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ORAL ABSTRACTS

Title: Y chromosome linked UTY modulates sex differences in valvular fibroblast methylation in response to nanoscale extracellular matrix cues

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Abstract: Clinical evidence suggests aortic valve stenosis progression is sexually dimorphic in disease presentation and outcomes. Male aortic valves tend to develop a calcified phenotype while female valves exhibit a distinct fibrotic phenotype. The calcified phenotype is characterized by stiff, spherical calcium-phosphate nanoparticles, where particle size and abundance increase with disease progression. Previous work also suggests that genes and epigenetic modifiers that escape X-chromosome inactivation may contribute to sex dimorphisms in valve disease. We hypothesize that sex chromosome-linked genes partially regulate sex-specific myofibroblast activation in response to nano-scale stiffness cues in the valve tissue microenvironment. To test this hypothesis, we describe a bioinspired hydrogel cell culture platform to interrogate how nanoscale stiffness cues (polystyrene nanoparticles, or PS-NPs) modulate the valvular interstitial cell (VIC) to myofibroblast transition in male and female cells. We observed that male and female VICs deactivate on soft hydrogels in response to the presence of PS-NPs on the surface of the hydrogel. Female VICs maintained significantly higher levels of activation relative to male VICs. Since we showed that VICs are mechanosensitive at the nanoscale, we then sought to investigate changes in our male and female VICs at the transcriptional level. We showed that global methylation states in male VICs in response to both PS-NP sizes were significantly less relative to female VICs. Interestingly, methylation states in female VICs remained largely unaffected by the presence and concentration of PS-NPs. To confirm this observation, we knocked out a Y-linked demethylase, UTY, and observed increased methylation states uniquely in males following culture on PS-NP hydrogels. Ongoing work seeks to further characterize the role of sex chromosome-linked demethylases in VICs through transcriptomics analyses. Overall, we have potentiated a role of the Y-linked UTY lysine demethylase in causing sex-specific effects that guide male VICs towards calcification. Taken together, our study implicates the importance of sex chromosome-linked genes in the progression of disease-driving phenotypes in AVS.

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In vitro models of acute and chronic cardiac injury: characterization and differences in the cardiac troponin I and T release

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Clinical practice and guidelines assume that systemic cardiac troponin I (cTnI) and T (cTnT) concentrations reflect the identical pathophysiological processes and can be used interchangeably in clinical practice. However, a recent international multicenter clinical study has provided evidence of differences in the release of cTnI and cTnT according to cardiac pathology. Specifically, aging and chronic cardiac diseases lead to a predominate release of cTnT, while acute cardiac diseases cause primarly the release of cTnI. Defining the specific type of cTn released based on the type of cardiac damage may provide significant value in differentiating between acute and chronic conditions. Further clinical and biological investigations are required to address this fully.

In the present study, we aimed to generate two different *in vitro* cardiac injury models, a mild-long term ischemic and an acute-intense ischemic injury condition, to study the relationship between the type of cell damage and the dynamics of troponin release. A three-day exposure to glucose-serum-free media in a hypoxic environment resulted in a mild-injury characterized by a high apoptotic cell rate and a low rate of sudden cell death. An acute-intense injury model was realized by subjecting cardiac cells to serum-glucose-free culture medium in a hypoxic environment for one day. The high rate of cell mortality confirmed the severity of the acute injury model. The results showed that supernatant cTnT levels predominated following a mild insult, while an acute-intense insult resulted in more cTnI release. These data confirm that cTnI and cTnT are released differently depending on the type of injury (mild-long term or acute-intense). These distinct troponin release patterns make the two cardiac ischemic models useful for revealing the mechanisms underlying the different release of TnI and cTnT.

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A Balancing Act: Platelets as the Initiators of Both TEVG Neotissue Formation and Stenosis

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A tissue-engineered vascular graft (TEVG) that grows with the patient could transform the surgical management of children requiring congenital heart surgery. However, clinical trials revealed a high incidence of TEVG stenosis. Small animal models showed that platelet-rich thrombi develop on the TEVG surface and remodel into collagen-rich neotissue, persistently narrowing or occluding the lumen. Our goal was to understand how platelet signaling contributes to the microenvironment of remodeling TEVGs and in excess, causes stenosis. We previously observed improved TEVG performance in mice with a global mutation in the lysosomal trafficking regulator (LYST). We implanted TEVGs into platelet-specific LYST mutant mice. Using ultrasonography and Micro-CT, we found that platelet-specific mutants had near complete resistance to 3-day narrowing and remained free of stenosis by 14-days. Platelet functional assays revealed that LYST mutations did not affect biomaterial adherence, alpha granule secretion, or aggregation. However, LYST mutations impaired dense granule exocytosis of molecules, including ATP and ADP. ADP binds to the P2Y12 receptor, leading to platelet activation, thrombus stabilization, and immune cell recruitment. We hypothesized that excessive purinergic signaling, which requires LYST for releasing dense granules and the P2Y12 receptor for binding secreted ADP, initiates stenosis and influences the downstream immune response. We observed widespread patency in complete P2Y12 KO mice and mice treated with an irreversible P2Y12 antagonist, prasugrel, prior to implantation. This indicated a central role for purinergic signaling in stenosis. Further studies are necessary to determine how platelet-mediated purinergic signaling influences the ensuing foreign body response to TEVGs and sets the foundation for neotissue formation. Collectively, our findings highlight the interplay between LYST-mediated and purinergic platelet signaling in TEVG stenosis, providing insights for potential therapeutic interventions to improve graft outcomes in the clinic.

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XABG: A Restorative Polymeric Coronary Artery Bypass Conduit

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Saphenous veins are included in 80% of coronary artery bypass graft (CABG) procedures, despite suboptimal chronic patency and painful complications associated with vein harvesting. No synthetic coronary artery bypass grafts are currently approved for clinical use. The Xeltis XABG is a coronary artery bypass graft composed of an absorbable polymer, designed to enable cell infiltration and new tissue formation, resulting in functional endogenous tissue restoration (ETR). We describe ongoing preclinical and clinical studies towards demonstrating potential of this off-the-shelf synthetic approach to CABG that harnesses the body's innate healing capacity.

A GLP preclinical study was performed to assess the safety and performance of this polymeric graft in a challenging ovine CABG model. Fifteen cm long, 4mm inner diameter XABG conduits (N=13) were electrospun from a supramolecular polyurethane and compared to autologous saphenous vein grafts (SVG; N=3) for a maximal follow-up of 12 months.

Implantation was successful in 11 of 13 XABG animals. Chronic aggregated patency was 73% (8/11) at 12-months. After an initial decrease in lumen diameter, the XABG had a stable luminal size at 3-months and onward, while SVG progressively dilated with time. At 12-months follow-up the XABG were characterized by a uniform graft diameter and wide open anastomoses. Mid graft SEM and histology demonstrated progressive graft replacement and a smooth lumen, focally covered by cells having vWf-positive immunohistochemical staining, suggesting endothelial cells.

Based on these results, a first-in-human trial is currently underway.

These studies demonstrate the potential of restorative polymer-based approaches to CABG in humans, potentially offering an off-the-shelf alternative to harvesting veins from a patient's leg.

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Tailored Endothelialization Accelerates Remodeling of Small-diameter Vascular Grafts

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Current gold standard for the replacement of small-diameter blood vessel (ID<4 mm) is still to utilize the autologous vessels of patients due to the limitations of small-diameter vascular grafts (SDVG) on weak endothelialization, intimal hyperplasia and low patency. Herein, we create a SDVG with the tailored endothelialization by applying the engineered endothelial cell vesicles to camouflaging vascular grafts for the enhancement of vascular remodeling. The engineered endothelial cell vesicles were modified with azide groups (ECVs-N_3) through metabolic glycoengineering to precisely link the vascular graft made of PCL-DBCO via click chemistry, and thus fabricating ECVG (ECVs modified SDVG), which assists inhibition of platelet adhesion and activation, promotion of ECs adhesion and enhancement of anti-inflammation. Furthermore, *In vivo* single-cell transcriptome analysis revealed that the proportion of ECs in the cell composition of ECVG surpassed that of PCL, and the tailored endothelialization enabled to differentiate endothelial cell into some specific EC clusters. One of the specific cluster, Endo_C5 cluster, was only detected in ECVG. Consequently, our study integrates the engineered membrane vesicles of ECVs-N_3 from native ECs for tailored endothelialization on SDVG by circumventing the limitations of living cells, and paves a new way to construct the alternative endothelialization in vessel remodeling following injury.

Tissue Engineered Blood Vessels to Examine Inflammation in Cardiovascular Diseases

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We developed tissue engineered blood vessel (TEBV) microphysiological systems to examine the role branched chain amino acids (BCAA) and rheumatoid arthritis (RA) in vascular inflammation and atherosclerosis. To address the effect of BCAA on vascular function and inflammation, TEBVs were perfused for 7 days in media replicating normal BCAA levels then perfused with U937 monocytes in 1X or 5X BCAA and 0, 10, or 50 μg/mL oxidized low-density lipoprotein (oxLDL) for 48 h. 5X BCAA and 10 μg/mL oxLDL treatment led to significantly lower vasodilation compared to 1X BCAA. Additionally, 5X BCAA and 50 μg/mL oxLDL treatment had significantly lower vasodilation compared to 1X BCAA with or without 50 μg/mL. Treatment with 5X elevated BCAA and oxLDL significantly increased monocyte adhesion in TEBVs. The results suggest that BCAA have an additive or synergistic effect on endothelial health and early events in atherosclerosis. To examine the role of RA muscle inflammation on vascular functions, TEBVs and engineered human skeletal muscle (myobundles) were fabricated separately and perfused together for 96 h. TEBVs integrated with RA myobundles had greater monocyte accumulation than TEBVs integrated with age-matched control myobundles or TEBVs cultured alone, suggesting higher inflammation in TEBVs integrated with RA myobundles. After 96 h perfusion, RA myobundles alone or integrated with TEBVs had much higher IL6 levels in the perfusion media than age-matched control myobundles perfused with or without TEBVs, whereas TNFα and IL1β were not detectable under either condition. IL6 plays a key role in inflammation and plaque development in atherosclerosis, suggesting that elevated levels of IL6 in RA influence the initiation of atherosclerosis. These studies show that human microphysiological systems can identify specific inflammatory interactions that affect vascular disease. Supported by grants from UH3TR002142, F31HL162539 (RJJ), U2CAG088071, AHA 24PRE1189799 (MX) and the International Federation for Ethical Research.

