteoglycan accumulation, fibrosis, and evidence of calcification.

Thus, impaired CNP signaling produces valvular/LV dysfunction and increases BAV incidence. These findings reveal a functional role for CNP in the aortic valve, suggest therapeutic strategies, and establish a novel BAV mouse model. They also broadly implicate guanylate cyclase family signaling in valvular development, complementing evidence from other BAV mutant mouse models.

C16

Pharmacological Antagonist Of Serotonin Receptor 2A Reduces The Pathological Activation of Human Mitral Valve Interstitial Cells: Implication For Myxomatous Mitral Valve Disease

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Myxomatous mitral valve disease (MMVD) is a pathological condition resulting in mitral regurgitation (MR) and is only treated surgically at present. Increasing evidence suggests that mitral valve interstitial cells (MVICs) play a critical role in the pathological remodeling of the MV. Serotonin (5HT) is a neurotransmitter, and if present at increased levels can cause a valvulopathy. This suggests that 5HT-receptor (5HTR) signaling plays a physiologic role, and that increased 5HTR activity could mediate the pathophysiology of valve disease. Here we test the hypothesis that 5HTR antagonism can alter the pathological prolapse of the MV *in vitro* and *ex vivo*.

Using microarray (N=4/group) and histological analysis on human specimens we show upregulation of serotonin receptors (5HR) 2A and 2B on MMVD patients vs. controls (+12.5 and +28.4 folds, respectively; p-value <0.001) Immunological analysis on surgically explanted tissues show pathological remodeling of the MV leaflets (increased thickness and ECM accumulation) with concomitant activation of 5HT signaling and MVIC activation (5HTR2A+, 5HTR2B+, OPN+, SMA+, RUNX2+). *In vitro* assay on human isolated MVICs (6 days), as well as *ex-vivo* biomechanical testing of human MV (6 days at 15% and 1 Hz using a uniaxial tensile bioreactor), were performed with results showing that VICs activation is associated with 5HTR activation and that 100 nm Ketanserin (5HTR1A antagonist) reverts these effects.

It is concluded that the pathological remodeling of the MV in the setting of myxomatous prolapse is associated with 5HT signaling. These results could have important implications for testing therapeutic candidates based on 5HT antagonism.

C17

Effect Of Mitral Regurgitation Severity And Extent Of Leaflet Tethering On Leaflet Remodeling In Ischemic Mitral Regurgitation

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Mitral leaflet remodeling, i.e. its expansion and fibrosis has been recently demonstrated in ischemic heart disease(IHD), and the leaflet remodeling capacity has been attributed to chronic leaflet tethering. In our study, we compared the extent of leaflet remodeling in human patients with IHD , and compared against patients with aortic stenosis(AS) with similar-sized ventricles and valve tenting. Cardiac gated phase contrast MRI was performed in 76 humans with ischemic heart disease with mitral regurgitation (MR severities: mild-45; severe-31) and compared with 10 humans with severe aortic stenosis with equivalent dilated ventricles. Left ventricular volume (LVV), mitral regurgitant volume (MRV), tenting area (TA) were measured in either groups, and leaflet remodeling was quantified as diastolic anterior and posterior leaflet lengths (ALL,PLL) normalized by the body surface area. LVV did not differ between patients with IHD and AS. In IHD patients, ALL and PLL increased significantly from mild MR to severe MR. A 25% increase in ALL from 12.5 ± 3.3 mm/m² in mild MR to 15.6 ± 3.4 mm/m² to severe MR ($p=0.004$) was measured, while a 42% increase in PLL was measured ($p<0.001$). Both ALL and PLL has a significant correlation with MRV (ALL: $r=0.37$, $P=0.001$; PLL: $r=0.42$, $P=0.0002$), and TA. AS patients with identical LV size, but without an infarction or regurgitation had shorter leaflets as compared to MR patients. Leaflet remodeling is an active process in IHD patients, and seemed to rely on the presence of mitral regurgitation and/or a myocardial infarction.

C18

Filamin-A Mutations Causing Mitral Valve Prolapse Alter Integrin Expression And Cellular Mechanotransduction

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Mutations in the gene encoding Filamin-A, a cytoskeletal protein, were identified in patients with familial mitral valve prolapse(MVP). In this study, we sought to investigate the impact of these mutations on cultured cells in comparison with wild type Filamin-A, to delineate the mechanisms underlying MVP.

Four immortal cell lines were established - Filamin-A KO, Filamin-A WT, G288R mutated Filamin-A, and P637Q mutated Filamin-A. Cells were cultured on plastic petri-dishes for a week, and at confluence the cells were imaged under a microscope, then their 3D morphology and mechanical stiffness measured with scanning ion conductance microscopy, and then the cells were assayed for integrins and counted using flow cytometry.

Filamin-A KO cells developed blebs on the surface, were flat and did not sufficiently attach to the cell culture substrate, while WT cells demonstrated excellent attachment and formed extensive lamellapodi and filipodia. Both G288R and P637Q mutated cells had reduced extensions, with a cell structure that is small in circumference but large in height. $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 5$, $\beta 1$, $\beta 3$, $\beta 4$, $\alpha 5\beta 3$, $\alpha 2\beta 1$ integrin expression was drastically reduced in both G288R and P637Q mutations, compared to WT. A 24 hour substrate attachment assay demonstrated 30% attachment of G288R cells were attached and viable, 50% of the P637Q cells were attached and viable, and 98% of WT cells were attached and viable, with similar results seen in gel contraction studies.

Filamin-A mutations associated with mitral valve prolapse seem to alter fundamental cellular mechanisms essential for cell attachment to matrix, motility and mechanotransduction.

C19

Paracrine TGF- β Signaling Amplifies Mechanical Force-Regulated Pulmonary Valve Leaflet Gene Expression

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The extracellular matrix (ECM) determines multiple aspects of tissue biology. Greater understanding of ECM as an extracellular source of cytokines mediating paracrine regulation of tissue function will better inform tissue engineering strategies for replicating ECM biology. During response to injury, extracellular latent TGF- β is activated and released to mediate remodeling in many tissues. To understand how mechanical forces regulate leaflet homeostasis, we study responses of native heart valves to chronic changes in flow. Using an ex vivo model in which multiple rat pulmonary valves are cultured 7-days together under flow (cycling) or static (non-cycling) conditions, we find that leaflets remodel in response to a chronic change in flow, accompanied by marked changes in the expression of multiple genes. Intriguingly however, we observed that TGF- β gene expression was unchanged between flow and static conditions. To investigate whether activation of latent TGF- β modifies gene expression changes induced by altered mechanical force in leaflets, valves were cultured in the presence of the TGF- β -Receptor-1/Alk-5 kinase inhibitor LY2157299. We found that the expression of genes highly regulated (4 to 16-fold) in response to chronically altered flow was markedly reduced by LY2157299 treatment: $63 \pm 15\%$ (mean decrease \pm SD; n=8) from the non-drug fold-change in gene expression. The effects of TGF- β receptor signaling inhibition on force-regulated gene expression demonstrate the presence of leaflet tissue-level paracrine TGF- β signaling. Amplification of mechano-sensitive gene expression by extracellular TGF- β reveals a robust and complex signaling network for the regulation of leaflet homeostasis, and demonstrates the critical importance of the ECM in directing valve biology.

C20

Role of Shear- and Side-dependent MicroRNAs in Aortic Valve Biology

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Aortic Valve (AV) experiences dynamic mechanical environment, which when altered can lead to inflammation and calcification, preferentially on fibrosa side. Using porcine AV, our *ex vivo* studies showed that altered-shear conditions induced inflammatory markers (ICAM-1, VCAM-1, BMP-4) preferentially on fibrosa. This shear- and side-dependent AV pathophysiology is attributable, in part, to the altered gene expression. MicroRNAs (miRs) are short noncoding RNAs that post-transcriptionally regulate gene expression and have been shown to regulate cardiac development and disease. Previously, our microarray studies on human AV endothelial cells showed that miRs-181a, 181b, 199a, and 214 responded differently to altered-shear conditions. It is hypothesized that these miRs are shear- and side-dependent and regulate AV pathophysiology by modulating inflammation, remodeling or cell death. For *ex vivo* studies, fresh porcine AV tissues (n=9) were cultured in a shear stress bioreactor for 48 hours. For altered-shear studies, fibrosa was exposed to unidirectional pulsatile (FL: 0-80dyne/cm²) shear stress and ventricularis was exposed to bidirectional oscillatory (VO: +/- 5dyne/cm²). For side-specific studies, fibrosa was also exposed to bidirectional oscillatory shear stress. Following shear, total RNA was isolated from 3 pooled samples and expression of miRs was determined by qPCR (n=3). Altered-shear (FL vs VO) significantly (p<0.05) upregulated miR-181a and downregulated miR-199a and 214, indicating these miRs are shear-sensitive. Also miR-181a was significantly (p<0.05) upregulated in fibrosa exposed to oscillatory shear compared to that of ventricularis, showing side-dependency. In summary, miR-181a, 199a and 214 are shear-sensitive but not miR-181b. MiR-181a is also side-specific which may regulate shear- and side-dependent AV pathophysiology.

C21

Influence of Aortic and Ventricular Shear Stresses on the stiffness of Valve Endothelial Cells

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Aortic valve endothelial cells (VECs) function in different hemodynamic environments. The influence of shear stress patterns on the biomechanical characteristics of VECs on each side of the valve has not been investigated. We assessed the relative expression of actin filaments and mechanical properties of VECs on intact porcine valve and cultured VECs which were exposed to aortic and ventricular shear stresses by a Cone-and-Plate machine. Immunofluorescent staining and western blot analysis of actin filaments revealed a great expression in VECs on the ventricular surface. Exposing the cells to the physiological or reverse-side pattern of flow did not influence the heterogeneous expression of actin on each side of the valve. By using the scanning ion conductance microscopy, measurements of membrane compliance of VECs demonstrated that VECs on the ventricular side ($0.020 \pm 0.001 \mu\text{m/kPa}$) were significantly stiffer than those on the aortic side ($0.032 \pm 0.002 \mu\text{m/kPa}$). Moreover, the relative differences in the mechanical properties of VEC on each side of the valve were not affected by varying the pattern of shear stress exposed to the VEC on either side of the valve. Under aortic and ventricular flow, membrane compliances of VECs on the ventricular side were maintained at $0.004\text{-}0.005 \mu\text{m/kPa}$, whereas those on the aortic side were retained at $0.011 \mu\text{m/kPa}$. This study has shown side-specific differences in the biomechanics of VECs that is independent of the forces generated across valve's surfaces. These findings further highlight the heterogeneous nature of VEC and may have important implications understanding how the valve functions in health and disease.

C22

Valve Interstitial Cell Remodeling Under Abnormal Mechanical Stress is Mediated by 5HT and FGF2 Signaling

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Altered remodeling is often an early indicator of valvular heart disease, however its link with valve mechanics or structure is not well understood. Our previous work demonstrated that 5HTR_{2A} and 5HTR_{2B} expression was upregulated when valve interstitial cells (VICs) experienced elevated stress. Immunohistochemistry of normal and calcified human valves showed that 5HTR_{2A}, 5HTR_{2B} and FGF2 were highly expressed in calcified cusps, while FGF1 was expressed in normal cusps. Taken together, we hypothesized that VICs respond to altered mechanical stress by changing their matrix remodeling activities and that this feedback is mediated via 5HT and FGF2 signaling in valve disease. In the current study, we forced single VICs to self-assemble into rectangles with 1:3, 1:5, 1:7 aspect ratio, to simulate 0%, 10%, 20% cyclic mechanical loading, respectively. After 48 hours, immunofluorescence showed strong expression of F-actin, FGF2, 5HTR_{2A}, 5HTR_{2B}, FGFR1 and FGFR2 at increased stretch. Interestingly, we observed the localization of FGFR1 and 5HTR_{2A} to actin cytoskeletal filaments while FGF2 localized to microtubule structures. We will quantify gene and protein expression in this model with the addition of serotonin and different combination of receptor inhibitors. Valves in a mouse model for hypertension and serotonin overload (3 weeks treatment) demonstrated increased leaflet thickening and thickness of collagen fibers as evidenced by picrosirius red staining. We expect our results to highlight the interplay between FGF and 5HT signaling in the progression of valvular heart disease and the key signaling molecules that can be targeted for early identification and treatment of stress-mediated valve disease.

C23

Peptide Amphiphiles Induced Collagen Production In Adipose Derived Stem Cells In Relation To Tissue Engineering Application

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A synthetic scaffold for heart valve tissue engineering offers a high level of reproducibility, low risk of infection and easy to tailored architecture such as mechanical properties and porosity. However a purely synthetic scaffold suffers a major drawback in that it offers little or no biomimetic surface to direct cell adhesion, migration and function. Biomimetic peptides are often simple, short peptide sequences that can exert bioactivity. Through computer modelling we have designed and validate different peptide amphiphiles (PA) tails that enhance coating ability of the PA onto a synthetic polymer, poly ϵ -Caprolactone (PCL), which is commonly used as a tissue engineered scaffold. The PA platform offers a single polypeptide chain that contains both polymer anchoring site and functional peptide region for a simple method to coat PCL scaffold with specific bioactivity. In this study, we use collagen stimulating peptide/peptide amphiphiles (PA) consisting of hydrophobic alkyl tails and hydrophilic head that covalently bind to the hydrophilic active peptide regions. Transmission scanning microscopy shows that the PA are able to self assemble into nanofibers. Cell studies show that the PA has low toxicity and enhances collagen production in adipose derived stem cells between 6-13%. Further development of PA that confer desirable cellular functions represents an exciting step toward the construction of intelligent scaffolds suitable for a range of tissue engineering applications including heart valves.

C24

A Collagen-Glycosaminoglycan-Fibrin Scaffold For Heart Valve Tissue Engineering Applications

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Non-coaptation of tissue-engineered heart valves (TEHVs) is due to cellular contractile forces and remodelling within the matrix, which act to decrease the overall size of the leaflets. We propose to address this problem by combining the advantages of fibrin gel as an autologous cell carrier material with the contraction-buffering capability of a freeze-dried, cross-linked collagen-glycosaminoglycan (CG) scaffold. The aim of the present study was to assess the contraction of the CG-fibrin scaffold (CGF) when seeded with human vascular smooth muscle cells (SMCs), and to develop a tri-leaflet TEHV scaffold using the same material. Disc-shaped CG scaffolds (n=4) were cast and subsequently crosslinked using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. Cells were seeded into the scaffold at a density of 3,000cells/mm³ using a fibrin carrier material. A control group consisted of cell-seeded fibrin without the CG backbone material. After 7 days of culture, fibrin-only scaffolds contracted to 10% of their original diameter, while the CGF scaffolds maintained their original diameter. PicoGreen assay on the CGF scaffold showed a significant increase in cell proliferation after 5 days, while live-dead staining revealed few dead cells. Masson's trichrome staining demonstrated that SMCs were homogenously distributed throughout the CGF scaffold and that CG and fibrin were still present after 7 days. Through the development of a mould and optimisation of freeze-drying and infiltration parameters, a tri-leaflet TEHV CGF scaffold was successfully produced. This CGF scaffold shows great potential for use as a HV scaffold due to its ability to maintain structural integrity and resist the contractile forces of seeded cells.

C25

Towards Designing Multifunctional Biomimetic Scaffolds for Tissue Engineering Heart Valves

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Bio-mimetic peptides are extremely useful to decorate bio-signals and to attract cardiac cells on scaffolds for tissue engineering (TE) heart valves. However, customizing the bio-mimetic peptides for cardiac TE and tailoring them to implant on the surface of the scaffolds require understanding of their structural properties such as stability and self-assembly. Here, we use computational bioengineering to model bio-mimetic peptides with unique design for collagen stimulation in TE construct and utilize molecular dynamics to study their structural properties. The modeling results signify that the studied bio-mimetic peptides form β -sheet for structural stability and self-assemble like twisted ribbons, in which, it is interesting to see the arrangement of collagen stimulation motifs as borders to attract cell receptors. The potential bio-mimetic peptides models are synthesised to examine under experimental conditions for ordered self-assembly, cell toxicity, collagen production and coating on polycaprolactone scaffold. These ongoing wet-lab experiments are essential to apply this novel design for TE heart valves. The structural basis from this study will be helpful to design a multifunctional bio-mimetic scaffold for TE heart valves and to attract different types of cells towards the scaffold.

C26

Surface-Immobilized LL37 Exhibits Mitogenic Effect On Endothelial Cells

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Scaffolding materials for tissue engineering should meet some basic requirements, e.g. being degradable, support cellular adhesion and provide adequate stimulation, i.e. growth factors (GFs). In this context immobilized GFs bear great potential and GF-mimicking peptides would be even more valuable. In this study we investigated the biological performance of LL37 - originally described as an antimicrobial peptide - as a VEGF-mimic.

LL37 was covalently grafted to a gold surface as a model substrate, either directly or via polyethyleneglycol-mediated (PEG) coupling. Proliferation of endothelial cells (ECs) was monitored using the Electric Cell-substrate Impedance Sensing (ECIS) system, based on label-free detection by resistance measurements.

PEG-immobilized LL37 was able to stimulate EC-proliferation over a period of 72 h whereas directly coupled peptide showed no activity. The mitogenic effect was comparable to soluble VEGF.

LL37 exhibits multiple biological functions of which the VEGF-mimicking ability is currently under investigation. Conjugation of this peptide might contribute to the vascularization problem of tissue engineering scaffolds while circumventing the application of entire GF proteins. In this way LL37 may add further biological features to cardiovascular tissue engineering constructs.

C27

In-Vitro Evaluation Of A Newly Developed Bio-Hybrid Tissue Engineered Aortic Valve Scaffold For TAVI-Application

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The aim of this study was the development and evaluation of a new cell seeded bio-hybrid aortic valve scaffold based on human origin and synthetic materials. Scaffolds (n=6) were manufactured using decellularized homograft leaflets and polyurethane patches that were sutured together on a conventional CoCr-Stent. Fibroblasts (FBs) and endothelial cells (ECs) were isolated from saphenous vein segments and expanded in culture. Scaffolds were seeded with FBs (7.5×10^5 cells/cm²), followed by static cultivation for 6 days. Subsequent EC-seeding (7.5×10^5 cells/cm²) was realised under the same conditions. Pre-conditioning was performed by an exposure to pulsatile low-flow (750 ml/min) for 5 days in a standard incubator at 37°C and 5% CO₂. Implantation was simulated by crimping the valve with an available crimping device followed by dilatation with a balloon catheter and finally exposure to higher flow conditions (1500 ml/min) for 2 days. Valve performance was documented by endoscopy. Samples were taken before and after each processing step, and analysed by scanning electron microscopy (SEM), immunohistochemical (IHC) and life-dead staining. SEM evaluation and IHC staining against TE-7 (FB-specific) and CD31 (EC-specific) proved the successful cell seeding process. Establishment of an extracellular matrix was detected by staining against collagen IV and fibronectin. The newly developed bio-hybrid scaffold showed excellent performance under endoscopic visualization and presented promising cell colonization characteristics, however, crimping and balloon dilatation induced significant cell damages. In summary, it seems that the crimping process has a big negative effect on biological tissue and must therefore be critically evaluated in further research studies.

C28

A New Single-Body Silicone-Polycarbonate-Urethane/PDMS Heart Valves: Material Characterization And Prototype Realization

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Polymeric heart valves (PHVs) have been investigated since 1960 but their success has been hampered by an overall lack of durability, due to leaflets calcification and thromboembolic complications. The aim of this work was to study a new elastomeric material for the development of a single-body PHV.

CarboSil[®] (CS) was dissolved in THF/DMAc 1:1 (v/v) to obtain a 2% (w/v) solution. CarboSil[®] solutions containing 10% (CS10) and 30% of PDMS (CS30) were obtained by a reaction under stirring and nitrogen flow for 6 h at 82 °C. For each materials planar patches were obtained by a spray, phase-inversion technique on a rotating cylindrical mandrel. In vitro tests were carried out to evaluate: bio/hemocompatibility, calcification, hydrolytic degradation, oxidative degradation, environmental stress cracking (ESC) degradation, and mechanical properties.

Materials extracts showed no cytotoxic effects on mouse fibroblasts respect to untreated cells. CS30 induced a lower in vitro platelets adhesion than the CS one. Coagulation times were similar for all tested materials. CS10 and CS30 showed less calcification spots compared to CS (SEM). IR demonstrated no significant differences among CS, CS10 e CS30 samples after hydrolytic degradation. CS30 was less susceptibility to oxidative degradation and to ESC. No mechanical differences were found between all materials. A single-body PHV prototype, with an incorporated stent, was successfully obtained by spray, phase-inversion technique with CS30.

CarboSil[®]-PDMS processed by the spray material deposition on a 3D mould, allowed the feasibility of a new low-cost and potentially long-term performing PHV.

C29

Off-the-shelf Decellularized Tissue-Engineered Heart Valves Repopulate and Remodel Rapidly in Pre-clinical Animal Models

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Tissue-engineered heart valves that contain living cells are associated with logistical hurdles. In contrast, decellularized xenografts and allografts lack growth and remodeling capacity and either risk immunogenic reactions and disease transmission or suffer from limited availability. Therefore, we recently introduced decellularized tissue-engineered heart valves (dTEHVs), based on biodegradable synthetic materials and homologous cells, as an alternative off-the-shelf valve replacement. Subsequently, we studied the functionality and host repopulation capacity of such dTEHVs in non-human primate and ovine models up to 24 weeks. Remarkably, the valve leaflets became rapidly repopulated with endogenous cells, starting within 5 hours post-OP. Excellent in-vivo performance of dTEHVs was demonstrated up to 8 weeks in the ovine model, where after mild to moderate insufficiency developed. The small valvular insufficiency was also detected in the non-human primates. Post-mortem the dTEHVs revealed mobile and thin leaflets, however, relative leaflet shortening was observed in the primates and beyond 8 weeks follow-up in sheep. Mechanical analyses of the ovine leaflets showed a trend towards increased anisotropic properties over time, resembling remodeling towards native-like valvular properties. Remodeling was confirmed by increasing collagen density, presence of elastic fibers, and endothelialized surfaces, starting in the valvular wall, going into the leaflets over time. To overcome the observed reduction in leaflet size, changes in valve geometry are necessary in order to improve physiological loading of the leaflets. Nevertheless, these homologous, non-immunogenic, off-the-shelf dTEHVs with good initial valve performance, rapid cellular repopulation and remodeling capacity have great potential as alternative to currently used valve replacements.

C30

Improved geometry of decellularized tissue engineered heart valves for future enhanced long term *in vivo* performance

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Currently used valvular prostheses lack growth potential. This is a major problem for young children that need staged interventions to accommodate growth of the valve with increasing risk of morbidity and mortality.

Driessen-Mol et al.^[1] demonstrated the unique regenerative capacity of decellularized tissue-engineered heart valves (DTEHVs) *in vivo*. Proper valve function was demonstrated up to 8 weeks, but was gradually lost toward 24 weeks. Loss in valve functionality was observed in accordance with increased host cell repopulation. Recent computational simulations by Loerakker et al.^[2] showed that when the current valve geometry was subjected to hemodynamic loading, it resulted in radial leaflet compression rather than the expected radial extension. Therefore, we hypothesize that contractile host cell repopulation in combination with hemodynamic leaflet compression, eventually may have resulted in leaflet shortening and valve insufficiency.

Based on the computational results, the valve geometry was adjusted to facilitate radial leaflet straining in diastole. Controlling DTEHV geometry has been challenging so far, because tissue compaction during culture always resulted in flattened leaflets. Therefore, a physical constraint was introduced in the bioreactor system to enforce curvature of the leaflets. During heart valve culture, cells compacted the leaflets around this insert, which guided the valvular geometry while allowing nutrient and oxygen supply to the valve. This resulted in excellent *in vitro* functionality of the valves, matching the exact imposed geometry.

[1] Driessen-Mol et al., *J Am Coll Cardiol.*, 2014

[2] Loerakker et al., *Journal of Biomechanics*, 2013

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C31

Tubular Heart Valves Fabricated From Decellularized Engineered Tissue Tubes

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Clinically available tissue valves commonly employ the use of chemically-fixed tissues that present minimal recellularization potential and are prone to calcification. Utilizing an allogeneic, decellularized engineered tissue valve could potentially satisfy these shortcomings by enabling tissue maintenance and homeostasis due to their high propensity for recellularization. We present here a novel tissue-engineered heart valve design based on a collapsing tissue tube (Cox et al, JTCS, 2005), using a decellularized engineered tissue tube. Tissue tubes with strong circumferential alignment were grown by fibroblast remodeling of fibrin gel over 5-7 weeks in vitro, including cyclic distension conditioning to promote collagen transcription and deposition, followed by decellularization. When such tubes grown from ovine fibroblasts were implanted for 6 months as interpositional grafts in the sheep femoral artery, they exhibited complete endothelialization and recellularization with no dilatation, narrowing, mineralization, or immune response.

When mounted on a custom frame (3-pronged crown), the tubular valves made from 22 mm diameter ovine tissue tubes performed well in pulmonary and aortic conditions of a pulse duplicator: >95% EOA, <5% regurgitant fraction, <3 mmHg systolic pressure drop. Pre-clinical testing of these valves is commencing. Performance of tubular valves made from human fibroblast-derived tissue tubes is compared; like the ovine tubes, the human tubes also possess mechanical strength / stiffness in the 5 MPa range and mechanical anisotropy.

A frameless version of this tubular heart valve that has potential for somatic growth and thus suitable for pediatric pulmonary applications is also presented, including pulse duplicator studies.

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C32

Influence Of Small Intestinal Submucosa (SIS) Configuration On Tricuspid Valve Remodeling After Three Months In An Ovine Model

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CorMatrix has designed a tricuspid valve from small intestine submucosa (SIS) with the ability to remodel into patient tissue. This design demonstrated excellent functional results two years post-implant in an ovine model. An earlier feasibility study with 2-ply SIS yielded better results with more rapid remodeling. Thus, the study objective was to evaluate this valve design using different SIS configurations. Valve function and remodeling were evaluated at three months (n=14). The valve was sutured into a cylindrical sleeve (30mmX30mm). SIS configurations included 2-ply, 2-ply pressed, and 4-ply. The native valve, including chordae, was excised, distal end of the SIS valve sutured to the papillary muscles, and proximal end sutured to the annulus. Echocardiography and angiography evaluated in vivo valve function. Histology included H&E, von Kossa, and Movat. Immunohistochemical staining with Vimentin and SMA identified interstitial cells, and vWF and eNOS identified endothelial cells. Echocardiography and angiography showed complete coaptation of the leaflets with mild/no regurgitation. Grossly, explanted valves appeared to be significantly remodeled at three months. The 2-ply valves had consistent cellular infiltration and confluent endothelial cell lining. The 4-ply valves contained microscopic intracuspal thrombus and less consistent or less advanced cellular infiltration. The 2-ply pressed configuration had excellent results in some valves, but microscopic intracuspal thrombus in others. These results demonstrate reproducible function of this valve design and influence of SIS configuration on valve remodeling. The 2-ply valve, which demonstrated excellent results in an earlier feasibility study, was superior to the 4-ply and 2-ply pressed configurations.