Title: Prognostic Value of ^{18}F-NaF PET/CT Imaging for Predicting 1.5-Year Progression of Vascular Calcification in Peripheral Artery Disease

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Background: Vascular calcification in peripheral artery disease (PAD) decreases muscle perfusion, limits options for revascularization, and contributes to poor clinical outcomes. Thus, there is a need for methods that predict risk of vessel-specific calcium progression in PAD. ^{18}F-NaF PET/CT imaging detects the active process of arterial microcalcification, but its ability to predict disease progression in PAD remains understudied.

Objective: We sought to determine if arterial retention of ^{18}F-NaF on PET/CT images measured on a vessel-by-vessel basis would be associated with and predict increases in CT-detectable arterial calcification 1.5 years later in PAD patients.

Methods: PAD patients (n=28) underwent baseline ^{18}F-NaF PET/CT imaging and follow-up CT imaging 1.5 years later. Arteries of interest (femoral-popliteal, anterior tibial, tibioperoneal trunk, posterior tibial, peroneal) were manually segmented and arterial retention of ^{18}F-NaF was quantified for individual arterial segments. Serial images were co-registered to quantify slice-by-slice changes in calcium mass. Each arterial segment was categorized as having 1) no calcium progression or 2) calcium progression. Multivariate linear regression was used to assess the association between baseline retention of ^{18}F-NaF and CT-detectable calcium progression after adjusting for age, sex, and body mass index. Area under the curve (AUC) for the receiver operator characteristic (ROC) curve of each artery was calculated to assess the discriminability of predicting calcium progression using ^{18}F-NaF uptake.

Results: ^{18}F-NaF uptake was significantly and positively associated with calcium progression in each artery of interest (each artery, $p<0.0001$). ROC analyses revealed variable vessel-specific results for PET imaging discriminating calcium progression: AUC=0.89 (anterior tibial), 0.87 (posterior tibial), 0.77 (peroneal), 0.75 (tibioperoneal), 0.63 (femoral-popliteal).

Conclusions: ^{18}F-NaF PET/CT imaging detects the active process of vascular calcification in PAD and is associated with and discriminates vessel-specific calcific disease progression 1.5 years later, thus providing an imaging tool for monitoring PAD pathophysiology and responses to emerging anti-atherogenic therapies.

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EGFR inhibition prevents CAV1-dependent calcifying extracellular vesicle biogenesis in the vasculature at different time points both *in vivo* and *in vitro*.

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Vascular calcification represents the most significant predictor of cardiovascular events with no current therapeutic options for prevention or treatment. Osteogenically-differentiated vascular smooth muscle cells (VSMCs) release calcifying extracellular vesicles (EVs), which nucleate nascent mineral. Caveolin-1 (CAV1), a plasma membrane scaffolding protein residing in caveolar domains, plays a critical role in the formation of calcifying EVs. Previous studies have reported interactions between CAV1 and epidermal growth factor receptor (EGFR) in cancer pathology. Given the nature of these reported interactions, we hypothesized EGFR inhibition may prevent the biogenesis of calcifying EVs by altering CAV1 trafficking. We assessed the potential of EGFR tyrosine kinase inhibition (AG1478 and PD153035, 2.5 uM, N = 3) to prevent calcification in vitro using VSMCs cultured in osteogenic media (OS) for 7, 14, or 21 days. In vitro, EGFR tyrosine kinase inhibition significantly prevented calcifications in OS cultures treated for 21 days ($p < 0.0001$ and $p < 0.0001$), 14 days ($p < 0.0001$ and $p < 0.0001$), and 7 days ($p < 0.01$ and $p < 0.01$). In vivo, EGFR inhibition (PD153035, 10 mg/kg, N = 40) was administered to treat calcification using a chronic kidney disease (CKD) diet model to induce medial calcification at different progressions of the disease. In vivo, calcification was significantly decreased by EGFR inhibition ($p < 0.01$) when administered as a preventative measure. Our results suggest that EGFR interferes with trafficking mechanisms that are required for calcifying EV biogenesis no matter when administered in the progression of calcification. EGFR inhibition effective at preventing calcification prior to pro-calcific stimuli in vitro and in vivo and following initiation of calcification in vitro. Future studies will investigate the interactions of EGFR and CAV1. Given that EGFR inhibitors exhibit clinical safety, the current data show that EGFR may be a propitious target in preventing vascular calcification.

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Mitochondrial and Metabolic Therapies for Heart Failure Based on the Regenerative Potential of the Pediatric Heart

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Heart disease due to ischemia is the leading cause of death worldwide. In a failing heart, metabolism often shifts from oxidative phosphorylation (OXPHOS) to non-oxidative glycolysis, reverting to a fetal phenotype. Restoring an ATP-rich oxidative metabolism could improve cardiomyocyte function and offer a novel therapy for heart failure. Mesenchymal stromal cell-derived paracrine factors (secretome) have shown benefits in heart disease models, both pre-clinically and clinically. Our study aimed to assess the impact of secretome from pediatric cardiac mesenchymal stromal cells on the metabolic phenotype and contractility of cardiomyocytes in an *in vitro* cardiac ischemia model. A secondary objective was to identify secretome proteins and determine if individual proteins could potentially replicate these effects.

Cardiac tissue samples from pediatric hearts (collected during open heart surgery) were cultured, and their secretomes were collected. In the ischemic model, iPSC-CMs(iCMs) were incubated under 1%O_2 with minimal media for 24 hours with or without patient secretome. Mitochondrial activity was measured using respirometry, and iCM contractility was assessed with a custom video processing assay. Protein constituents of the secretomes were identified using mass spectrometry (MS) and lentiviral vectors were generated with the potential candidates for overexpression of the protein in iCMs.

Secretome treatment significantly enhanced iCM contractility($p<0.0001$). Coupled with the improvements in contractility, we observed enhancement of mitochondrial respiration including basal respiration($p<0.05$), maximal respiration($p<0.05$), and mitochondrial ATP production($p<0.05$) after ischemic stress. Protein candidates overrepresented in the secretome proteome were delivered to iCMs using lentiviral vectors. This overexpression resulted in a 10% increase in iCM contractility.

Our results indicate that pediatric cardiac mesenchymal stromal cell-derived secretome can enhance oxidative capacity and cardiomyocyte function. Preliminary data suggest that therapeutic proteins from the secretome, when overexpressed, improve iCM contractility. Future experiments will focus on encapsulating these proteins in LNPs for delivery to evaluate contractility and metabolic function in iCMs.

A minimal-invasive implantation of haem-scavenging microsponges protects heart against ferroptosis-induced reperfusion injury

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Background: Myocardial ischemia/reperfusion injury with a high incidence of intramyocardial haemorrhage (IMH) contributes to enlarged infarct size by inducing additional cell death, and predisposes risk of heart failure, However, the risk factors in blood have not been completely verified or addressed.

Objective： We regarded the pivotal role of haem in IMH-induced cardiac ferroptosis, and developed a haem-sequestering implant, ferroptosis-inhibiting lactoferrin microsponges (FILMS), for treating I/R injury.

Methods: To find the main risk factor in blood, the different components in blood were administered to ischemia left ventricle (LV) to reproduce l/R injury in an MI model. We examined all reported haem-scavenging proteins in terms of their haem-binding affinity and capacity through the surface plasmon resonance (SPR) technique. Apo-lactoferrin was screened out and modified into a UV-curable engineering protein for the microfluidic fabrication of ferroptosis-inhibiting lactoferrin microsponges (FILMS). To show its potential in clinical translation, a surgery of transendocardial FILMS implantation through the marketed transcatheter injection system was displaced in the endocardium of a Chinese white pig (65 kg). The anti.ferroptotic effects of FILMS were confirmed in both rat and porcine cardiac l/R models.

Results: Haem was confirmed as the major source of exogenous iron contributing to I/R-induced ferroptosis. The modified Apo-Lf with the record-breaking high haem affinity and binding capacity, and its final product, FILMS, showed an excellent haem-trapping capability. Intramyocardially injected FILMS locally scavenged haem from IMH, hence lowering levels of free haem and intracellular iron and inhibiting lipid peroxidation (a land marker of ferroptosis) in both rat cardiac I/R models. Transendocardial FILMS implantation in the left ventricle wall was successful without leakage, bleeding or wall perforation. FILMS recovered the infarcted size, level of myocardial enzyme markers, and cTnI in serum, the amount of haem, the expression of HMOX1 and FTL, the cardiac total iron content and lipid peroxidation nearly to

Long-Term Tolerance to Heart Allografts Through Selective Expansion of regulatory T cells

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Introduction
Regulatory T cells (Tregs) are essential for suppressing auto- and alloimmune responses, and their expansion can promote immunological tolerance. Selective *in vivo* expansion of Tregs can be achieved by treatment with interleukin-2 (IL-2) complexed to a specific anti-IL-2 antibody (IL-2 cplx). This study aimed to induce long-term heart allograft tolerance in mice by combining IL-2 cplx treatment by preventing acute T cell mediated rejection and the development of donor specific antibodies (DSAs), which are associated with cardiac allograft vasculopathy and late graft failure.