C33

Tissue-Engineered Heart Valves with a Tubular Leaflet Design for conventional and minimally invasive implantation

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Historically, tissue-engineered heart valves (TEHVs) have been designed to mimic the shape of the native valve to recreate the natural haemodynamics. This implies the fabrication of leaflets to ensure the unidirectional blood flow. However, the inadequate leaflets' functionality ultimately determined the failure of TEHVs in preclinical studies. Here we propose a tubular leaflet design as alternative to the classical design, abandoning the idea of mimicking the complex native leaflets' shape. Instead, a simple tubular tissue-engineered construct is sutured at three distinct sinotubular commissural points and along a circumferential line at the annulus level, so that it collapses inwardly and closes the conduit when exposed to diastolic backflow. The valve can be either implanted orthotopically in the aortic/pulmonary root by conventional surgical replacement or it can be sewn into a self-expandable nitinol stent for minimally invasive transcatheter delivery.

The valves were moulded in fibrin gel embedding human umbilical vein cells and reinforced by a warp-knitted textile mesh for mechanical stability. After three weeks of conditioning in bioreactors, the valves were fully functional with unobstructed opening and complete closure. Tissue analysis showed deposition of oriented collagen I and III and a confluent endothelial cell layer on the surface of the valves. The valves underwent crimping for 20 minutes to simulate the catheter based-delivery, with no effect on their functionality, on the extracellular matrix organization nor on the mechanical properties. These results show the potential of the tubular leaflet design as an attractive alternative to the conventional design of semilunar valve prostheses.

C34

Mechanical Conditioning Of Cell Seeded PCL Fibers Under Pulsatile Pressure

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Biological scaffolds have been assessed for tissue engineering purposes, however the lack of long-term stability due to degradation in some situations limits their use. Synthetic materials can overcome this issue by offering longer stability with slower degrading rates. We have previously developed a novel method to create synthetic anisotropic fibers that can promote cell proliferation and matrix production. This study aims to analyse the behaviour of the matrices in a dynamic environment where pulsatile pressure is applied. Poly ϵ -Caprolactone (PCL) aligned fibers made by using a jet spraying method were tested with and without human mesenchymal stem cells (hMSCs) using a pulsatile bioreactor. Aligned fibers are able to withstand pulsatile pressure with a gradient of 0-80 mm Hg for up to 3 weeks without ultrastructural damage as shown by scanning electron microscopy. hMSCs were dynamically seeded for 7 days within the fibres to ensure cell-spreading throughout the thickness as shown by DAPI staining of transverse sections. The seeded scaffolds were then moved into a bioreactor to test their durability. Pulsatile pressure was applied and maintained at a gradient of 0-80 mm Hg for a further 7 days to mimic aortic valve systemic conditions. Preliminary data show cells still present inside the scaffold after exposure to pressure synthesized more collagen than samples not exposed to pulsatile pressure. These results suggest that under systemic pulsatile pressure, jet sprayed anisotropic PCL fibres do not lose integrity and support cell function, proving to be good candidate for further studies toward valve tissue engineered constructs.

C35

Minimization of Human Allograft Immunogenicity by Ice-free

Cryopreservation

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Previously, we demonstrated *in vivo* that ice-free cryopreservation (IFC) reduced T cell infiltration heart valve leaflets compared with controls. We hypothesized that IFC human tissues will exhibit reduced allogeneic immune stimulatory properties compared with fresh untreated control grafts. Human engineered blood vessels (TEBVs) were generated by culturing smooth muscle cells on a polyglycolic acid scaffold, cryopreserved with an 83% formulation (DMSO, propanediol and formamide) and stored. Fresh and rewarmed, washed IFC TEBVs were compared by incubation with human peripheral blood mononuclear cells (hPBMCs) for 3 and 7 days. Proliferation was assessed by resazurin assay and cytokines by ELISA. Statistical significance was determined by t-test, $p < 0.05$. There was no stimulation of hPBMC proliferation by IFC TEBVs. Significant proliferation of hPBMCs occurred with fresh TEBVs. Significantly lower IL-6, IFN- γ , TNF- α and IL-10 release from IFC groups was observed compared with fresh tissue groups. IL-2, IL-12, and IL-18 release were not in either IFC or control TEBV groups. Additional culture controls with fresh and IFC tissues demonstrated that cytokines were not detectable without the presence of PBMCs. We conclude that ice-free cryopreservation of allogeneic TEBVs significantly reduces the hPBMC proliferative response and cytokine release (IL-6, IFN- γ , TNF- α and IL-10) compared with untreated, fresh TEBVs. The source of cytokines was the PBMCs not the allograft tissues. These results suggest that ice-free cryopreservation may be an improved method for processing and cryopreservation of allogeneic human heart valve and arterial tissues for surgical applications. Future studies will compare IFC with decellularization methods. NIH Grant #R43EB014614

C36

Histological Change of A Novel Autologous Tissue Engineered Heart Valve after Implantation

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It is not clear how tissue engineered materials for heart valvular substitutes change in histogenesis after implantation. We are developing a novel autologous aortic valve (Biovalve), using simple, safe and economical in-body tissue engineering which is based on a tissue encapsulation phenomenon in the living body. In this study, we investigated key factors in histogenesis when the Biovalve was implanted in a large animal model.

Biovalves were prepared by 2-month embedding of the plastic molds in the subcutaneous spaces of goats. After extracting the molds and removing the plastic rods only, Biovalves were constituted from completely autologous connective tissues. We combined them with a metallic stent and implanted in the aorta in situ with transcatheter valve implantation technique.

The Biovalve Stents were successfully implanted in the aortic position. They were extracted 1, 2 or 5 months after implantation. The leaflets of the Biovalve kept their shape and elasticity even after 5 months and neither calcification nor thrombi were observed. Histological examination showed the cell migration inside the Biovalves' body and laminar endothelialization on the surface of the valve leaflets even in 1 month after implantation. Their histogenesis have gradually advanced in 5 months, but were interrupted when the Biovalve tissues were not attached enough on the native vascular wall tissue.

the Biovalve satisfied the higher requirements of systemic circulation in goats for 5 months with the histogenic potential that was mainly induced by cell migration from the native vascular tissues contacting with the Biovalve.

C37

Dynamic Relationship between the Mitral Annulus and the Basal Left Ventricular Myocardium in the Beating Ovine Heart

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Mitral valve closure is preceded, during atrial systole, by reduced mitral annular (MA) size. This reduction requires little inward force from contracting atrial myocytes because the valve is open and left ventricular pressure (LVP) is low. But this annular size reduction, important to valve competence, is maintained by some inward-directed force during ventricular systole and the source of this force is not well understood. One possible candidate is contraction of the basal left ventricular myocardium pressing inward on the annulus which requires tight coupling between the MA and the basal LV myocardium. To test this possibility, MAseptal-MAlateral and MAseptal-LVlateral dimensions were computed from 4D marker data obtained every 16.7ms for 3 sequential beats in each of 12 ovine hearts. The group mean Pearson Correlation Coefficient between these dimensions for all data in all 12 hearts was 0.59 (range 0.04 to 0.80), thus the LV myocardium is not tightly coupled to the MA. But basal LV dynamics did have a precise relationship to MA dynamics. In late diastole, the MA moved inward while the LV base was relatively fixed; in systole, the MA was relatively fixed, while the LV base moved inward. During isovolumic relaxation, the MA moved outward as LVP fell, while the LV base was relatively fixed. Thus the inward force maintaining the annular size reduction is LVP acting on the posterior leaflets; the LV pulls back, not presses in, on the annulus. Consideration of this elegant mechanism may be important to the design of tissue-engineered valves.

C38

Canonical and Non-canonical TGF β Inhibition Differentially Regulate Matrix Homeostasis in Porcine Aortic Valve Interstitial Cells

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Misexpression of collagens and glycosaminoglycans (GAGs) is a hallmark of aortic valve disease (AVD), and is frequently associated with TGF β pathway abnormalities. We investigated the effects of exogenous TGF β , pathway inhibition (Smad/canonical and ERK1/2/non-canonical), aging, and stretch, on aortic valve interstitial cells (VICs) regulation of ECM content and specifically, homeostasis of GAG hyaluronan (HA). VIC cultures from suckling (1-3 month old) and aged (2 year old) pigs were treated with TGF β \pm canonical (SB431542) and non-canonical (U0126) pathway inhibitors for 48hrs, under 0% or 5% cyclic strain. Secreted collagen, GAGs, and HA were quantified using biochemical/electrophoresis assays. Expression of α SMA (VIC activation marker) was assessed using RT-PCR. In suckling VICs, TGF β elevated GAG synthesis and reduced collagen synthesis. In aged VICs, both collagen and GAGs increased with TGF β addition in 0% and 5% strain conditions. Both inhibitors rescued collagen levels but not GAG levels, with or without TGF β , for suckling VICs but not for aged VICs. TGF β activated suckling VICs; quiescence was partially restored with U0126, but not SB431542. Neither inhibitor rescued cell phenotype for aged VICs. TGF β increased HA secretion 3-5X into culture medium and 10X within the cell layer; SB431542 prevented HA retention in the cell layer but HA secretion into the medium was unaffected. Results suggest differential VIC regulation of ECM due to TGF β and its inhibitors with biomechanical stimulation. We speculate inhibition of ERK1/2 activation reverses the defective regulation of valve ECM homeostasis, particularly HA, early on, and reduces the potential for AVD later in life.

C39

A Computational Approach For In Situ Estimation Of Aortic Valve Interstitial Cell Mechanical State From Tissue Level Measurements

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We hypothesize that deriving the biomechanical state of AVICs in-situ using an inverse modeling approach will reveal more accurate information regarding AVIC adaptations to various stimuli. To achieve this, a novel, integrated numerical/experimental methodology was developed to estimate AVIC mechanobiological state in-situ. Flexural deformations of intact AV leaflet specimens were used to quantify the effects of AVIC stiffness and contraction at the tissue level. Next, we incorporated tissue micromorphology in a down-scale framework that simulates AVIC-Extracellular matrix (ECM) interactions as a function of layer location. AVIC size, shape, distribution and orientation were quantified from histological data from all three layers. A representative volumetric element was optimized statistically for each layer to represent the native 3D structure. The optimized RVE size in each layer defined a single mesh element within the macro model. Cellular inclusions and surrounding ECM were defined as neo-Hookean materials. Experimental flexure data from three different activation states (thapsigargin, basal tonus and hypertensive) were used in conjunction with the model to probe the effects of AVIC stiffness and contraction on the tissue level response. The computed differences between the computed macro and micro stress was attributed to cell stiffness and contractile changes. This numerical/experimental methodology was used to deduce AVIC properties under various pathophysiological conditions. We are currently using the desired approach to the effects of statins.

C40

Mimicking Heart Valve Disease In An Ex Vivo Flow Model

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Heart valve disease is a major cause of mortality and morbidity in the Western population. More than 25% above the age of 65 suffers from a form of heart valve disease. The only current therapy is the replacement of the diseased valve with a prosthetic valve. Little is known about the cellular and molecular mechanisms underlying the initiation and progression of heart valve disease. This is mainly due to the absence of a heart valve disease model, which allows manipulation of the environmental conditions of the valve in its normal position in the heart. We have adapted the existing Miniature Tissue Culture System (Lieber et al., 2010) and created culture conditions supporting the initiation and progression of heart valve disease in the mouse aortic and mitral valve. Thickening of the leaflets, proliferation and activation of the valvular interstitial cells, disruption of the extracellular matrix organization are observed. In addition, we have findings that suggest the endothelial to mesenchymal transformation of the endothelial cells of the valves. This model allows the modification of the hemodynamic, chemical and metabolic conditions, which are main determinants of the valvular organization, and therefore, could provide major insights in the mechanisms underlying heart valve disease.

C41

Bicuspid Aortic Valve: Determining Susceptibility for Dilation of the Ascending Aorta by Histopathology

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Bicuspid aortic valve (BAV) is often complicated by thoracic aortic dilation. Since aortic diameter as a criterium for surgery is only decisive at population level, it would be valuable to have tailored risk stratification at patient level. Therefore, we investigated structural differences between the aortic wall of BAV and tricuspid aortic valve (TAV) and identified immunohistochemical markers predictive of aorta dilation in BAV.

Biopsies of the ascending aorta of BAV (n=36) and TAV (n=23) both without (BA, TA) and with (BAD, TAD) dilation were compared. Differentiating and mature vascular smooth muscle cells, lamin A/C, and progerin were studied immunohistochemically and with western blot. Further an activation cascade was investigated, featuring c-Kit, phosphorylated c-Kit (pc-Kit), mmp9, Hif1alpha and eNOS, to unravel possible differences within the BA group.

BAVs showed significantly less expression of differentiated smooth muscle cells: α SMA, SM22 α , calponin and smoothelin as compared to TAVs. Lamin A/C and progerin expression was significantly lower in BAV than TAV ($p < 0.05$). Progerin expression increased significantly in TAD as compared to TA. The activation cascade with pc-Kit as a keyfactor, was present both in a subset of patients with BA and BAD.

Our study showed that the aortic wall with BAV is less well differentiated as compared to TAV. Phosphorylation of c-Kit plays a crucial role in aortic dilation in BAV rather than inflammation and accelerated aging seen in TAV. For the first time, a subgroup is distinguished in BAV patients without aorta dilation suggestive of a different state of vulnerability for future aortopathy.

C42

Second Harmonic Generation Microscopy For Analysis Of Collagen Fiber Alterations In Calcific Aortic Valve Disease

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Extracellular matrix (ECM) remodeling is one of the earliest hallmarks in the development of calcific aortic valve disease (CAVD). However, traditional histochemical analysis does not provide information about higher-order ECM structure, such as collagen fiber organization. Characterizing the dynamic behavior of these structures is important for not only understanding disease-related changes in valve architecture and its impact on valve mechanics, but also for potentially developing imaging approaches that allow earlier CAVD detection. We performed confocal second harmonic generation microscopy (SHG) on aortic valves from healthy and diseased humans and found numerous changes in collagen fiber architecture that were visible using SHG but not detectable via histochemistry. In contrast to the collagen-sparse spongiosa in healthy valves, diseased valves possessed randomly-aligned collagen fibers throughout the leaflet mid-section, with a sudden transition to tightly and uniformly aligned collagen fibers in the fibrosa. Despite similar density, the fiber directionality in the diseased fibrosa was substantially different from that in the healthy fibrosa. Analysis of forward-scatter SHG intensities, forward/backward scatter ratio, and Monte Carlo modeling of the SHG data revealed additional differences in collagen fiber density, length, diameter, and crimping at different disease stages. Interestingly, there was low standard deviation of collagen organization patterns within both the healthy and diseased valve groups, which was unexpected given the heterogeneity of CAVD. This study advances our understanding of ECM remodeling in CAVD, and may provide a basis for use of SHG in the detection or evaluation of CAVD progression utilizing real-time in vivo SHG techniques.

C43

Comparative *in vivo* analysis of ovine tissue-engineered heart valves re-endothelialized with ECs derived either from umbilical cord blood or peripheral blood

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Dezellularized valve allografts re-endothelialized with autologous endothelial cells (EC) from vessels or differentiated from peripheral blood have shown promising results. To circumvent this invasive, respectively, low-success harvest of EC, we utilized autologous umbilical cord blood EPC derived EC for re-endothelialization of ovine decellularized pulmonary valves (UCBEC-DPV) and compared them to those re-endothelialized with peripheral blood derived EC (PBEC-DPV). UCBEC-DPV and PBEC-DPV (n=6, each) were implanted orthotopically in sheep (Ø 72 kg, age 2 y). Umbilical cord blood was kept frozen until EPC isolation for re-endothelialization. Valve function was investigated at implantation and at explantation 8 months *post OP* by echocardiography. Cusps' surface cell coverage was analysed by phalloidin stain. Cellular repopulation and matrix integrity was analysed by histological stainings, while the identity of cells in the matrix was characterized by immunological staining.

One animal (UCBEC-DPV) did not recover from anaesthesia. No valve related problems occurred during implantation period. Valves exhibited no insufficiencies, cusps were thin and translucent at explantation time. Cusp surfaces showed no significant difference concerning cell coverage among groups. In both groups matrix repopulation was mainly limited to ventricular sides of cusps and to adventitial sides of valvular walls. Cells covering cusps revealed expression of vWF, eNOS, sm- α -actin and sm heavy chain 2.

Re-endothelialization with ECs from both sources showed to be equally beneficial for function and repopulation of valves with host cells. As no cell type revealed to be superior, cell source could be chosen individually from case to case.

C44

Expression and activity of extracellular ectoenzymes (CD39 and CD73) in aortic valves cells and in endothelial cells under the shear stress conditions.

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Extracellular nucleotides have major impact on thrombosis, inflammation and immune responses, but their expression and activity in valve is poorly studied. Normal aortic valves and calcified valves were analyzed for ectonucleoside triphosphate diphosphohydrolase 1 (CD39) and ecto-5'-nucleotidase (CD73) gene expression and immunocytochemical localisation. Nucleotide metabolism was characterised by incubation with AMP and ATP in both cultured human aortic valve endothelial (VECs) and interstitial cells (VICs). In addition, the influence of ventricular and aortic side flow patterns on CD39 and CD73 was assessed. CD39 was highly expressed in VECs and VICs with reduced levels of expression in calcified valves. In contrast CD73 was more highly expressed in calcified regions of the valves. When the VECs were exposed to their physiological pattern of flow the activity of CD39 and CD73 increased significantly ($p < 0.05$). In conclusion, ventricular and aortic flow could play an important role in ectoenzyme expression in endothelial cells. The presence of CD39 and CD73 in valves suggests that valve have the ability to degrade extracellular nucleotides, which may contribute to the protective effect of their degradation product adenosine against inflammation and calcification.

C45

Reoperation Rate After The Ross Procedure Compared To Bioprostheses In Different Age Groups - A Report From The German Ross Registry

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An increasing number of young patients are choosing bioprostheses for aortic valve replacement. In this context the Ross operation deserves renewed consideration as an alternative biological substitute.

Reoperation rates after the Ross procedure and after bioprosthetic AVR, however remain a concern in these patients.

We report freedom from reoperation after the Ross procedure and bioprostheses for different age groups.

The reoperation rates of 1.925 patients (mean age 41.2 ± 15.3 years, 1.444 male) from the German Ross registry with a mean follow-up of 7.4 ± 4.7 years (range 0.00 - 18.51 years, 12866.6 patient years) were analyzed and compared to bioprostheses in three age groups (Group I < 40 years, Group II 40-60 years, Group III > 60 years).

At 10 (resp. 15) years of follow-up freedom from reoperation was as follows: Ross Group I 83% (85%), Ross group II 90% (90%) and Ross group III 81% (81%). Bioprostheses had the following results: Group I 51% (35%), Group II 63% (25%) and Group III 92% (81%).

There is some evidence that at least in the first 10 and 15 years after aortic valve replacement the Ross procedure provides a lower reoperation rate in younger and middle-aged patients compared to patients with bioprostheses. This may be of interest to patients' or physicians' for decision making for aortic valve surgery.

C46

Decellularized Valves For RVOT Reconstruction During The Ross Operation - Should Cryopreservation Be Abandoned?

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Evaluate the mid-term clinical and echocardiographic results of fresh decellularized versus cryopreserved decellularized pulmonary allografts for RVOT during the Ross operation.

Between 2005 and 2013, 166 Ross operations were performed using SDS decellularized pulmonary valve allografts. Of these, 124 were fresh decellularized allografts (mean age = 30±15 years) (Group 1) and 42 were cryopreserved decellularized allografts (mean age= 34±11 years) (Group 2). Conduit dysfunction was defined as any peak gradient > 40 mmHg or insufficiency grade III or IV. Comparisons were made with Kaplan Meier survival estimate and the mean of peak gradients over time by the unbalanced two way repeated measures ANOVA.

Peak gradients at hospital discharge (Group 1 = 14±5 mmHg and Group 2 = 10±5mmHg) and at the latest follow-up (Group 1 = 14±5 mmHg and Group 2= 17±13 mmHg) were similar between groups (p=0,78). Peak gradient >40mmHg was never present in Group 1 but in 3 patients in Group 2. Moderate or severe regurgitation was present in one case of each group. Freedom from conduit dysfunction at 6 years was significantly better in Group 1 (97% - CL95%, 85-99%) versus Group 2 (85% - CL95%, 78-90%) (p=0,014) at six years. Reoperation due to SVD was needed in only one patient in Group 2.

Fresh decellularized allografts were associated with better medium term results when compared to cryopreserved decellularized allografts for RVOT reconstruction up to six years of follow-up. These results indicate that cryopreservation is unnecessary and may be even detrimental during the processing of decellularized allografts.

C47

A multicenter evaluation of the autograft procedure for young patients undergoing aortic valve replacement: Results from the German Ross Registry

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Conventional aortic valve replacement(AVR) in the young, active patient represents a suboptimal solution, in terms of long term survival and quality of life. Aim of the present work is to present our multicenter results with the pulmonary autograft in young patient undergoing aortic valve replacement.

Between 1992-2013, 1967 patients (1786 adults;41.6±15.1 years) underwentAVR with the pulmonary autograft principle in 9 centers. All patients underwent prospective clinical and echocardiographic examinations, annually. Mean follow-up was 7.7±4.8 years (range 0-23) with a total cumulative follow-up of 14571 years with 635 patients having a follow-up of at least 10 years.

In-hospital mortality was 1.37%(n=26). Late survival of the adult population was comparable to the age and gender matched general population (observed deaths:90, expected deaths:70; p=0.439).

Freedom from autograft reoperation at 5, 10, and 15 years was 97.8%, 95.9% and 89.6% respectively whereas freedom from homograft reoperation was 98.4%, 96.6% and 94.6% respectively. Overall freedom from reoperation was 96.2%, 92.8% and 86.2% respectively.

Longitudinal modeling of functional valve characteristics revealed a low(<5%) probability of a patient being in higher autograft regurgitation grades throughout the first decade.

For the young, active patient requiring aortic valve replacement, the autograft principle results in postoperative long-term survival comparable to that of the age and gender matched general population and reoperation rates within the 1% / patient*year boundaries. The autograft principle for the treatment of the aortic valve disease in young, active patients who want to avoid the shortcomings of conventional prostheses should be strongly considered.

C48

An Assessment of Shape and Function of the Pulmonary Autograft 10 or more Years after the Ross Procedure

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There is continuing concern about the risk of long-term autograft dilatation following the Ross procedure. The objective of this study was to characterise the shape and function of neo-aortic root both in dilated and nondilated states.

Among 108 Ross patients undergoing yearly echocardiographic assessment in our unit for 16.5 ± 1.9 years after operation (range 13 - 20), 11 patients with dilated autografts (DG, $>45\text{mm}$, age 47 ± 10) and 10 patients with non-dilated autografts (NDG, $<45\text{mm}$, age 59 ± 7) were consented for cardiac multi-slice cine CT. Dimensions and distensibility of the aortic root were derived from the cine-CT images, and were compared with 11 age-matched normal controls (age 47 ± 9). The aortic valve function was also analysed by echocardiography.

Additionally to the difference in the sinus diameters between DG, NDG and C (50 ± 3 mm vs 41 ± 3 mm vs 31 ± 4 mm, $p < 0.01$), the non-coronary sinus of the DG tended to have larger height than that of NDG and C groups. DG patients had similar distensibility compared to control (25 ± 14 vs 34 ± 14 MPa⁻¹, $p = 0.20$) whereas NDG patients showed trend towards lower distensibility (13 ± 7 MPa⁻¹). Echocardiographic data showed only 2 severe and 2 moderate regurgitations, with no clear correlation between regurgitation severity and sinus dimension.

These data support the notion that the autograft root has similar physical properties to the normal aortic root despite significant dilation, which was reflected in its haemodynamic function.

C49

Decellularized valve allografts combined with vascularized autologous small intestine for the reconstruction of the right ventricular outflow tract (RVOT)

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Infundibular stenosis occurring in “tetralogy of Fallot” has been found to be a very challenging abnormality to repair. We replaced the pulmonary valve with a combination of decellularized allogenic valve together with autologous vascularized matrix (= AutoVaM) as patch material to enlarge the RVOT.

RVOT reconstruction was performed in minipigs. A decellularized allogeneic valve conduit was used in place of the pulmonary valve and AutoVaM obtained from the small intestine with preserved vessels as a patch to enlarge the outflow tract. Control animals received non-vascularized autologous pericardium instead. Valve function was analysed by MRI. At explantation 3 and 6 months *post OP*, phalloidin was used to visualize cell coverage on the cusp surface. Repopulation and ECM integrity were examined by histological stainings and cell identity by immunofluorescence stainings.

Allograft developed more severe insufficiencies when combined with a pericardial patch as observed by MRI. The cell layer on the cusp did not cover more than $\frac{1}{4}$ of the ventricular surface at explantation. Matrix-repopulation with cells was moderate in the valvular wall and minimal in the cusps' base. Cells between valvular wall and cusps consisted of cells positive for vimentin and sm α -actin. Clusters of smooth muscle cells (sm α -actin and MYH11 positive) were still detectable 6 months *post OP* within AutoVaM patches but never in pericardial patches. Few Troponin-T-positive cells were found in the patches towards the ventricular anastomosis.

Clinical outcome and MRI analysis showed a better outcome of valve and heart function when allogeneic valves were combined with AutoVaM.

C50

Outcome Of Pregnancy After The Ross Operation - A Report From The German-Dutch Ross Registry

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Young women wishing to become pregnant are choosing Ross operation for aortic valve replacement as an alternative to mechanical or biological substitute. But there are limited data on maternal and perinatal outcome of pregnancy.