Methods
C57BL/6 mice were transplanted with fully mismatched BALB/c hearts and treated with IL-2 cplx, rapamycin, and anti-IL-6. Cardiac allograft survival was monitored using the palpation score, with the loss of a palpable heartbeat indicating the day of rejection. Treg frequency in the blood during the follow-up period was analyzed by flow cytometry. Flow cytometry crossmatch (FCXM) was performed to detect donor-specific antibodies (DSA).

Results
Notably, the IL-2 cplx protocol treatment group exhibited indefinite survival (>150 days), while the mean survival time (MST) for fully mismatched untreated recipients was only 7.5 days ($p < 0.0001$). Treg frequencies within CD4+ cell populations in the blood were markedly increased compared to untreated heart graft recipients on both day 21 post-HTX ($p=0.01$) and day 28 post-HTX ($p=0.008$). Furthermore, on day 150, treated allograft recipients had significantly lower levels of DSA IgG1 ($p=0.0002$) and almost no IgG2ab ($p<0.0001$) compared to untreated graft recipients.

Conclusion
Recipient mice treated with short term IL-2 cplx, rapamycin and anti-IL-6 exhibited prolonged graft survival and significantly reduced levels of DSA even after 100 days, suggesting the induction of humoral tolerance. Our findings indicate that the IL-2 cplx-based treatment promotes immune tolerance in heart transplant recipients without continuous immunosuppressive treatment.

Sex-Based Machine Learning Models for Prediction of Abdominal Aortic Aneurysm Patient Outcomes

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Abdominal aortic aneurysm (AAA) is a localized dilation of the infrarenal aorta that is devastating when ruptured, with up to a 90% mortality rate reported. Although male patients have a higher prevalence rate of AAA, female patients have an AAA rupture rate 3-4 times higher that of males. Clinicians utilize the maximum diameter for deciding when to repair AAAs, but ruptures below the threshold have been reported. Our group has trained machine learning (ML) classification models that predict AAA outcomes using clinical, biomechanical, and morphological indices with better discriminability than maximum diameter. However, these models did not account for sex-based differences. We aim to test whether striating ML models based on sex improves predictions. Sex-specific ML models were trained, and 20% holdout testing was performed. Receiver-operator characteristic curves and confusion matrices were generated to assess model performance. All area under the curve (AUC) values reported are from the testing group. For this cohort of patients (n=150 female, 378 male), the sex-specific models produced an AUC of 0.98 and an accuracy of 83% for females and an AUC of 0.83 and an accuracy of 79% for males. This compares to an AUC of 0.86 and an accuracy of 78% for a general model for both sexes. This general model applied to females alone had an AUC of 0.72 and an accuracy of 79%, and for males alone an AUC of 0.71 and an accuracy of 77%. Further, the sex-specific model was better at predicting unstable AAA in females, with an accuracy of 100% compared to 60% accuracy for the general model. All models outperformed diameter alone, which has an AUC of 0.65 for females and 0.58 for males. ML models show promise in improving outcome prediction for AAA.

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Title: Hemodynamics of Acute Type B Aortic Dissections with 4D Flow MRI

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Background: Type B aortic dissections (TBAD) develop from a tear in the intimal layer of the descending thoracic aorta. In cases of surgical intervention, treatment for TBAD after the acute phase (> 14 days) is associated with worse outcomes. Identifying patients at risk for growth before the chronic phase could improve intervention timing. Therefore, the goal of this study is to extract blood flow metrics for risk stratification during the acute TBAD phase using 4D flow MRI. We identified possible markers of interest: entry tear velocity, pulse wave velocity (PWV), and wall shear stress (WSS).

Methods: TBAD patients (n = 7; 3 F) from Emory University Hospital were enrolled. We acquired 4D flow MRI during the acute phase (3T Prisma Fit; Siemens Healthcare). We created image-derived 3D models of lumens and measured velocity at entry tears. We estimated PWV using cross correlation of waveforms at perpendicular planes throughout the TL. We estimated WSS throughout the FL using a previously developed method (Matlab, Ansys EnSight). Growth rates were measured from follow up CT exams.

Results: Three of the patients (P2, P3, P7) did not grow, while P1, P4-P6 grew 2.1, 1.2, 5.3, and 2 mm/month, respectively. Peak entry tear velocities were higher in growth (143 cm/s) than non-growth (90 cm/s) cases, with P5 having the highest (180 cm/s). PWV measurements between growth and non-growth were similar (4.5 and 3.5 m/s). Both averages were lower than PWV for non-dissected aortas. Average WSS values revealed no trends between cases. However, regional examination revealed regions of high WSS in TL and FL near tears, particularly in the FL opposite the tear in the largest growth case (P5).

Conclusion: We anticipate stronger trends as enrollment continues, but our preliminary findings demonstrate the first MRI study to investigate acute TBAD subjects and identify markers of interest.

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Mechanical Behavior of the Dissected Aortic Media During Radial Extension

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Background. Radial tensile strength of the aorta may be significantly reduced due to pathological conditions that predispose patients to dissection – one of the most devastating complications of thoracic aortic disease. The mechanical behavior of arteries under such loading has rarely been investigated.

Objective. We compare how the global and local mechanical response of the aortic media differs between dissected human and healthy porcine aorta using optical coherence tomography (OCT) and digital volume correlation (DVC).

Methods. Specimens of the aortic media were mounted on the holders, the test chamber was filled with 0.9% PBS for rehydration, and a 4 × 1 × 1.6 mm (radial × circumferential × axial) volume was imaged with OCT. After 5 preconditioning cycles, radial displacement was applied in 100µm increments until failure and the volume was imaged immediately after each increment while force was recorded. DVC was then applied to the volumetric images using DaVis 8.4 (LaVision) to obtain the displacement fields, and Green-Lagrange strain fields were derived using a custom Matlab code.

Results. The loading curves initially showed a plateau, then the stress increased continuously to failure with the subsequent loading steps. Dissected tissues failed at lower loads, voids that merged into larger defects appeared before the peak force during the initial loading steps. DVC revealed complex deformations and early strain localizations.

Conclusions. Healthy porcine aortas can withstand significant radial stretches, likely caused by sucking up fluid. Large radial stretches also lead to induce localization effects and ultimately to mechanical damage, which, like GAG pools, can play an important role in triggering a dissection. Ongoing microstructural studies can provide information about the influence of pathological structural changes on the global response and the local deformations.

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Conflict of Interest. None

Title: “Attractive” Treatment for Abdominal Aortic Aneurysm Repair: Magnetic Localization of Silk-Iron Packaged Extracellular Vesicles

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Background: Abdominal aortic aneurysm (AAA) features a degradative environment and loss of vascular elastin, which we believe could be treated with an engineered regenerative therapy. Our group and others have investigated using mesenchymal stem cells (MSCs) and MSC-derived extracellular vesicles (EVs) for regeneration, but a key barrier to successful implementation is delivery. To address this problem, we have developed an EV carrier and delivery system that can incorporate, magnetically localize, and release EVs.

Hypothesis: Silk-iron packaged extracellular vesicles will display magnetic properties and release EVs for uptake by vascular smooth muscle cells (SMCs).

Methods: Silk and iron oxide nanoparticles were chemically conjugated and mixed with potassium phosphate buffer, with or without EVs isolated from MSCs, to create silk-iron packaged extracellular vesicles (SIPEs) and silk-iron microparticles (SIMPs), respectively. SIMPs were tested for cytotoxicity and for magnetic movement through solution and hydrogel. EVs were characterized with Western blot and nanoparticle tracking analysis. SIPEs and SIMPs were incubated over 7d and releasates analyzed with flow cytometry for CD63, a marker for EVs or co-cultured with SMCs in fibrin gels for 6h and 24h to assess EV uptake.

Results: SIMPs showed enhanced magnetic movement in both solution and hydrogel compared to silk microparticles. SIMPs also showed low cytotoxicity compared to cell death controls (94.7±5.3% live cells, $p=0.0010$ vs. 73.3±5.9). EVs were CD63+ and showed expected size and morphology. SIPEs showed increased release of CD63+ particles over 7d compared to SIMPs. Co-culture of SIPEs with fibrin gels demonstrated better uptake compared to SIMPs, and higher uptake signal at 24h compared to 6h.

Conclusions/Interpretations: Silk and iron can form magnetic microparticles, and EVs can be released from SIPEs to be taken up by SMCs. A magnetic silk delivery system is a novel approach to localizing and releasing EVs to target tissue for regenerative repair.

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Cardiac Tissue Engineering with Metamaterials to fabricate Robust and Contractile Cardiac Tissue Patches

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Ventricular Septal Rupture (VSR) is a challenge in cardiac medicine, with a high mortality rate of 45-60%. Current treatment methods use bovine pericardial patches (BPPs), which are non-contractile, tend to calcify over time and fail to integrate effectively with the myocardium. Therefore, patients do not tend to recover cardiac function fully. To address these limitations, we are engineering a cardiac tissue patch that uses human stem cell-derived cardiomyocytes in the hydrogel, reinforced with a metamaterial lattice. This approach allows us to tune the patch's mechanical properties and contractility, while enabling stable implantation within the intraventricular space. Here, we will showcase our current results on metamaterial design and manufacturing, mechanical characterization (tunable stiffness and anisotropic ratio), and biological characterization (biocompatibility, cell maturation, and tissue contractility). In summary, we will show how metamaterials can be combined with engineered cardiac tissues to fabricate centimeter-scale, three-dimensional, and implantable cardiac tissues.

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Fish swim bladders as valve leaflets enhance the durability of transcatheter aortic valve replacement devices

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Background: Transcatheter aortic valve replacement (TAVR) has emerged as an effective therapy for the inoperable patients with severe aortic stenosis (AS). However, the calcification induced limited durability has restricted its application in younger patients. Fish swim bladders (FSB) which are composed of collagen, GAGs and elastin, in addition exhibiting well hemocompatibility and resistance to calcific degeneration offer a viable solution to this challenge.

Purpose: To verified its feasibility of fish swim bladders as valve leaflets for TAVR device, we test its mechanical properties including suture retention strength, ultimate stress. In addition, the hydrodynamic properties and *in vitro* durability, as well as *in vivo* performances and size selection of this TAVR valve were assessed by experimental study and finite element analysis.

Methods: First, the FSB films were prepared by decellularization and crosslink process. And mechanical properties were assessed. Then self-expandable TAVR device was fabricated by suturing the FSB films into a 23 mm nitinol alloy framework. Further, hydrodynamic performance, such as EOA, TPD and RF, and durability were tested by the pulsatile flow test and accelerated fatigue test, according to the ISO5840:2021. Further, the effect of compression on the hydrodynamic performance was also investigated by using experiment study and finite element analysis. At last, the performances of this TAVR device *in vivo* were examined by a porcine implantation model.

Results: The strength of FSB films met the requirements for valve leaflets. The hemodynamic performance of FSB self-expandable TAVR device was that EOA about 1.82 cm^2, TPD 10.80 mmHg, and RF 5.36%. After 400 million fatigue tests, FSB was no fiber loss, torn, perforated or other valve failure phenomena. After implanted into 2 adult pigs by a transapical route, the devices were positioned well, good functionality and no stenosis immediately after operation. And the mean transvalvular pressure gradients were very low.

Conclusions: we successfully developed a TAVR device with fish swim bladder as valve leaflets, which has good fatigue resistance and maintains good performance after a certain degree of compression.

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DESIGN, DEVELOPMENT, AND FABRICATION OF A BIORESORBABLE POLYMERIC HEART VALVE STENT USING 3D PRINTING

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Transcatheter aortic valve implantation (TAVI) has been developed as a minimally-invasive valvular replacement method for high, intermediate, and now also low-risk patients suffering from severe aortic valve stenosis. Stent devices are crucial for a successful TAVI, for a correct valve delivery, and for its structural support after deployment. Currently, metallic stents are the preferred choice in clinics, but they lack growth and remodeling capacities, leading to long-term drawbacks, such as hyperplasia, thrombosis, and infections. Here, we used bioresorbable polymers to develop a transcatheter aortic valve stent with fine-tuned mechanical properties and a biodegradation rate that favors growth, engraftment, and low risk of thrombus formation and infections. For this purpose, a mixture of two established polymers for medical applications was selected and characterized. Results showed that this blend is a good candidate, because of its promising mechanical properties, appropriate weight loss, biocompatibility, and hemocompatibility. Next, the copolymer was used as bioink for stent fabrication using a four-axis 3D printer that features a single rotational axis which allows the production of precise and customizable stents with different sizes and shapes. Two different stent designs were developed and showed crimpability while maintaining suitable mechanical properties compared to the control metal stent. To evaluate the feasibility and safety of the polymeric heart valve stent, stents were successfully crimped, loaded into a delivery device, and implanted transapically in an acute pig in-vivo study. Angiography and echocardiography revealed no signs of stenosis, and the stents remained securely anchored in all animals. These findings confirm the potential of 3D-printed biodegradable polymeric stents for use as heart valve stents in TAVI. The successful application of this technology opens new possibilities for interventional therapies, offering patients safer and more effective options for heart valve replacement.

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In vitro and in vivo testing of a novel, fully absorbable magnesium-based annuloplasty ring with inert antibacterial properties

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Abstract

Introduction

Infective endocarditis poses an increasingly prevalent, often fatal complication frequently associated with severe SARS-CoV-2 infections. Currently used annuloplasty rings for surgical valve repair do not offer antibacterial features, therefore leaving systemic antibiosis as the only additional treatment. In this study we aimed to develop a fully absorbable magnesium (Mg)-based annuloplasty ring covered with silver nanoparticle (AgNP)-loaded polycaprolactone (PCL) providing potent antibacterial activity. Initially, in-vitro and in-vivo biocompatibility, mechanical and degradation studies of the Mg alloy ZX00 and the AgNP-PCL coverage were performed. Finally, long-term functionality of the annuloplasty rings is being assessed in a sheep model.

Methods

In-vitro biocompatibility, inflammatory, degradation and hemocompatibility assays were performed. Human umbilical vein endothelial, human foreskin fibroblasts and macrophages were utilized. ZX00 was implanted (as a rod) subcutaneously in rats (5 months follow-up) for histopathological, degradational (µCT), immunohistochemistry, inflammatory (qPCR) and mechanical bending assessments. Electrospun AgNP-PCL was tested for antibacterial activity using two endocarditis pathogens. To ensure implant functionality, cardiopulmonary bypass surgery was performed to implant the rings in sheep hearts (n=5, 1 year follow-up). Diastolic and systolic heart function (echocardiography) and blood chemistry were assessed.

Results

Ultimate iin-vitro and in-vivo biocompatibility of ZX00 and AgNP-PCL and antibacterial activity of AgNP-PCL were demonstrated. Implants degraded homogeneously. Rings were implanted successfully in a sheep model with no implant failure. Follow-up for one year is ongoing. Echocardiography shows preserved mitral valve function. Blood chemistry outcomes were positive.

Protein-engineered Elastin-Like Fibers for Biomedical Textile Applications

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Elastin plays a vital role in the functionality of tissues, facilitating their ability to expand and contract. The absence of elastin in medical implants often leads to poor performance, as these implants failing to emulate the dynamic properties of natural tissues. This work tackles this issue by creating elastin fibers through recombinant technology and assembling them into textiles that emulate the hierarchical and anisotropic characteristics of natural tissues.

In this study, elastin-like fibers were produced through a process involving using elastin-like recombinamers (ELRs) through microinjection molding and a catalyst-free click-chemistry cross-linking process. These fibers' microstructures were analyzed with confocal microscopy and scanning electron microscopy (SEM), while their mechanical properties were investigated through tensile testing. The interaction between the fibers and primary endothelial cells was evaluated in both static and dynamic conditions. The potential for sterilizing the elastin-like fibers and incorporating them into textile assemblies was also examined.

The resulting engineered elastin-like fibers achieved lengths of over 3 meters and were able to be adapted for salt-leaching and gas-foaming techniques, offering control over their molecular configuration, length, diameter, and porosity. These fibers exhibited exceptional stretchability (up to 500%) and recoil properties. They also showed flexibility, thermal stability, and supported cell adhesion and alignment. The textile assembly of these fibers enabled the creation of intricate patterns, providing a foundation for sophisticated textile constructs. Moreover, the fibers are capable of being autoclaved and have a storage lifespan exceeding two years, highlighting their potential for clinical use.

By merging recombinant ELRs with textile assembly, our approach enables the creation of elastin-like textiles that are customizable, reproducible, and storable, thereby broadening significantly the possibilities for next-generation medical textiles.

Acknowledgements:

Customized *In Situ* Small Caliber Vascular Graft Manufacturing with Focused Rotary Jet Spinning

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Cardiovascular disease remains the leading cause of death and revascularization strategies are common treatment options. Therefore, the demand for off-the-shelf vascular grafts for replacement or bypasses is high and continues to rise. Focused rotary jet spinning (FRJS) is a technique that can produce customized fibrous structures depending on the shape of the collector. In this study, vascular grafts were manufactured from the biodegradable polymer poly(l-lactide-co-ε-caprolactone) (PLCL) using FRJS. Customized grafts were fabricated across ranges of inner diameters (0.5-6 mm), wall thicknesses, (100-300 um), and lengths (up to 21 cm). Internal pressure, tensile properties and suture retention were investigated to confirm structural stability prior *in vivo* implantation. FRJS vascular grafts maintained or showed improved functional performance compared to the native vessels. In a rat model, vascular conduits were implanted into the femoral vein (n=6) or femoral artery (n=6) for a maximum of 28 days. Blood flow rates, hemoglobin content and oxygen saturation levels showed the functional performance. Histological evaluation confirmed initial cell infiltration.
These findings indicate that FRJS is a viable method for manufacturing customized small caliber vascular grafts that are off-the-shelf available and may improve patient's outcome.