We report outcome of pregnancy after the Ross procedure.

42 Pregnancies among 31 women (mean age at Ross operation 25.0±6.1 years) with a mean follow-up of 10.5±4.8 years (range 2.5 - 22.4 years, no lost of FU, no death) were analyzed.

24 women gave birth to one child, 5 women to two, one woman to three and one woman to four children. One baby was a stillbirth. There have been five reoperations in three women (four homografts for SVD, one autograft for NSVD in combination with one homograft for SVD). Two out of these five reoperations have been after the birth of a child in two women due to SVD of the homograft in one patient and due to one autograft for NSVD in combination with one homograft for SVD in the other patient. Three reoperations have been in one patient before childbearing due to SVD of the homograft three times. In last FU after birth there has been non aortic valve-regurgitation or pulmonary valve regurgitation > II° in all women.

Ross procedure seems to be a possibility for uncomplicated and successful pregnancy in women with aortic valve disease.

C51

Haemodynamics in Anatomically Shaped Sinus Prostheses Analysed by 4D Flow MRI

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Valve-sparing aortic root prostheses with anatomically shaped sinuses ("sinus prosthesis", Uni-Graft® W SINUS, Braun) imply near-physiological conditions. Therefore, we sought to test their hemodynamics in comparison to straight grafts and volunteers by use of 4D flow MRI. Scans of 16 patients after David operation (13 sinus prostheses: "SP", 1f, 54±14y; 3 straight grafts: "SG", 1f, 51±13y) and 13 age-matched healthy volunteers ("Vol", 11f, 55±6y) were examined at 3T (Philips Achieva) after written informed consent. Using GTFlow (GyroTools, CH) data were visualized with streamlines and particle traces. Occurrence and magnitude of secondary flow patterns (vortices, helices) in the thoracic aorta and its sinuses were graded and correlated with aortic geometry.

Whereas sinus vortices in SP resembled those of Vol, SG revealed abnormally formed vortices aligned orthogonal to the vessel wall. Sinus vortices were graded small or medium in 91% (SP) and 98% (Vol) analysis of 6 datasets ongoing due to aliasing; In SG, 22% showed no sinus vortex, 78% only small vortices. Patients revealed more aortic secondary flow patterns than Vol (SP: 2.6±0.8, SG: 3.0±1.0, Vol: 1.4±0.8; p<0.05), potentially due to steeper postoperative curvature angle. Round aortic arch configuration predominated in Vol (11/13) compared to cubic (SP: 8/13, SG: 2/3) and gothic (SP: 3/13; SG: 1/3) forms in patients.

Near-physiological sinus flows in sinus prostheses is reassuring. The increased number of secondary flow patterns distal to prostheses, however, is hemodynamically of concern and should trigger follow-up studies and the discussion, whether curved grafts could mitigate those clearly abnormal blood flow patterns.

C52

Aortic Valve Dynamics After Valve-sparing Procedure With The New 'Sinus' Prosthesis Used For Valve Sparing Aortic Root Replacement

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Durability of aortic valve sparing root reconstruction depends on root dimensions and leaflet stress. A new "Sinus prosthesis" imitates the anatomical root considerations (Unigraft® W SINUS, B. Braun Melsungen AG, Germany). Aim of this study is the evaluation of aortic valve hemodynamics after valve sparing root reconstruction with this prosthesis.

In the first 38 patients (32 m/6 f; mean age 52 ± 13.1 years; range 24-70 years) who underwent valve sparing procedures (02/2009-04/2012) according to the David reimplantation technique using the new Sinus prosthesis (SP), aortic valve movement was examined echocardiographically and compared to 20 healthy controls (C). Opening and closing motion patterns as well as distensibility of the aortic root and function of the valve were studied.

Aortic valve opening and closing characteristics were as follows: rapid valve opening velocity was comparable between groups (SP: 40.9 ± 9.1 vs. C: 36.8 ± 21.9 cm/s; $p=0.24$), rapid valve closing velocity was faster in SP than in controls (26.4 ± 11.0 vs. 14.6 ± 13.9 cm/s; $p=0.002$). Percent change of diameter, as a parameter of distensibility, was comparable to controls at the level of annulus and sinutubular junction and lower at sinus level (3.2 ± 2.7 vs. $4.7 \pm 2.6\%$, $p=0.048$).

The new sinus prosthesis imitates the native sinuses with normal dimensions of the sinuses. Closing velocity of the cusps is faster after SP implantation than in native roots of controls. Distensibility is limited according to the material. The dynamic behavior of the AV after root reconstruction with the SP is not completely physiological yet. However, mid-term results of valve function are suitable. The aim should be the development of a more elastic material for this prosthesis.

C53

In Vitro Analysis of The Alterations Produced by Transcatheter Aortic Valve in The Valsalva Sinuses

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Transcatheter Aortic Valve Implantation (TAVI) has rapidly become the preferred solution for high-risk patients. However, despite the excellent short and medium term results, this approach has recently been associated with high rates of ischemic lesions and dementia. In most cases, these cannot be directly correlated with catheter manipulation during implantation, or with the severity of calcification. Hence, the source of the complications is reasonably to be sought in the flow perturbations produced by transcatheter valves. In fact, contrary to surgical solutions, TAVI devices are positioned inside the leaflets of the diseased valve, which are widened into the sinuses of Valsalva, producing alterations of the physiological hemodynamics.

In this work, in order to gain a better understanding of the fluid dynamic perturbations introduced by transcatheter valves, particle image velocimetry was used to measure the flow in a mock aortic root made of transparent silicone. To isolate the effects of transcatheter valves, the operating valve was encased in a transparent cylindrical wall, mimicking the expanded native leaflets after TAVI. The experiments were carried out in a pulse duplicator, under physiological conditions, using a fluid solution providing optimum refractive index matching with the root and dynamic viscosity similar to blood.

Direct comparison of the mean flow dynamics indicated that TAVI produces stagnation regions at the bottom of the Valsalva sinuses, which may promote thrombus formation and contribute to ischemic lesions. The study also provides accurate estimates of local shear and dissipative rates and turbulence levels, that are typically associated to blood damage.

C54

Aortic valve replacement with decellularized aortic allografts: First clinical results

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The use of decellularized valved allografts shows promising results in replacement of the pulmonary valve. Here, we report about our clinical results of aortic valve replacement using decellularized aortic homografts (DAH) in children and adults. From February 2008 to February 2014, 34 patients with a mean age of 23 years (range, 0.19 to 65) were prospectively assessed after aortic valve replacement using DAH. Mean time from homograft harvesting to implantation of DAH was 46 days (range, 25 to 69). The mean DAH diameter was 23 mm (range, 10 to 27). Mean extracorporeal circulation and aortic cross-clamp time was 211 and 120 minutes respectively (range, 113 to 397 and 64 to 180) using DAH for aortic root replacement, including coronary transfer. There was one early, none conduit related, death (3,5%). Echocardiography and magnetic resonance imaging were used for postoperative evaluation. None of the patients required re-intervention or conduit explantation so far. In two patients (7.41%) a moderate aortic valve insufficiency was seen, no severe valve stenosis was observed so far. No dilatation of the aortic root was seen, however the follow up is short so far (45 years in total, mean 1.5 yrs., max. 6 yrs). DAHs satisfy the requirements of the systemic circulation. For selected patients, DAH may represent an interesting alternative to conventional other grafts.

C55

Patient-Specific Finite Element Modeling of Ascending Thoracic Aortic Aneurysm Using In Vivo Magnetic Resonance Imaging

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Elective ascending thoracic aneurysm (aTAA) repair is based on family history, connective tissue disorder, growth rate, but primarily size. Rupture/dissection carries a high mortality and occurs in many patients who had not met size criteria for surgical repair. Wall stress may be better predictor than size for adverse events, but cannot be directly measured *in vivo*, rather determined from finite element analyses (FEA). Current FEA models are fraught with assumptions that limit result accuracy. The purpose of this study was to develop the first patient-specific aTAA model using *in vivo* magnetic resonance imaging (MRI) to assess wall thickness, material properties, and 3D-geometry. ATAA patient underwent 4D-flow DENSE MRI. Lumen geometry and wall thickness were used to create surface contour meshes of aTAA geometry. DENSE measured circumferential and longitudinal aortic wall strain and wall material property was derived using inverse analysis. Zero-stress geometry was derived using a novel fast pre-stress approach. Peak von Mises stress of FEA model with zero-pressure correction was 465kPa, while without zero-pressure correction the model predicted 272kPa. Average von Mises stress predicted by zero-pressure correction FEA model was 98 ± 18 kPa while that for non-zero-pressure correction was 86 ± 16 kPa. ATAA models in literature have not accounted for zero-stress configuration or patient-specific material property. We demonstrate that *in vivo* MRI can be used to obtain patient-specific parameters that significantly impact wall stress results. Future computational models that use wall stress to predict aTAA adverse events must take into account zero-stress geometry and patient material property for accurate wall stress determination.

Abstracts – Poster Presentations

P1

Chondrogenic Pathways Involved In The Development of Myxomatous Valve Disease

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Myxomatous mitral valve (MMV) prolapse is the second most common heart valve disease and the leading cause of mitral regurgitation. The hallmark of myxomatous disease is an excessive accumulation of proteoglycans leading to thickened heart valve leaflets. Moreover, focal areas, which display chondrogenic features, are observed indicating that myxomatous disease processes mimic chondrogenesis. However, the underlying molecular mechanisms driving chondrogenic gene expression in diseased valves are still poorly understood. Therefore, two murine models with skeletal defects were examined as potential models of myxomatous disease. Osteogenesis imperfecta murine (Oim) mice have a spontaneous mutation in *Collagen1a2* leading to altered collagen content and structure. Adult homozygous mice display thickened aortic valves primarily due to increased proteoglycan deposition as indicated by pentachrome staining. Sox9, Collagen 2 and Hapln1 also are increased. Similarly, mice deficient in Axin2, a negative regulator of Wnt/ β -catenin signaling, also develop thickened proteoglycan-rich aortic valves with increased chondrogenic markers, including increased aggrecan expression. These markers are consistent with chondrogenic features observed in myxomatous area of human MMV. Interestingly, chondrogenic differentiation appears to occur also in MMV from patients with Marfan syndrome and in the *Fbn1C1039G* mouse model for Marfan Syndrome. Together these mouse models indicate that chondrogenic pathways are active in myxomatous disease and can be used for future mechanistic studies of MMV progression and prevention.

P2

Versican Is Markedly Elevated During The Early Pathogenesis Of Murine Aortic Valve Disease

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The initial pathogenesis of aortic valve disease is poorly understood. Proteoglycans are putative early therapeutic targets, as they mediate initiation of atherogenesis and surround calcified valvular nodules. We examined the impact of a mildly atherogenic diet on proteoglycan content/composition in the mouse aortic valve.

Male C57Bl/6J wild-type mice were fed control or high-fat (HF) diets for 4 or 16 months. Valve function was assessed by echocardiography, and matrix composition/morphology by (immuno)histochemistry. Cultured porcine aortic VICs were treated with/without 5 ng/ml TGF- β 1 for 5 days. At 4 months, valve function was unchanged between diets ($p = 0.75$), and all leaflets were negative for α SMA, CD45, and ApoB. HF leaflets were distally thickened (+126%, $p < 0.05$) due to proteoglycan deposition (+110%, $p < 0.01$), not collagen accumulation ($p = 0.87$). Only versican was elevated in HF leaflets (+138%, $p < 0.05$), with no change in decorin ($p = 0.69$) or biglycan ($p = 0.59$). Differential proteoglycan expression was mirrored in culture: TGF- β 1 elevated total proteoglycan synthesis (+114%, $p < 0.05$), but only versican mRNA was increased (+53%, $p < 0.05$) while decorin and biglycan were reduced (-105% and -162% respectively, $p < 0.01$). By 16 months, HF mice developed impaired valve function with increased transvalvular velocity (+48%, $p < 0.05$). HF leaflets were proteoglycan-rich, α SMA-positive, and fibrotic, with all leaflet regions (proximal/medial/distal) thickened vs. controls (+493%, +472%, +77% respectively, $p < 0.001$).

Thus, diet-induced valve dysfunction is preceded in mice by early versican synthesis that leads to leaflet thickening and fibrosis.

P3

Non-Linear Optical Visualisation Technologies For Characterisation Of Aortic Valve Sclerosis In A Murine Model

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Degenerative heart valve disease like aortic valve stenosis (AVS) is characterized by disorganization of fibers in the extracellular matrix and cell invasion. Currently most histological-based methods for characterization of AVS demand for extensive processing of extracted valve material with impact on tissue morphology. We present a novel approach combining coherent anti-Stokes Raman scattering (CARS), endogenous two-photon excited fluorescence (TPEF) and second harmonic generation (SHG) to characterize AVS on aortic leaflets from apoe^{-/-} and wildtype mice. In addition optical coherence tomography (OCT) enables thickness measurements and 3D visualization of murine aortic valve leaflets dynamics. OCT demonstrated increased leaflet thickening from 30µm up to 80µm, aortic valve insufficiency and decreased opening angle in apoe^{-/-} mice. TPEF illustrated decreased and fragmented elastin fibers (ventricularis), SHG showed increased and disorganized collagen fibers (fibrosa) and CARS displayed adipocytes infiltration in apoe^{-/-} whereas the wildtype did not show any of those pathological changes.