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Title: Engineering Bioelectric Threads from hiPSC-derived Cardiomyocytes to Direct Cardiac Conduction Pathways

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Heart attack survivors live with permanent damage in their hearts and are at higher risk of developing heart failure. Implanting engineered cardiac tissues (ECTs) to restore contractility to the damaged myocardium is a promising therapy but is challenged by poor integration with the host heart. To address this issue, we engineer a bioelectric thread capable of propagating electrical signals to improve coupling between the two interfaces. We differentiated high purity cardiomyocytes (CMs, >85% $cTnT^{+}$) from human-induced pluripotent stem cells (hiPSCs) and seeded them onto fibrin threads. We investigated how cell density (low vs high), electrical stimulation (constant vs ramp pacing), and media compositions (RPMI/B27 vs maturation media) impacted electrical maturation over 3 weeks of culture. Optical mapping was performed to characterize electrical properties and assess *in vitro* coupling. Bioelectric threads are electromechanically active with action potential durations (APDs) ranging from 350 to 650 ms and conduction velocities (CVs) ranging from 25 to 65 mm/s. Increasing pacing frequency shortened APD and increased CV in a rate dependent manner. Low density threads cultured in maturation media under ramp pacing protocol produced the highest quality threads, with the shortest APDs, fastest CVs, and highest maximum capture rates (MCRs) that mimic those reported in the human heart. α-actinin and connexin-43 staining revealed increased hiPSC-CMs alignment along the thread with significant gap junctions, suggesting an electrical syncytium. Bioelectric threads sutured to two separate ECTs electrically coupled within 3 days, enabling directed electrical propagation from one tissue to the other. Ongoing work focuses on an injection-based delivery system for *in vivo* translation. Taken together, bioelectric threads have tremendous potential for revolutionizing treatments of cardiac conduction disorders as they hold potential to facilitate electrical integration, reduce arrhythmias, and improve cardiac function. This work is funded by an AHA Transformational Project Award and NIH R21. **Financial Disclosures:** None.

Bioengineering Immune Shielded Living Heart Valves for Pediatric Valve Pathologies

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Background: Children with heart valve disorders risk the development of heart failure and require valve replacement with cryopreserved donor valves as the current best alternative. However, these are avital and cannot grow. Living donor valves would offer excellent outlooks for growth but face immune rejection. We have previously shown human pancreatic cells implanted in the murine kidney can be shielded from immune rejection by introducing human cytomegalovirus encoded US2.

Objective: Here, we pioneer its adaptation in living human heart valve tissue to yield a more durable transplant in children.

Methods: Using lentiviral (LV) delivery of viral genes that mediate immune evasion (US2/Serpin 9), we transduced human heart valves and assessed transduction efficiency, viability, and HLA expression throughout the valve leaflet and assessed valve immunogenicity *in vitro*.

Results: Human aortic and pulmonary valve endothelial cells (hVECs) and interstitial cells (hVICs) were efficiently transduced (HLA-A2 hVIC: 29±3% [US2-Spi9] vs 77±5%[Control]; $p<0.001$). Heterogeneous HLA cell populations were observed in both older donors (Donor 1-3, >70y) and younger donors (Donor 4-5, <20y) where HLA class I and class II expression could be decreased using two-time centrifugation-based transduction while standardized transduction yielded high variability in HLA suppression amongst donors. HLA levels after heart valve leaflets transduction show efficient HLA-ABC downregulation in the deepest valve layers (700µm: 5.5±1.3% [US2-Spi9] vs 38±5.1%[Control]; $p<0.0001$) without affecting cellular viability (TUNEL/trypan blue). Transduction decreased immune-mediated valve degeneration (decreased activation of HLA-reactive CD8+ T-cells and peripheral blood mononuclear cells (PBMC)).

Conclusions: Here, we pioneer a new technology to improve donor heart valve durability. When successful, this new technology will have large implications for modern transplant technology and may prevent the use of immunosuppressants/anticoagulants and extensive animal testing.

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Preclinical Evaluation of Cell-Assembled Extracellular Matrix Sheets for Surgical Repair of Tetralogy of Fallot

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Tetralogy of Fallot (ToF) is the most common cyanotic heart malformation in newborns. The surgical correction involves enlarging the right ventricular outflow tract (RVOT) using a transannular patch. This approach generates pulmonary valve regurgitation, causing right ventricular arrhythmia and the patient's sudden death in adulthood. A promising surgical technique to limit regurgitation involves inserting a monocusp valve below the transannular patch made of synthetic material or chemically treated animal tissue. However, these materials are ineffective in the short and mid-term because of thromboembolic complications and/or infectious endocarditis. In addition, they cannot grow with the patient, resulting in supplementary surgeries. This project aims to develop and evaluate an innovative, non-chemically denatured, non-living, and completely biological monocusp valve using Cell-Assembled extracellular Matrix (CAM) sheets.

We first demonstrated that a CAM sheet cultured for 16 weeks is compatible with surgical manipulations and sutures required to create a monocusp valve in an explanted animal heart *ex vivo*. Using an organosynthetic dynamic heart model, we evaluated different designs of CAM-based monocusp valves to prevent pulmonary regurgitation and restore baseline hemodynamic pressures. The optimal CAM-based monocusp valve design was implanted in clinically relevant ovine models (n = 3). The effectiveness of the valve was evaluated using epicardial echocardiography, magnetic resonance imaging, and hemodynamic pressure measurements. The CAM-based monocusp valve was hemodynamically effective (transvalvular pressure gradient: 4.3 ± 1.4 mmHg) and provided a competent pulmonary valve (regurgitation: 4.6 ± 0.9 %) after seven days of implantation. Histological staining indicated that the CAM material has integrated well with the surrounding tissue. Immunostaining for CD64 showed only a few macrophages at the boundary between the native tissue and the CAM material, confirming a minimal early immune response.

Finally, we demonstrated the potential of CAM sheets to be used in designing new repair strategies for congenital heart defects.

COMPLIANT AORTIC STENT-GRAFTS ATTENUATE LEFT VENTRICULAR MASS AND PULSE WAVE VELOCITY INCREASES COMPARED TO STIFF COMMERCIAL TEVAR STENT-GRAFTS IN A PRECLINICAL PORCINE MODEL

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Thoracic endovascular aortic repair (TEVAR) using stent-grafts (SG) is the preferred treatment for aortic pathology and trauma, but its long-term effects remain poorly understood.

Yucatan mini-pigs were used to evaluate the effects of TEVAR with conventional and compliant SGs manufactured from electrospun elastomeric material. Animals were randomized into Control (N = 5), Conventional SG (CSG, N = 8), and compliant Nanofiber SG (NSG, N = 5) groups. Devices were deployed in the descending thoracic aorta (DTA). Pressure waveforms were recorded in the mid-ascending thoracic aorta (ATA) and the aorta near the celiac artery pre- and immediately post-implantation, and again at 16 weeks. Aortic and left ventricular (LV) anatomies were assessed via gated Computed Tomography Angiography. Pulse Wave Velocity (PWV) was calculated using pressure waveforms and ATA-celiac distance.

Pre-procedure, there were no differences in PWV (p=0.900), LV mass (p=0.486), or aortic strains (p=0.164) across groups. Following SG implantation, PWV increased 83.9% in the CSG group (4.73m/s to 8.69m/s, p=0.001) and 16.2% in the NSG group (4.69m/s to 5.45m/s, p=0.049). At 16 weeks, PWV was 40.2% higher in the CSG group compared to controls (7.33 vs 5.22m/s, p=0.018) but similar in the NSG and control groups (p=0.981). LV mass of control, NSG, and CSG animals increased respectively by 10%, 11%, and 21% (p=0.086, p=0.072, p=0.002) at 16 weeks. The average LV growth rate was 2.52g/month, 3.42g/month, and 4.86g/month in Control, NSG, and CSG groups, respectively. Aortic strains at 16 weeks were consistent across groups in ATA (p=0.942) and DTA between Control and NSG animals (p=0.201). Strains in the DTA were much smaller in the CSG than in control animals (p=0.001).

TEVAR with conventional SG significantly increased PWV and LV mass and reduced aortic strain. The compliant SG moderated these changes, demonstrating the potential for improved hemodynamics and reduced LV load.

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Discovering the Potential for Using Stromal Vascular Fraction to Form Lymphatic Vessels

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Microvascular therapies involve growing blood and lymphatic vessels. Adipose-derived stromal vascular fraction (SVF) has emerged as a heterogeneous cell population for de novo blood vessel growth, but the potential for SVF to form lymphatic vessels remains unknown. The objectives were to characterize the presence of lymphatic endothelial cells (LECs) in mouse SVF and evaluate vessel formation over time. SVF was isolated from C57BL/6 mouse inguinal adipose tissue and analyzed via flow cytometry for PECAM and lymphatic markers (Prox1, Podoplanin, Lyve-1). For vessel formation studies, SVF was seeded onto avascular mouse mesentery tissues and cultured in MEM + 10% FBS for up to 9 days (0.5 x 10^6 SVF cells/tissue). Tissues were labeled for PECAM plus Lyve-1 or Prox1. To probe non-endothelial cell specific contributions, SVF was harvested from neuron-glial antigen 2 (NG2) -/- mice and seeded onto tissues from WT C57BL/6 mice. The presence of LECs in SVF is supported by the percentages of PECAM positive cells that are also lymphatic marker positive (Prox1: 4 - 11 %; Podoplanin: 15 – 41 %; Lyve-1: 1.2 – 1.3 %). By day 1, SVF cells formed PECAM positive segments. Day 3 tissues displayed cell clusters with segment extensions. At later time points, segments were connected in a network pattern. Lymphatic marker positive structures, termed “blebs,” were characterized by large diameters, connected with SVF derived PECAM positive segments, and changed shape over time. By day 9, structures with typical lymphatic vessel morphology were observed. NG2 -/- SVF resulted in fewer lymphatic structures indicating the importance of non-endothelial cells. The presence of LECs and discovery of lymphatic structures provoke a new research area focused on the ability for SVF to form lymphatic vessels. Our results also suggest that lymphatic vessel formation is dependent on the heterogenous SVF cell milieu. Funding source: NIH grant R21HL159501.

Sex dependent differences in baseline extracellular matrix composition and chronic kidney disease induced aortic valve calcification in mice.

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Background: Recent studies have suggested a sex-dependent differences in the manifestation of calcific aortic valve disease (CAVD), but mechanistic differences remain uncertain. Further, given the difficulty in obtaining non-diseased human aortic valves, sex-dependent differences in baseline extracellular matrix (ECM) composition and structure are unknown.
Objective/Hypothesis: Through this study we seek to elucidate baseline ECM composition differences that may contribute to the sexually dimorphic onset of aortic valve disease in a chronic kidney disease (CKD) mouse model of CAVD.

Methods: Male and female (N = 22 & N = 32 respectively) wild type mice (C57BL6J) were placed on an adenine-rich diet for 6 weeks and a high adenine/high phosphate (CKD+HP) diet for an additional 6 weeks to induce CAVD. Age matched chow-fed mice were used as controls. A day prior to euthanasia, mice received tail vein injections of OsteoSense 680x to visualize and quantify aortic valve leaflet (AVL) calcification. We stained tissues with fluorescent collagen and elastin probes (CNA35 OG 488 & Alexa Fluor 633 Hydrazide). A custom MATLAB script was used to quantify calcification, elastin, and collagen abundance.

Results: Irrespective of diet condition (CKD+HP or chow), males had more abundant elastin and collagen as quantified by mean fluorescence intensity (MFI) normalized to tissue thickness ($P<0.0001$ & $P=0.0281$, respectively). Male leaflets trended thicker (30.8 um) compared to females (23.6 um) but did not reach significance ($P=0.126$). On the CKD+HP diet, males presented with more calcification (0.061 ± 0.061) compared to females (0.021 ± 0.057) as quantified by fraction positive OsteoSense signal over the area of the AVLs ($P=0.0336$). Compared to chow fed controls, the CKD+HP diet led to a reduction in elastin abundance ($P=0.006$) in male mice only. There was no significant difference or appreciable trends in sex dependent collagen abundance nor in elastin abundance in females between CKD+HP and chow fed conditions.

Conclusions: Baseline differences in ECM composition, such as higher elastin and collagen abundance in males, may result from difference in the biomechanical behavior of the tissues under load as males exhibit higher average systemic blood pressures compared to females. It is possible that elastin fragmentation, because of higher loading conditions in males, serve as nucleation sites for calcific mineral deposition. Further studies are needed to fully assess sex-dependent differences in aortic valve physiology and remodeling.

Funding Sources: Florida Heart Research Foundation Doctoral Student Grant

Unravelling Neointima Hyperplasia: the Role of Flow Disturbances in Endothelial Cell Activation and Matrix (Dis)organization

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Chronic kidney disease significantly reduces life quality, forcing patients to hemodialysis for blood detoxification, thus requiring long-term vascular access grafts. *In-situ* tissue-engineered vascular access grafts (TEVAGs) offer durable solutions, potentially circumventing high occlusion rates caused by neointima hyperplasia (NIH). NIH results from hyperproliferation and migration of (myo)fibroblasts and vascular smooth muscle cells (VSMCs), especially at vein-anastomotic site. However, recent studies still highlight the risk of NIH in TEVAGs, with a critical causal role for the local hemodynamic environment. Non-physiological flow can activate endothelial cells (ECs), further triggering VSMCs migration, proliferation, and promote adverse matrix (dis)organization, thus increasing NIH risk.
Our research aims to understand the role of flow disturbances and neo-tissue organization in NIH emergence in *in-situ* TEVAGs. Our studies concentrate on (i) developing an *in-vitro* model of *in-situ* vascular access graft remodeling, and (ii) understanding the role of the endothelium in early-stage tissue formation and NIH. This is crucial as endothelium is virtually absent in *in-situ* neo-tissue formation, while NIH can still develop.
To set up the vascular graft model, tubular polymeric biodegradable scaffolds were electrospun and seeded with human monocytes (THP-1), with or without a monolayer of primary human umbilical vein endothelial cells (HUVECs) in a fibrin-gel environment. Constructs were cultured statically for 7 days, and subsequently dynamically for 72h under flow conditions mimicking vein-graft local hemodynamic: low shear stress, high shear stress, and high, oscillatory shear stress.
After flow stimulation, constructs showed a near-confluent endothelium layer and homogeneous monocyte penetration, confirming model effectiveness. Current studies are focusing on flow-activated ECs secretion profile and will include, later on, analyses on VSMCs activation and matrix remodeling to understand the role hemodynamics in neo-tissue development and its (dis)organization.

This study is financially supported by the Gravitation Program “Materials Driven Regeneration,” funded by the Netherlands Organization for Scientific Research, grant#024.003.013

Rat infection model supports improved infection resistance of restorative aXess conduits compared to ePTFE controls

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Background: Infection risk is one of the most common and challenging problems in vascular access grafts which are cannulated routinely for dialysis. The synthetic Xeltis access (aXess) graft has a porous structure which allows endogenous cell migration and tissue formation into the scaffold which may prevent and facilitate response to infections.

Methods: A subcutaneous rat infection model was used to assess aXess infection resistance using ePTFE as control. Samples (~2cm^2) of the aXess and ePTFE were implanted bilaterally into dorsal subcutaneous pockets of six Sprague-Dawley rats. Each pocket was injected with *Staphylococcus aureus* (1 x $10^{8 \pm 0.5}$ log colony forming units / 0.25 mL) at implantation. After 14 days the scaffolds were explanted, bisected, and assessed with Microbiology and Histopathology. Microbial recovery was assessed by vortexing, and sonication followed by culture procedure. Microbial recovery results were evaluated using a non-parametric t-test (Mann-Whitney test).

Results: Explanted aXess samples contained significantly less *S. aureus* bacteria than ePTFE controls ($p=0.013$). This finding was supported by Histopathology showing fewer residual bacterial cocci around and within the implant material in aXess implant sites in comparison to ePTFE. Histopathology showed that the aXess graft allowed inflammatory cells, endogenous tissue and neovascularization to infiltrate the structure of the device, while the lower porosity of the ePTFE implants inhibited substantial cell ingrowth. The attraction and migration of inflammatory and immune cells into the aXess material likely contributed to better exclusion of bacteria and reduction of densities of visible bacteria around and within the material at aXess sites compared to ePTFE sites.

Conclusion: The study supports superior infection resistance of aXess compared to ePTFe controls, presumably due to increased permeability of the aXess microstructure for the biological host response. These findings are consistent with low infection rates reported during ongoing clinical investigations of the aXess conduit.

Funding sources: The preclinical study has been funded by Xeltis.

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Spontaneous multifocal metastatic mammary tumors induce gradual development of cardiac remodeling and dysfunction in mice

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Background. Carcinomas alter regular cardiac function through paracrine signaling.

Hypothesis. We hypothesized that advanced growth of murine multifocal metastatic mammary tumors may result in cardiac structural remodeling and functional deterioration.

Methods. A murine model of breast cancer induced by overexpression of polyoma middle T-antigen driven by mouse mammary tumor virus promoter (MMTV-PyMT) was analyzed at early, advanced, and metastatic phases of tumor development, while wild-type littermates served as controls (CTRL). Transthoracic echocardiography was performed under isoflurane anesthesia at all phases. Left ventricular (LV) contractility at the metastatic phase was evaluated using isolated working heart examinations in the absence or presence of OR1896 (500 nM), an active metabolite of calcium-sensitizer levosimendan. Myocardial immune cell infiltration was assessed using flow cytometry and metabolic adaptations were measured by glucose- and fatty acid-related conversion assays in parallel to histopathologic, proteomic and immunoblotting analyses.

Results. Echocardiography revealed LV diastolic dysfunction and preserved ejection fraction in the metastatic phase. Analyses on a working heart apparatus showed significantly lower maximal rate of pressure development, prolonged isovolumic relaxation time, inferior contractile reserve after exposure to gradually increased afterload in the LVs of MMTV-PyMT compared to CTRL. The latter effect was reversible by OR1896 supplementation. Cardiac sarcoplasmic reticulum calcium ATPase (SERCA2a), phospholamban, and mitochondrial calcium uniporter were downregulated in MMTV-PyMT. Cardiac interstitial fibrosis was not increased, but tumor development was associated with significant metabolic reprogramming to favor myocardial glycolytic and suppress fatty acid oxidation rate by concomitant depletion of ATP-generating capacity and infiltration of CD8+ T-cells, dendritic cells, and macrophages.

Conclusions. Advanced mammary tumor development is accompanied by a significant myocardial metabolic shift, impaired LV diastolic function in vivo and suppressed contractile and relaxation capacity ex vivo. Energy-sparing calcium sensitizers that improve LV function warrant new investigation in tumor-burden associated cardiomyopathy.

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The design of a dynamic Arteriovenous Fistula, a Vascular Access only when the patient needs it.

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The durability of arteriovenous fistulae (AVFs) in providing high-flow vascular access is far from optimal. A major limiting factor affecting durability is stenosis and occlusion of the venous outflow tract induced by the supraphysiological and turbulent flow. Another limitation of AVFs in the substantial cardiac burden of the constantly elevated cardiac output. The high flow caused by the AVF is always present, but only necessary during dialysis sessions. By enabling opening and closing of the AVF, the necessary high flow for dialysis will only be present during the ~12 hours of dialysis per week. Circulation can return to close to normal outside these sessions, removing the core issue in vascular access: continuous high flow and turbulence.

A novel, fully implantable device to allow opening and closing of the AVF has been developed. A short piece (<1cm) of standard vascular graft is anastomosed between the vein and artery creating a configuration similar to an side-to-side AVF. The device is sutured to the outside of thegraft and can completely close-off the cross-sectional area between the artery and the vein, normalising the circulation and minimising the risk of thrombus formation. A magnetic drive mechanism placed directly subcutaneously allows actuation with an external set of magnets. This enables non-invasive control of the anastomotic flow.