Using non-linear optical microscopy cusp constituents can be examined simultaneously, three-dimensionally and without extensive manipulation of the sample. These technologies using high resolution imaging methods require no staining procedures, enable the analysis of the progression of AVS and give impressive insights into a complex disease.

P4

Patient-Specific Fluid Structure Interaction Simulation of Ascending Thoracic Aortic Aneurysm Using In Vivo Magnetic Resonance Imaging

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Elective ascending thoracic aneurysm (aTAA) repair is based on family history, connective tissue disorder, growth rate, but primarily size. Rupture/dissection carries a high mortality and occurs in many patients who had not met size criteria for surgical repair. Wall stress may be a better predictor than size for adverse events, but cannot be directly measured in vivo, rather determined from finite element analyses (FEA). FEA analysis of aortic stress faces many unique challenges. Reference (unloaded) geometry of aortic wall can't be readily measured; in vivo wall material property is often unknown. In this work we implement novel techniques to address these challenges. 4D flow MRI imaging was used to measure patient specific aortic blood flow information and displacement encoding with stimulated echo (DENSE) MRI was used to measure cyclic aortic wall motion. Aortic lumen geometry was contoured from MRI images and was used to create a numerical model of the aTAA. DENSE measured wall motion was used in an inverse analysis to characterize aortic material property. Reference geometry of the aortic was derived using a novel pre-stress approach. These patient specific aortic wall characteristics were then used to conduct a fluid structure interaction (FSI) simulation to calculate the in vivo wall stress. In vivo blood flow conditions measured from the 4D MRI were used as inlet boundary condition. We achieved unprecedented level of patient specificity in terms of in vivo aneurysm wall stress prediction. Further work on validation could pave the way for clinical applications of aTAA wall stress analysis.

P5

Tissue-Engineered Textile-Reinforced Mitral Valve - Second Generation TexMi

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Tissue engineering a complete mitral valve substitute is challenging because of the anatomical complexity of the native valve and the high load conditions it has to withstand. Recently we realised the proof-of-principle of the first tissue-engineered, fibrin-based, textile-reinforced mitral valve - TexMi - recapitulating the main anatomical features of the native valve i.e. the annulus, the asymmetric leaflets and the chordae tendineae, a crucial element to preserve the ventricular pumping efficiency which is, however, disregarded in the commercial available (non-viable) substitutes. The TexMi valve relies on a hybrid scaffold consisting of fibrin gel as a cell carrier and a warp-knitted mesh that defines the 3D geometry and provides mechanical stability. Here we present the second generation of the TexMi, with a load-optimised textile co-scaffold and improved design with four fully tissue-engineered chordae tendineae to be sutured at the papillary muscles. The valves were moulded with human cells isolated from the vein of the umbilical cord and cultivated under dynamic conditions over three weeks. Immunohistochemical staining showed the presence of aligned collagen fibres which appear striated in transmission electron microscopy. The collagen content was approximately 50% of the content found in the native mitral valve and the burst pressure reached 1000 mmHg. The considerable extracellular matrix formation and the high burst strength are encouraging results towards the realisation of a living prosthesis specifically designed for implantation in the mitral position.

P6

Novel CALP Pericardium Shows Superior Hydrodynamics and Fluid Structure Interaction Compared to Current Bioprosthetic Valve Tissues: A Computer Simulation

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We developed a novel pericardial based tissue (CALP) for bioprosthetic and tissue engineered heart valves. The influence of its material properties on blood flow, as well as its fluid structure interaction when fixed and decellularized for bioprosthetic valve manufacture were numerically simulated. Glutaraldehyde fixed and decellularized CALP was compared to that of Glutaraldehyde (Glu) fixed and decellularized bovine pericardium (BP) and porcine (PV) heart valve leaflets. Tissues were fixed in 0.6% Glu and stored in 0.2% Glu for testing. A two-dimensional fluid structure interaction was modeled using Comsol Multiphysics finite element package (version 4.4, Comsol Ltd., London, UK). Calculated values for Young's modulus from uniaxial tests were: 10, 8, 8 and 5 MPa for fixed PV, BP and CALP and decellularized CALP, respectively. The calculated Young's modulus of decellularized BP and PV was close to their fixed samples (approximately 8 and 10 MPa), and hence, simulations were not performed for these samples. Maximum valve opening was calculated as 82.26, 74.52, 71.13% of annulus diameter, for decellularized and fixed CALP and fixed PV, respectively. The time of maximum valve opening was similar (13.17, 13.01, 13.02% cardiac cycle). The peak pressure gradient was 0.49, 0.51 and 0.53 kPa, and peak Von Mises stress was 0.47, 0.68 and 0.78 MPa, for decellularized and fixed CALP and fixed PV, respectively. Peak shear rate was 21.13, 18.68 and 16.67 × 10³ 1/s. CALP could be considered as a superior alternative for valve fabrication as fixed CALP opening and structural behavior were similar to BP and decellularized CALP showed superior structural and opening behaviors compared to other materials.

P7

Characterization and Validation of a 3D PEG-NB Screening Platform for Improved MSC-Based Heart Valve Tissue Engineering

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Substrate properties (physical and biochemical) as well as external mechanical and soluble chemical cues are important microenvironmental factors for directing the differentiation of bone marrow mesenchymal stromal cells (bmMSCs) in vitro. Select combinations of these factors have been applied to bmMSCs (mostly in 2D) for the purpose of heart valve tissue engineering. However, the integration of environmental factors in 3D culture has yet to be appropriately considered, despite being necessary for functional valve tissue development. To address this, we sought to characterize and validate a 3D screening platform that enabled the combinatorial assessment of chemical and stiffness factors affecting bmMSC viability, morphology and differentiation to a valve phenotype. Human bmMSCs, encapsulated in 4–11 wt% (5–30 kPa) polyethylene glycol norbornene (PEG-NB) hydrogels, were cultured for 1 week. Viability and morphology of bmMSCs were assessed as a function of polymer wt% and RGD concentration. Factorial central composite designs modeled the viability and morphology responses, demonstrating an optimal wt% and RGD concentration for maximal viability and spread morphology. Separately, the effect of polymer wt% on the promotion of a synthetic valve phenotype (indicated by enhanced α -SMA staining) was investigated by supplementing media with 5 ng/mL TGF- β 1 for 1 week. The intensity of α -SMA staining significantly increased 1.6 fold in response to TGF- β 1 treatment in both 4.7 and 7.5 wt% PEG-NB gels, but remained constant for 10.3% gels. This study highlights a novel platform with which to identify specific aspects of the 3D valve environment that direct bmMSCs to produce valve tissue.

P8

Assessment of Parylene C Thin Films for Heart Valve Tissue Engineering

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Scaffolds are main components of the proposed model of the tissue engineered heart valve (TEHV), which provide mechanical support, and guide cell growth and tissue formation. Thus, we investigated the compatibility of parylene C (PC) for use as scaffolds in TEHV by studying its biocompatibility and mechanical properties. Plasma oxidized PC slides were coated with type I collagen or fibronectin, and their adsorption was estimated by protein staining. To investigate the compatibility of PC with valve cells, porcine pulmonary valve endothelial and interstitial cells were grown on plasma oxidized PC with different types of ECM coating and their adhesion, viability, apoptosis, and phenotype were determined. Also, the mechanical properties of PC were measured and compared to porcine aortic valve, and the effect of film thickness on the mechanical properties was studied. PC showed compatibility with protein adsorption, and valve endothelial and interstitial viability and phenotypic expression. In addition, PC exhibits high yield strength and ultimate tensile strength, indicating its high resistant to fracture and plastic deformation. All of these properties indicate that PC could be suitable for use as scaffold for tissue engineered heart valves. The high stiffness of this material could be a drawback, so PC should be tailored to reduce the stiffness.

Surface Functionalization Of Anisotropic Nanofibers For Driving Human Mesenchymal Stem Cell Behaviour

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Synthetic scaffolds for tissue engineering have proven to be good alternative to biological ones due to their flexibility in the manufacturing process and their controllable biodegradability. However, they lack biological cues for improved cell adhesion, migration and function. Poly ϵ -Caprolactone (PCL) films and aligned PCL fibers were coated with Dendri-Graft Poly-L-Lysine (DGL) and RGD peptide to investigate the effect on human mesenchymal stem cells migration and proliferation. Films and aligned fibers were coated with DGL-bound RGD peptide and compared with uncoated scaffolds. Cells were incubated for 15 minutes or 2 hours, stained with DAPI and phalloidin to quantify cell numbers and cell adhesion by means of total cell area. After 15 minutes incubation cell adhesion was higher when the DGL was bound to RGD peptide compared to both uncoated scaffolds or DGL only coated. This difference was even greater after 2 hours. Using specially designed chamber slides, a cell free area was created on surface of PCL fibers to study cell migration. Preliminary results show that even in a 3D structure the DGL-RGD coating improves cell migration by a faster cell growth/migration rate toward closing the gap compared to uncoated fibers. The use of DGL and extracellular matrix mimicking peptides such as RGD, could prove to be a good strategy to increase cell adhesion and induce specific cellular response on a synthetic scaffolds.

P10

PNGase F removes glycoalyx structures of decellularized porcine pulmonary heart valve matrices

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Implantation of xenografts leads to graft rejections. So far the α Gal epitope was identified as strongest xenoantigen present on non-human grafts. Carbohydrates in general are believed to be xenoantigens, since the glycoalyx is species-specific. To address and circumvent the limited availability of human decellularized heart valve matrices for heart valve replacements in future, we investigated the removal of glycoalyx structures of porcine pulmonary heart valves (PPHV) by decellularization and glycolytic treatment.

PPHV were decellularized by different detergent-based protocols and enzymatically treated by PNGase F. Potential carbohydrate removal was investigated by histochemistry using isolectin B4 (IL-B4) staining α Gal epitops, wheat germ agglutinin (WGA) staining N-acetylglucosamines, datura stramonium lectin (DSL) staining β - 1,4 linked N-acetylglucosamines and N-acetylactosamines, and ricinus communis agglutinin I (RCA I) staining N-glycosides. Samples decellularized only served as controls.

All lectins stained native PPHV, whereas a reduced lectin stain was observed on decellularized PPHV matrices in dependence of the decellularization protocol used. Compared to native tissue, SDS/TritonX100 reduced IL- B4 but not WGA, DSL, or RCA I. The other protocols reduced IL-B4, WGA and DSL stains. PNGase F abolished IL-B4, WGA, and DSL stains independent on the type of decellularization. Thus, decellularization *per se* reduced α Gal whereas decellularization by SDS/Na-deoxycholate and TritonX100+SDS, but not by SDS/TritonX100, additionally led to a removal of N-acetylglucosamines. Cleavage of GlcNac β (1-N)Asn sites by PNGase F eliminates detergent-independent carbohydrate structures like α Gal and N-acetylglucosamines. In summary, detergent based decellularization followed by PNGase F treatment resembles an efficient way to remove potentially immunogenic epitopes from PPHV.

P11

BMP-pSmad1/5/8 Pathway Activation in a Novel Mouse Model of Calcific Aortic Valve Disease

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Calcific Aortic Valve Disease (CAVD) affects >2% of the population over the age of 65, for whom the only clinical therapy available is valve replacement surgery. Furthermore, the underlying pathogenic mechanisms of CAVD, which could serve as potential therapeutic targets, remain unknown. Klotho-null mice exhibit accelerated aging and aortic valve calcification similar to human CAVD. Notably, phosphorylation of Smads1/5/8, Bone Morphogenetic Protein (BMP) pathway effectors essential for bone calcification, is detected prior to the onset of calcification and later localizes with calcified nodules in Klotho-null aortic valves. Our hypothesis is that activation of the BMP-pSmad1/5/8 signaling cascade promotes aortic valve calcification in CAVD. Our preliminary studies demonstrate that osteo-chondrogenic factors are significantly increased in Klotho-null aortic valves, and pSmad1/5/8 activation precedes their induction in the region of the valve where calcification occurs. Moreover, increased BMP2/4 ligand expression is detected prior to, as well as during, calcific nodule formation. Together these data support an active role for BMP signaling as a critical mechanism in the development of CAVD. Our ongoing work includes: 1) Identifying the requirements for the BMP pathway in aortic valve calcification in vitro, and 2) Determining if BMP pathway inhibition can prevent or inhibit aortic valve calcification in vivo.

P12

Mild Hyperlipidemia Induces Aortic Valve Disease And An Osteoporotic Phenotype In Aged Mice

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Calcific aortic valve disease is paradoxically correlated with osteoporosis in humans. Hyperlipidemia has been independently linked to both of these pathologies, and may represent a unifying causative factor between these two conditions. We examined this relationship by feeding male C57Bl/6J wild-type mice a control or a high fat (HF) diet (58.7% kcal from fat, cholesterol < 0.1% w/w) for 16 months ad libitum. Echocardiography was used to examine valve function, and blood testing determined serum cholesterol levels. Mouse bone mineral density (BMD) was measured using a Piximus bone density scanner. Overall weight of the HF group was 45% ($p < 0.001$) higher than the control, indicating obesity, and corresponded with elevated non-HDL cholesterol levels (+62%, $p < 0.05$). This coincided with the onset of valvular dysfunction, with a 48% increase in transvalvular velocity ($p < 0.05$). The BMD of mice on the HF diet was reduced in the femur, humerus, and whole skeleton by 7-16% ($p < 0.05$ vs. control diet). Bone mineral content in HF mice was also reduced 23-24% from controls ($p < 0.001$). The whole-body and femoral BMD was inversely correlated ($r^2 = 0.43 - 0.49$, $p < 0.01$) with transvalvular velocities in all mice. Overall, our results support the notion that mild hyperlipidemia leads to both valve dysfunction and altered bone metabolism in obese mice. Further investigation of the paradoxical progression of cardiovascular disease with osteoporosis under hypercholesterolemic conditions should be considered when examining long-term mouse models of valve disease.