The system has been tested in a benchtop setting and implemented in a cadaver, illustrating feasibility of non-invasive control. Through a 5mm layer of silicone skin model, the graft can be non-invasively manipulated to block fluid exceeding pressures of 200mmHg, and accurately controlled to any value between 0 and 100% luminal area.

If proven valid, the device should improve AVF outcomes, and reduce cardiac burden and costs of AVF interventions due to stenosis and high flow issues. Ongoing preclinical studies are pivotal to establish long-term functionality and cardiovascular effects.

Clinical Vascular Tissue Engineering under GMP Conditions

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Purpose:

Tissue engineering products for clinical use are regulated as medicinal products in the EU. Therefore they have to be produced under GMP conditions (Good Manufacuring Practise). GMP is an extremly elaborate system which influences all production steps. We had a successful program of endothelialization of vascular grafts for peripheral vascular reconstruction for 24 years. We could demonstrate that endothelialized vascular grafts in femoro-popliteal and femoro-craural positions have a significantly higher long term patency rate than untreated grafts. When the EU regulation came into power, a new GMP facility had to be built, which was fully funded by the City of Vienna. After having obtained the GMP certificate, we could start production in September 2020.

Patients and Methods

Patients suffering from peripheral vascular disease and who had no suitable saphenous vein for surgical reconstruction, were eligible for an endothelialized vascular graft. Endothelialization of vascular grafts is a two stage procedure. In a first step a short segment of a subcutaneous vein was retrieved from patients. From these segments endothlial cell cultures were established and grown to mass cultures. The cells were then confluently seed onto fibrin glue-coated ePTFE grafts and implanted.

Results

73 patients received endothelialized grafts, produced in our GMP facility, in femoro-popliteal (n=63) or femoro-crural (n=10) position. In 43,3 % of cases these grafts were used for reoperation procedures. The overall primary patency rate for all endothelialized grafts was 88,0% at 1 year and 83,1% at 3 years.

Conclusion

The production of tissue engineering products for clinical use under GMP conditions is challanging but possible. We could repeat the outcome of our 24 yearl long endothililization program. We have recently also begun to produce MACT (Matrix Associated Chondrocyte Transplantation) products for cartilage and bone reconstruction.

19th Biennial Meeting of the ISACB
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ALLAN CALLOW MD YOUNG INVESTIGATOR AWARD FINALISTS ABSTRACTS

Novel Surgical Model of Thoracic Aortic Aneurysm Mediated by Vasa Vasorum Inhibition

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Objective:
The adventitial layer of the aorta contains the network of microvessels known as vasa vasorum. Previous work by our team revealed deficient vasa vasorum associated with medial hypoxia and upregulated adventitial thrombospondin-1 (TSP-1), an anti-angiogenic molecule, in human aortic aneurysm. We hypothesize that heightened TSP-1 in the ascending thoracic aorta induces aneurysmal degeneration through its anti-angiogenic effects on the vasa vasorum. We tested this hypothesis in a rabbit model via peri-adventitial delivery of ABT-510, a thrombospondin peptide mimetic.

Methods:
The ascending aorta was exposed through a median sternotomy in New Zealand White rabbits (n=16) and treated with ABT-510 proximally and a vehicle control distally. Echocardiography was undertaken immediately prior to the procedure and 14 days post-operatively. Adventitial vasa vasorum were quantified from H&E-stained sections. Elastin and collagen organization were assessed with Verhoeff-Van Gieson (VVG) and pentachrome staining as well as multiphoton microscopy. Immunodetection of Hypoxyprobe™ and alpha smooth muscle actin (αSMA) were also undertaken to identify hypoxia and smooth muscle cell content, respectively.

Results:
Echocardiography revealed an increase in aortic diameter over 14 days with ABT-510 treatment compared with control (42.27% ± 5.54 vs 20.99% ± 4.10, p=0.004). Histological analysis showed decreased vasa vasorum count (44.7 ± 9.19 vs 85.8 ± 12.89, p=0.048) and decreased vasa vasorum indexed cumulative area (0.013 ± 0.0037 vs 0.021 ± 0.0050 $\mu m^2/ \mu m^2$ adventitial area, p=0.019). Immunodetection of Hypoxyprobe™ showed evidence of increased hypoxia. VVG, pentachrome, and immunodetection of αSMA showed decreased elastin content, increased deposition of ground substance, and areas of smooth muscle cell loss. Multiphoton microscopy demonstrated disorganized and fragmented elastin fiber microarchitecture and decreased collagen content.

Conclusions:
Our study findings demonstrate a novel, clinically relevant model for thoracic aortic aneurysm through vasa vasorum inhibition that recapitulates tissue and cellular features of cystic medial degeneration. This translational model could enable development of targeted, pro-regenerative therapies that restore perfusion to the aortic wall and prevent the degenerative sequelae aortic aneurysm.

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PCSK9 inhibition promotes intraplaque angiogenesis in accelerated atherosclerosis independent of cholesterol

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Introduction
Alirocumab, a monoclonal antibody targeting proprotein convertase subtilisin/kexin type 9 (PCSK9), reduces cardiovascular events in patients with atherosclerosis and cardiovascular bypasses. While its cholesterol-regulating effects are well-established, PCSK9 is also thought to contribute to angiogenic and inflammatory mechanisms.

Objective
We studied the lipid-lowering independent effects of PCSK9-inhibition in accelerated atherosclerosis.

Methods
In 40,829 participants from the UKBiobank (55% women, mean 56.9±8.1 years), we analyzed associations between 2,921 plasma-proteins and incident coronary artery disease (CAD), using Cox-proportional hazard modeling, adjusting for considered confounders including low-density lipoprotein. ApoE3*Leiden mice received a high-cholesterol diet (HCD) with weekly Alirocumab (10mg/kg i.p., n=7) or control-IgG (10mg/kg i.p., n=9), or a moderate-cholesterol diet (MCD) with weekly control-IgG (10mg/kg i.p., n=7). After vein graft (VG) surgery, mice were monitored via ultrasound and photoacoustics. VGs were harvested at 28 days for further analysis. Migration and angiogenesis assays were performed with Alirocumab and TNF-α stimulation.

Results
From the UKBiobank, we identified 287 proteins that differentially associated with incident CAD between individuals with relatively lower ($PCSK9^{lo}$) or higher ($PCSK9^{hi}$) normalized plasma PCSK9. Direct PCSK9hi-to-$PCSK9^{lo}$ analyses revealed overrepresented pathways, including positive regulation of cell migration, cell-vascular wall interactions and cytokine-cytokine receptor interactions. Alirocumab treatment increased endothelial cell migration (36.5%, p=0.035) and aortic angiogenic sprouting (35.4%,p=0.031) in a dose-dependent manner, which TNF-α could negate. HCD-mice treated with Alirocumab and control MCD-mice showed similar plasma cholesterol levels, and reductions (31.8%, 37.0%) in VG wall size after 28 days compared to control-treated HCD-mice (p<0.001). Alirocumab-treated VGs showed an increase in neovessels (58.7%, 68.7%, p<0.05) and in vivo oxygen saturation (53.1%, 43.5%, p<0.05) relative to both control groups, independent of wall lipid content.

Conclusion
Alirocumab-mediated inhibition of PCSK9 in accelerated atherosclerosis reduced vessel wall thickening and increased oxygen saturation and neovascularization in a cholesterol independent manner.

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None.

ISACB 2024 – Call for abstract

First long-term preclinical evaluation of a fully biological, human, woven vascular graft.

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To meet the clinical need for small vascular graft, ***we propose a new textile-based Tissue-Engineered Vascular Graft (TEVG)***. Vessels were produced using Cell-Assembled extracellular Matrix (CAM) synthetized *in vitro*. Study objectives were to 1) develop a miniaturized graft, and 2) assess its behavior in the arterial circulation of immunosuppressed rats to evaluate its *in vivo* functionality.

CAM sheets were produced *in vitro* by human skin fibroblasts (20% bovine serum, 500 mM ascorbate), devitalized (frozen/thawed/airdried) and cut into 2-mm-wide ribbons. Mini-TEVGs (1.6 mm inner diameter) were assembled from CAM-ribbons (35/TEVG) with a custom-made circular loom, then mechanically tested or implanted into the abdominal aorta of immunosuppressed rats. TEVGs were explanted for up to 12 months, and echo doppler was performed before sacrifice. Hematoxylin & Eosin (HE) and Masson Trichrome & Verhoeff (MT) stanning were performed, as well as immunostaining of smooth muscle and endothelial cells, elastin, collagen and macrophages.

Mini-TEVGs with sufficient mechanical properties to support arterial circulation have been produced and successfully implanted in 22 rats, demonstrating graft implantability and absence of transmural leakage. ***After one year, ultrasound images revealed flow through the TEVGs*** (overall patency: 82%) and preservation of the weaving pattern. ***A neo-media*** filling the spaces between CAM ribbons and smoothing the inner surface of the TEVGs ***was observed as early as 1 month***. This neo-media was mainly composed of differentiated muscular cells (MHC+), collagen (col IV+) and elastin (elastin+), ***and was covered by a monolayer of confluent, oriented endothelial cells*** (VWF). The immune response to TEVGs was minimal, in contrast to that of synthetic sutures.

The woven assembly of CAM resulted in a highly promising vascular graft given its excellent stability and biocompatibility. The evaluation of these TEVGs in an allogeneic context is ongoing in sheep.