P13

Relationship Between Free Aldehyde Groups In Bioprosthetic Heart Valves And Calcification

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Glutaraldehyde is commonly used to crosslink the collagenous matrix of pericardium leading to increased durability and reduced antigenicity of bioprosthetic heart valves. Structural valve deterioration due to calcification is the primary failure mode in these valves. Calcification is believed to be caused by multiple factors with glutaraldehyde as one of the major factors. Anti-calcification technologies have been developed to address this specific factor, yet the mechanism is still not completely understood.

The objective of this work is to describe the amount of free aldehyde groups in glutaraldehyde-fixed bovine pericardium used for bioprosthetic heart valves and how it relates to *in vivo* interactions such as calcification. The concentrations of residual aldehyde groups found in bovine pericardium were determined by a novel test method. Glutaraldehyde fixed tissue contained a high amount of free aldehyde groups, while tissue produced using GLX, an advanced integrity preservation technology which permanently caps residual aldehydes, contained an 88% reduction in aldehyde values. Calcification studies showed significantly higher calcification in tissue containing high concentrations of aldehyde residuals compared to low aldehyde residuals, with a 99.4% reduction in calcium for GLX tissue. The relationship between aldehyde levels and calcification potential of bioprosthetic tissue may be used as a tool for explaining the mechanism of calcification due to glutaraldehyde of bioprosthetic heart valves.

P14

Investigating the Potential of Valve Interstitial Cells to Act in a Pericyte Manner by Promoting Angiogenesis and Invasion by Valve Endothelial Cells

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Calcific aortic valve disease (CAVD) is the third leading cause of heart disease in adults. It has been hypothesized that therapeutically stopping the formation of microvessels during early CAVD could prevent the pathological progression of the disease. However, the lack of understanding of valve cellular pathology has stymied discovery of potential treatments. Pericytes have been localized histologically along the exterior of these microvessels, but it is unknown whether these cells are derived from valve interstitial cells (VICs) or if VICs can differentiate into a proangiogenic pericyte phenotype. Therefore this study investigated the perivascular potential of VICs to stabilize valve endothelial cell (VEC) angiogenesis in a matrigel model and investigated potential VEC/VIC signaling pathways controlling their organization using small molecule inhibitors. Control vascular derived endothelial and pericyte cell lines formed stabilized networks in the model, but valve interstitial cells collapsed valve endothelial cell network formation over 24 hours into spheroids. Interestingly after spheroid formation, VICs began to promote valve endothelial cell sprouting into the matrix and were found wrapped around the VECs. These behaviors are suggesting both a pericyte-like and invasive phenotype for the VICs. This process was found to be Rho Kinase, protein kinase C, TGF-B, and Tie2 dependent and to be VEGF, NOTCH, NO, and PDGF independent at the concentrations tested in this study. The results of this study align with current hypotheses of the pathology of CAVD and offer new insights into the VEC/VIC dynamics during its progression.

P15

MG53-Mediated Protection in Heart Valve Biology

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Utilizing both overexpression and extracellular application, we aim to target MG53, an essential cell membrane repair protein, to valvular cells for treatment of tissue injuries associated with valvular heart disease (VHD). Our group has identified MG53 as an essential component of the cell membrane repair machinery. Membrane repair is important to maintain normal cellular physiology, and disruption of this process can result in disease in a number of different tissues. With the four heart valves experiencing a tremendous amount of mechanical stress, we have utilized microelectrode needle penetration and glass bead damage assays to assess the ability of MG53 to protect valvular interstitial cells from physical injury. Furthermore, we have begun calcification studies to investigate the ability of MG53 to protect against inflammatory and osteogenic changes associated with VHD. To date, we have observed MG53-mediated protection against membrane damage through both live cell imaging and LDH release. With preliminary results, we have also noted MG53-mediated protection against changes in osteogenic gene expression after LPS treatment. Ongoing tests will further characterize the mechanisms of protection in valvular cells and assess the effects of MG53 in in vivo models of VHD. We anticipate that, once all of our aims are completed, our experiments will greatly contribute to the study of cell biology, regenerative medicine, and cardiovascular disease, the number one cause of death in the United States and worldwide.

P16

Relevance of YAP/TAZ Transcriptional Co-factors on Mechanotransduction of Aortic Valvular Interstitial Cells

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Activity and nuclear vs. cytoplasm localization of the transcriptional co-factors YAP (Yes-associated protein) and TAZ (PDZ-binding motive, WWTR1) have recently been associated to ECM stiffness e.g. in mammary epithelial cells, mesenchymal stem cells and cancer cells. Therefore these factors may represent key mediators of cell differentiation in response to ECM elasticity and cell shape. Accordingly, it has been presented that activity and localization of YAP/TAZ was under control of RhoA and the functional state of the actin cytoskeleton independent of Hippo kinases. Since mechanical cues reportedly affect structure and function of aortic valves this study aims at potential roles of YAP and TAZ as mediators of mechanotransduction.

The expression of YAP/TAZ was analyzed in valvular interstitial cells (VIC) and quantified after culturing on soft gels with different stiffnesses. The total expression level of YAP and TAZ protein was elevated when VICs were cultured on surfaces with an elastic modulus of 25 kPa reflecting the matrix stiffness in fibrotic valves. On soft gels reflecting the stiffness of healthy valves (8 kPa) the protein expression was significantly lower.

Whereas the localisation was not clearly correlated to matrix stiffness immunofluorescence experiments revealed a partially co-localisation of YAP to focal adhesion kinase on collagen coated stiff matrices.

Aims of the project are now to investigate signaling cascades of cell-matrix interaction and/or stress fiber formation in relation to YAP as well as TAZ expression and localisation in VICs to define the relevance of YAP/TAZ in mechanotransduction and osteoblastic differentiation of aortic VICs.

P17

An *In Vitro* Model for the Investigation of Potential Mechanical Interactions Between Surgical Sealants and Aortic Tissue

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BioGlue[®] Surgical Adhesive is approved for use in cardiac, vascular, and pulmonary surgery, as an adjunct to standard methods of repair (such as sutures, staples, electrocautery, and/or patches) to bond, seal, and/or reinforce soft tissue. This study evaluated the potential mechanical effects of BioGlue when applied to reinforce a suture line surgical repair of a valved aortic conduit.

A defect was created in six aortic conduits, each conduit being bisected by a single incision just above the sinus. A 4-0 Prolene running suture line was then used to reattach the two halves of the conduit. BioGlue was applied to the suture line of three conduits. The remaining three conduits did not receive BioGlue.

The physiological motion of the conduit was simulated using a Dynatek Heart Valve Tester. All conduits were loaded into the tester and cycled at approximately 200 cycles per minute with a closure load of 100 mmHg.

Daily inspections were performed to assess any changes to the surgical repair of each conduit. Visual examinations show the suture underneath the regions where the BioGlue had been applied to remain intact and in its original condition. There was no impact on suture performance as a result of BioGlue application over 60 million cycles. The suture material and tissue showed no signs of dehiscence, suture fracture, fraying, tearing, or abnormality. The study will continue to run to a target of 120 million cycles.

P18

Optimization Of Cyclic Stretching For Acceleration Of Collagen Production In Fibrin-Based Engineered Tissue Equivalents

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Fibrin is a promising scaffold material for tissue engineered heart valves, as it is completely biological, allows for engineered matrix alignment, and is able to be degraded and replaced with collagen by entrapped cells. However, the initial fibrin matrix is mechanically weak, and extensive *in vitro* culture is required to create valves with sufficient mechanical strength and stiffness for *in vivo* function. Cyclic stretching has been shown to increase collagen production by cells entrapped in a fibrin scaffold, accelerating the strengthening of the tissue and reducing the required culture time. However, the entrapped cells adapt to constant amplitude cyclic stretching, suggesting that some perturbation to a constant amplitude regimen may maximize the benefit.¹⁻³ In this study, intermittent cyclic stretching (alternating stretching and rest) and incrementally increasing strain amplitude cyclic stretching were explored to overcome this adaptation and accelerate collagen production. Using a Flexcell Tension System, the effects of constant amplitude, intermittent, and incrementally increasing amplitude cyclic stretching regimens were investigated for neonatal human dermal fibroblasts entrapped in a fibrin scaffold. The cellular response was studied at the signaling level by monitoring the extracellular regulated kinase (ERK) and p38 pathways and at the gene transcription level by monitoring collagen transcription. Total collagen content and tensile properties of the resulting tissues were quantified. Collagen production was accelerated using an optimized cyclic stretching regimen.

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¹Paxton JZ, *Tissue Eng. Part A* 2012, 18(3-4):277-84.

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P19

Parameter Estimation of Heart Valve Leaflet Hyperelastic Mechanical Behavior Using An Inverse Modeling Approach

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In this work, we present a method for determining the functional properties of heart valve leaflets from non-invasive imaging techniques such as ultrasound and CT. The novelty of present approach is the use of collagen fiber maps to constrain the optimization and the use of a coupled surface fitting/in-plane strain approach to obtain new information for structural modeling of valve leaflets. As a first step, we use in-vitro experimental data from porcine bioprosthetic heart valve emulating an aortic valve put in a flow loop. The leaflet was imaged at three different static transvalvular pressures of 40, 80 and 120mm Hg using surface markers and dual-camera setup. This highly comprehensive data set with high resolution, marker positions at multiple pressures, valve specific fiber architecture and biaxial data was ideal for this study – to design the inverse model, validate it and calculate it's sensitivity to various input parameters and optimization constraints. This information when combined with the inverse model presented in this work will lead to an in-vivo assessment tool for heart valves and help diagnose problems in the mechanical functionality at an early stage. Additionally, this approach will have the potential to serve as a general-purpose in-vivo assessment tool for heart valves – for evaluating the performance of replaced prosthetic valves as well as monitoring the progression of valve diseases.

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P20

A Computational Framework for Multiscale Modeling of the Mitral Valve

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The mitral valve (MV) is one of the four heart valves which locates in between the left atrium and left ventricle and regulates the unidirectional blood flow and normal functioning of the heart during cardiac cycles. High-fidelity computer simulations provide a means to connect the cellular function with the organ-level MV tissue mechanical responses, and to help the design of optimal MV repair strategy. We present here the details of our first steps to create state-of-the-art of mitral valve modeling techniques, with an emphasis on what is known and investigated at various length scales. A modified left heart simulator was used for acquisition of the in-vitro mitral valve deformations at stress-free (referential), pressure loaded (~30 mmHg) and fully loaded (~70 mmHg) states. The segmented MV geometry at each state was imported into Geomagic Studio for generation of the corresponding finite element model. The 3D locations of the key points for representation of the MV chordae tendineae, such as MV PM attaching points, chordal branching points, and MV leaflet attaching points, were quantified for realistic reconstruction of chordae tendineae (297 three-dimensional truss elements). Moreover, spatially-varied and anatomically accurate MV leaflet thicknesses were determined from the Micro-CT data. Next, collagen fiber micro-structural information was mapped onto the finite element model. The numerical predictions of the deformed geometry agreed well with the in-vitro experimental data, with the total displacement errors. We thus conclude that the improved accuracy of the current mapping techniques do indeed provide more accurate modeling results of the MV.

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P21

A novel experimental/numerical method to assess BHV biomaterial fatigue response in-vivo

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There does not currently exist a means to evaluate and predict in vivo bioprosthetic valve response to long-term cyclic loading and blood contact interactions. Herein, we present a novel, integrated numerical/experimental technique to assess BHV biomaterial response in vivo in a completely device independent manner. GLUT treated porcine aortic valve leaflets are cut into shape and implanted in the anterior mitral valve leaflet of Dorset sheep. To explore the stress induced change in configuration, we implanted the PAV patch in 2 different orientations: with the circumferential direction of the patch aligned to the circumferential direction of the mitral valve (C-C aligned), and with the circumferential direction of the patch aligned to the radial of the mitral valve (C-R aligned) thus interchanging the loading boundary condition in the circumferential and radial direction of the patch. To quantify the in vivo deformation and subsequently determine the in vivo stress, 4 sonocrystals are placed at the corners of the implanted patch, with an additional one at the center. An inverse finite element model is applied to the implanted patch using shell elements. After four weeks in vivo, the tissue was explanted and further evaluated for mechanics and microstructure. Results demonstrate that when compared with the original cross-linked material, the patch implanted in the C-C orientation exhibited a nearly identical stress-strain response when subjected to biaxial mechanical testing. However, the patch implanted in the rotated C-R orientation was drastically increase in stiffness and exhibit significant permanent creep along the radial direction of the patch.

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P22

Adipose-Derived Stem Cells as a Potential Source of Valve Endothelial Cells

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Valve endothelial cells (VECs) have been demonstrated to have a role in the regulation of the mechanical and biological integrity of the valve. As part of tissue engineering of heart valve, endothelial cell (EC) source is required that will match VECs in terms of the ability to respond to the flow patterns found on the aortic and ventricular surface of the valve. This study focuses on using adipose-derived stem cells (ADSCs) as a potential source of VECs and differentiating them into site-specific VECs by aortic and ventricular shear stress patterns. After stimulation with endothelial media, VEGF and site specific shear stress, ADSCs were positively stained for CD31, vWF and NOS III, and exhibited the ability to take-up low-density lipoprotein. Semi-quantitative analysis of the level of the staining demonstrated that the aortic shear stress significantly increased the expression of NOS III (10.25 fold) and SM α A, (6.52 fold) as compared to the static control, whereas the ventricular shear pattern does not induce the same effects (n=3). These studies reveal that ADSCs have the potential to differentiate into VECs. Moreover the finding that the different flow patterns induce the different expression of the EC marker suggests the importance of specific flow pattern on the differentiation of ADSC-derived VECs. Further function of ADSC-derived VECs to communicate and regulate VICs will be investigated.

P23

A New Strategy for Heart Valve Decellularization for Tissue Engineering

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Tissue engineering of pulmonary heart valves represents an enormous advantage for the treatment of Tetralogy of Fallot. It is important to find out the best decellularization method to preserve native structure of the leaflets before seeding with autologous cells.

Porcine pulmonary heart valves were prepared and decellularized enzymatically. Six heart valves were treated with Trypsin/EDTA for 12 hours (group 1) and six heart valves with AccutaseTM for 96 hours (group 2). Heart valves were embedded in paraffin, analyzed histologically using H.E.-, Movat- pentachrome-, Elastica/van Gieson- and immuno-histochemistry-staining using anti-collagen I and III.

Movat-Pentachrome- and Elastica/van Gieson-staining demonstrated complete collagen structure in group 2 whereas in group 1 collagen structure was diffuse and not completely preserved. H.E.-staining showed remaining cell nuclei in group 1 revealing an incomplete decellularization. In contrast, group 2 revealed no cell nuclei. AccutaseTM incubation time was tested at different time points and demonstrated no cell nuclei and prevention of the structure after 96 hours whereas Trypsin/EDTA did not reveal complete structure after 12 hours. The collagen structure analyzed using anti-collagen I and III- staining indicated in group 1 destroyed cellular structure whereas in group 2 various cell structures were completely preserved.

In this study we demonstrated that AccutaseTM did not degrade the original structure of the leaflet while the original cells were completely removed. The rest of the DNA, we have found, will be analyzed by a cooperation group (Prof. Kaatsch, UKSH, Campus Kiel, Institute of forensic medicine).

P24

WITHDRAWN

P25

Adipose Tissue-Derived Stem Cell Sourcing for Heart Valve Tissue Regeneration; Selection of the Optimal Anatomical Site and Harvesting Procedure in Sheep

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The isolation and cultivation of stem cells from experimental animals has become a common research technique in the field of regenerative medicine. The aim of this study was to establish the most appropriate anatomical location for harvesting of adipose tissue derived stem cells (ASCs) with perivascular localization (pericytes) in sheep. The first anatomical study was performed postmortem; fragments of subdermal adipose tissue collected from different locations were processed by standard histological methods. All subsequent interventions (n=19) were performed intra-vitally on sedated animals. Samples of about 0.5 cm³ were harvested aseptically from the following anatomical locations: interscapular, paravertebral - lumbar and coccygeal, groin, perimammar, great omentum and perirenal. After harvesting, samples were placed in cell culture medium (DMEM/10% FBS10, 2% antibiotic/antifungal) and ASCs were isolated and cultured in vitro as described by Zuk (2001) and Gimble (2002) for subsequent use in studies of cardiovascular tissue regeneration. Microscopically we observed mature adipose tissue with the presence of small blood vessels in all tissues. Harvests from the paralombar areas did not yield ASCs. The vascular density was higher in the perivisceral and the somatic fat. However, due to the easier surgical approach, consistency of viable cells and to prevent postoperative complications, the interscapular area was chosen as the preferred site for isolation and culture of ASCs in adult sheep. Ongoing studies are testing the in vitro pluripotency of our sheep ASCs and their in vivo ability to sustain valve regeneration as autologous ASC-seeded valve scaffolds.

P26

Decellularisation of Allogeneic Aortic and Pulmonary Valves using Low Concentration SDS

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Over 5,000 patients benefit from heart valve replacement in the UK annually. Cryopreserved allografts are the "gold standard" but these are subject to immunological responses leading to calcification and stenosis resulting in multiple reoperations. We have developed methods to remove the immunogenic cells from porcine aortic valves whilst preserving the biochemical and biomechanical properties (1, 2) and shown these to regenerate in the pulmonary position in sheep (3). This project aims to translate this technology to human cardiac valves for the benefit of patients in the UK.

Five aortic and 3 pulmonary cryopreserved valves were treated sequentially with: hypotonic tris buffer, 0.1% (w/v) SDS in hypotonic buffer plus protease inhibitors and then treated with nucleases to remove nucleic acids. The histology and DNA content of four different regions of the acellular valves were compared to untreated cryopreserved valves. These studies were repeated on 4 pulmonary and 4 aortic valves to determine the robustness of the process, and these valves were also tested for biocompatibility using extract and contact cytotoxicity assays, and analysed for collagen and glycosaminoglycan content. H&E staining of acellular tissues showed no evidence of cell nuclei or cell remnants. Quantitative analysis for total DNA content showed that acellular valves had less than 15ng DNA /mg wet tissue. There was no evidence of cytotoxicity and acellular tissue showed loss of GAGs and retention of collagen.

Allogeneic cardiac valves successfully decellularised, whilst maintaining their histoarchitecture. The retention of collagen and loss of GAGs was expected based on previous studies.

P27

Comparative Analysis of the Chemoattractive Properties of Human Mesenchymal Stem Cells

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Human mesenchymal stem cells (hMSCs) have shown to release a plethora of soluble factors that are associated with paracrine therapeutic effects. In vascular tissue engineering, seeded cells including hMSCs have shown to be a major factor involved in the induction of in situ remodeling and neo-tissue formation of bioengineered constructs. In addition, functional improvement following myocardial infarction has been reported after injection of MSC conditioned media (CM).

Therefore the presented study aims at the in vitro comparison of different hMSC sources as to their chemoattractive properties. hMSCs were isolated from amniotic fluid, adipose tissue, bone marrow, wharton's jelly and vascular tissues as control cell source. For each source a phenotypic profile was achieved including expression of MSC markers, multilineage differentiation and proliferation capacities. CM of all cell sources were evaluated as to their chemoattractive potential to induce PBMC migration in a transwell migration assay.

Transanastomotic ingrowth was examined with an in vitro endothelial scratch assay.

The results pointed towards an increased monocyte migration in MSC CM with significantly higher values compared to controls. The comparative analysis showed that umbilical cord MSCs have the highest chemoattractive capacity. No significant differences between the cell sources were found in the endothelial scratch assay.

In conclusion, these preliminary results suggest that CM of hMSCs has a major potential in the recruitment of monocytes in vitro, while the attraction of endothelial cells was not enhanced. Future experiments will aim at a profiling of the secretoma of all hMSC sources to identify functionally relevant factors involved.

P28

In Vivo Investigation of Stented Monoleaflet PGA-P4HB Based Valves in The Arterial Circulation of a Rat Model.

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Cardiovascular tissue engineering aims at the creation of stem cell-based living autologous substitutes to overcome the limitations of currently used cardiovascular replacement materials for cardiovascular valve disease. Experiments in sheep, being a gold standard animal model for in vivo preclinical cardiovascular assessment, cannot be used to unravel underlying mechanisms of tissue formation and in situ remodelling. Therefore the investigation of implanted tissue engineered vascular grafts (TEVGs) in the venous/arterial circulation of rodents has been intensively initiated as this would allow for the assessment of constructs remodelling in immune-incompetent or transgenic strains. Nevertheless to extrapolate findings from vessel-like structures to more complex "dynamic" structures of valves, more hemodynamically relevant model must be created, which was the aim of this study. For this stented monoleaflet PGA-P4HB valves (n=5, length= 2mm, diameter=1.5mm) were created and successfully implanted infrarenally into abdominal aorta of Wistar Outbreed Rats (n=5) by catheter based technique. All animals survived the entire time of experiment and were stable during the surgery and post-operative ultrasound evaluation. Also no significant hemodynamic changes were observed. Nevertheless ultrasonographic evaluation revealed only minor leaflet movement (n=2 out of 5) in arterial circulation, most probably due to geometrical and hemodynamic reasons. Histological evaluation revealed some infiltration (within 3 hours) both into the wall and the leaflet and no signs for thrombus formation. These findings support the need for valve like structures assessment in the environment more similar to orthotopic cardiac one with more physiological flow conditions as well as structural adaptations of the bioengineered leaflets used.

P29

Acellular Mitral Valve Scaffold with Intact Annulus and Chordae Tendinae for Tissue Engineering

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The worldwide prevalence of degenerative mitral valve pathologies coupled with reparative and replacement strategies would benefit from improved solutions offered by tissue engineering strategies employing biological scaffolds and stem cells. In order to develop mitral valve scaffolds with optimal mechanical and biological properties, porcine fresh mitral valves were subjected to the removal of all cellular components, while leaving the extracellular matrix components intact. These acellular scaffolds were treated with penta-galloyl glucose (PGG), a polyphenol that binds to collagen and elastin and renders the extracellular matrix of the annulus, leaflets, and chordae tendinae resistant to rapid enzymatic degradation. Scaffolds were also evaluated for their mechanical properties, using biaxial tensile tests and thermal denaturation profiles. After seeding the annulus scaffolds with human smooth muscle cells and the leaflets with human adipose tissue derived stem cells, the constructs were mounted in a heart valve bioreactor. Cell proliferation and viability were evaluated after testing under static and dynamic bioreactor conditions for three weeks. Histological and immunohistochemical results indicated that there was complete removal of cellular components including DNA and cell proteins, yet a clear retention of both collagen and elastin. PGG treatment stabilized the extracellular matrix components of the scaffolds, improving the resistance to proteases and the mechanical properties of the leaflets and annulus. Cells were successfully injected within the scaffold and were maintained alive in vitro. Overall, we have developed a cytocompatible mitral valve scaffold, with adequate mechanical properties.

P30

Whole Aortic Root Decellularization and Recellularization: An Elusive Task in Heart Valve Tissue Engineering

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The long-term goal of this project is to generate living, tissue-engineered (TE) heart valves from biological scaffolds and autologous stem cells. At the basic level, a successful TE device must withstand mechanical stresses immediately upon implantation without posing risks of immunological rejection. The three main challenges to this objective are complete decellularization of porcine aortic valve roots (AVR) including the aortic root wall and sinus, efficacious seeding of adult stem cells within cusps, and the controlled dynamic progressive conditioning before implantation. We hypothesized that these challenges will not likely be overcome without using advanced bioreactor systems. We developed integrated platform technologies for complete AVR decellularization and cell seeding. Porcine AVRs were mounted into universal “no-touch” tissue-holding devices, treated with detergents and enzymes in a pulsatile perfusion system, and analyzed by histology and DNA quantitation. Human adipose-tissue derived stem cells (hASCs) were injected into cusps prior to surface recellularization in seeding chambers. The AVRs were progressively conditioned in heart valve bioreactors until reaching pulmonary conditions. Cell retention was assessed through surface imaging and histology. Decellularization of the entire porcine AVR (especially the aortic wall) was not possible through immersion techniques, but was successful using our pulsatile perfusion system. Utilizing the rotating seeding chambers resulted in more complete seeding as compared to dropwise seeding methods. Progressive conditioning regimens facilitated good initial cellular retention and stem cell pre-differentiation into cells resembling valvular interstitial cells. Overall, we have developed the basic equipment and expertise necessary to the successful development of TE cardiovascular products.

P31

Reasons Of Reoperation After The Ross Procedure In Different Age Groups - A Report From The German Ross Registry

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An increasing number of young patients are choosing bioprostheses for aortic valve replacement, but Ross operation deserves consideration as an alternative biological substitute. Reoperation rates after the Ross procedure and after bioprosthetic AVR, however remain a concern in these patients.

We report reasons of reoperation after the Ross procedure for different age groups.

Reoperation rates of 1.925 patients (mean age 41.2±15.3 years, 1.444 male) with a mean follow-up of 7.4±4.7 years (range 0.00 - 18.51 years, 12866.6 patient years) were analyzed in three age groups (Group I < 40 years, Group II 40-60 years, Group III > 60 years). Reasons of reoperation were figured out.

In Group I 12% (after 4.6±4.2 years), in Group II 6% (after 5.12±4.65 years) and in Group III 9% after (5.0±4.8 years) of the patients had to be reoperated. Mean interval to reoperation in bioprostheses was about 8 years in younger and about 13 years in middle aged patients. Most frequent reasons of reoperation were in: Group I SVD of the homograft (7%), Group II NSVD of the autograft (3%) and Group III SVD of the autograft (6.5%).

There is some evidence that in younger patients the reason of reoperation after Ross procedure is mainly SVD of the homograft and if there is a need for reoperation it is probably earlier than in patients with bioprostheses.

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