This work was supported by the European Research Council.

Title: Reverse remodeling of the left ventricle during recovery from pressure overload in a refined murine model

Authors: Elnaz Ghajar-Rahimi[1], Chloé Come[2], Chris Jopling[2], Thomas Moore-Morris[2,3], Pierre Sicard[2], Craig J. Goergen[1]

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Background: 10-15% of adults experience left ventricular (LV) hypertrophy, secondary to pressure overload and a precursor to heart failure [1]. Although treatment may enable recovery and block progression to heart failure, the accompanied reverse remodeling is poorly understood.

Objective: We aimed to establish an experimental model of reversible pressure overload to better characterize the recovery of cardiac function, mechanics, and fibrosis in the failing murine heart.

Methods: We refined a murine model (13-week-old, male, C57BL/6J, n=8) of transverse aortic constriction (TAC) that can be non-invasively reversed via de-banding (deTAC). We performed serial 2D and 4D ultrasound of the LV at baseline, 3-4 weeks (W) after TAC, 1W- and 4W- after deTAC. Ejection fraction (EF), stroke volume, and cardiac output were quantified from 2D images in VevoLab. Geometry and cardiac strain were measured from 4D ultrasound using a custom MATLAB script. Immunohistochemistry and single nucleus-transcriptomics were performed on excised hearts. Statistical significance was assessed by two-way ANOVA with Tukey's test for multiple comparisons ($p<0.05$).

Results: Aortic blood flow velocity immediately normalized from 3.73 $\pm$ 0.39 m/s at 3-4W TAC to 1.78 $\pm$ 0.44 m/s after deTAC. EF decreased from baseline during TAC and recovered by 1W post deTAC (63.39 $\pm$ 5.78% vs. 40.06 $\pm$ 7.35% vs. 54.02 $\pm$ 9.0%). Circumferential and longitudinal strain recovered to approximately 86% of their baseline values. LV mass increased after TAC and remained elevated after deTAC compared to baseline. Histology revealed the presence of activated interstitial cells and cardiac fibroblasts in the myocardium.

Conclusions: We characterized an improved murine model of reverse cardiac remodeling with functional recovery that does not require a second open-chest surgery. On-going transcriptomics analysis will pinpoint the underlying cell-signaling responses. Our findings may uncover cardiac adaptations to pressure overload and guide future treatment strategies.

[1] Cuspidi, *et al.,* J Hum Hypertens. 2012

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Disclosure: Craig Goergen is a paid consultant for FUJIFILM VisualSonics.

Title: Assessing Growth Adaptation of the IRIS Transcatheter Pulmonary Valve for Pediatric Patients: Histopathological Analysis after Six Months

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Background: Around 1% of newborns face congenital heart defects, frequently requiring pulmonary valve replacement due to right ventricular outflow tract dysfunction. However, existing solutions such as the Melody™ transcatheter pulmonary valve (Medtronic Inc., Minneapolis, MN) are not viable for children under 20 kg. Forcing smaller children to wait until they gain enough weight poses risks to the right ventricle. To address, we have developed and are testing the growth-accommodating IRIS transcatheter pulmonary heart valve, suitable for children weighing as little as 8 kg.

Objective: To assess valve integrity and monitor tissue response in Yucatan mini-pigs over a six-month timeframe.

Methods: The IRIS valve has been designed based on origami principles, ensuring its trileaflet form remains fully coapted even in larger sizes. Initial animal implantation studies involved seven Yucatan pigs weighing between 9 and 17 kg, employing both 12-Fr and 14-Fr delivery catheters. As the animals grew, the IRIS valve was balloon-expanded to accommodate growth up to 20 mm. About six months post-implantation, following our IACUC protocol, two animals were euthanized, and their hearts were harvested for histopathological analysis. We excised the right ventricular outflow tract (RVOT) and proximal pulmonary artery containing the IRIS Valve from each heart and embedded them into MMA blocks, followed by sectioning and grinding.

Results: Macroscopic examination reveals the valve leaflets to be intact and securely attached to the stent. H&E images show strong integration of IRIS Valve material into the RVOT wall, with diverse chronic inflammatory response featuring mixed lymphocytes and macrophages, and multinucleated giant cells reacting to both the ePFTE skirt and suture material.

Conclusions: Our findings suggest that the IRIS transcatheter pulmonary valve remains intact over the 6-month study and displays typical chronic inflammatory signs, including mixed lymphocytes, macrophages, and multinucleated giant cells.

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The endomembrane homeostasis controls the phenotype switching of vascular smooth muscle cells

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Background
Vascular calcification contributes to cardiovascular disease. One important process is the phenotypic transition of vascular smooth muscle cells (SMCs) from a contractile to a mineralizing phenotype, releasing calcifying extracellular vesicles (EVs). The endolysosomal system is involved in EV biogenesis and regulates essential cell physiological functions.

We hypothesize that alterations in endomembrane homeostasis affect SMC phenotypic identity and EV cargo, thereby regulating vascular calcification.

Results
In human calcified carotid plaques and calcifying SMCs, the abundance of FYVE-Type Zinc Finger Containing Phosphoinositide Kinase (PIKfyve), an essential lipid kinase, in the endomembrane maturation, is increased. Phosphatidylinositol 3-phosphate (PI3P) - the substrate of PIKfyve - was decreased in cellular membranes of calcifying SMCs (-40%) and recovered by Apilimod, a small molecule PIKfyve inhibitor. In vitro, Apilimod reduced the osteogenic features, like matrix mineralization (-77%), collagen secretion (-99%), and tissue non-specific alkaline phosphatase (TNAP) protein expression (-86%) in calcifying SMCs. Moreover, Apilimod-induced EVs exhibited fewer mineral-positive EVs and reduced aggregation potential (-80%), suggesting diminished EV calcification potential. A proteomic analysis of the EV cargo demonstrated that Apilimod reduces TNAP cargo, which was supported by western blot. In a murine atherosclerosis model, Apilimod reduced aortic calcification assessed by ex vivo osteosense imaging. Transcriptomics revealed phenotypic transitions of calcifying SMCs after PIKfyve inhibition. Apilimod promoted adipogenic markers (PPARG: +218%, FABP3: +2250%), fatty acid uptake (+86%), lipid droplets (OilRed, Raman spectroscopy), and increased the expression of macrophage-like SMC markers (CD68: +500%; LGALS3: +132%), suggesting an alternative SMC phenotype. Kinome and in silico analyses identified YAP1 deactivation as a potential mechanism. Re-activation of YAP1 partially antagonized Apilimod-dependent effects in calcifying SMCs.
Conclusion
PIKfyve inhibition inhibits the osteogenic transition of SMCs while promoting a phenotypic adaptation towards adipogenic/pro-inflammatory SMCs, which is partially mediated by YAP1. The phenotypic identity of calcifying SMCs is sensitive to alterations of the endomembrane homeostasis.

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PIEZO1 EXPRESSION INCREASES UNDER DISTURBED FLOW MODEL OF FEMORAL ATHEROSCLEROSIS

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Background: Peripheral arterial disease (PAD) is the 3rd leading cause of atherosclerotic morbidity. PAD regularly manifests with atherosclerotic plaque formation in the infra-inguinal arteries, severely impacting quality of life. While it is well known that low and oscillatory wall shear stress (d-flow, OS) occurs around and promotes blockages in PAD, *in vivo* mechanistic pathways that unveil therapeutic targets have not been tested. Piezo1 is a mechanosensitive ion channel associated with regulating the endothelial cell (EC) response to d-flow. However, the role of Piezo1 in flow-mediated plaque deposition in femoral arteries is unknown.

Objective: The main objective of this project was to develop a d-flow murine model of atherosclerotic plaque in the femoral arteries (FAs) and to investigate how Piezo1 is involved in flow-mediated PAD.

Methods: S129 mice underwent partial femoral ligation (PFL) imposing d-flow in the left FA (LFA) and conserving the right FA (RFA) as an *in situ* stable flow (s-flow) control. A week prior to PFL, atherogenic conditions were imposed with AV-PCSK9 infection and high fat diet. Ultrasound was used to evaluate the hemodynamic environment. Immunohistochemistry was used to quantify Piezo1 expression. The effect of inhibiting Piezo1 on human aortic endothelial cells (HAECs) exposed to OS was evaluated using an in vitro model.

Results: Post-PFL, blood flow velocity in the LFA was dampened inducing low wall shear stress. Four weeks following PFL, atherosclerotic plaque was formed in the LFA of atherogenic mice. EC expression of Piezo1 increased in the LFA following PFL. Moreover, our in-vitro model showed that acutely inhibiting Piezo1 with GsMTx4 decreased CCN2, a fibrosis marker.

Conclusions: We successfully developed a flow-mediated model of atherosclerotic plaque in murine FAs. This is associated with the upregulation of Piezo1 gene expression. Ongoing work is examining the role of Piezo1 on endothelial response to d-flow.

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POSTER ABSTRACTS

ISACB 2024 ABSTRACT

Loss of NOTCH1 Promotes Impaired Vascular Smooth Muscle Cell Response to Hemodynamic Stress and Ascending Aortic Aneurysms

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Background/Objective: NOTCH1 is a critical cardiovascular developmental gene. Mutations in NOTCH1 are linked to BAV, Ascending Aortic Aneurysms (AscAA), and other of cardiovascular defects in humans. This study sought to investigate the potential mechanisms by which NOTCH1 haploinsufficiency produces the AscAA phenotype and the impact of NOTCH1 haploinsufficiency on vascular smooth muscle cell(VSMC) response to hemodynamic stress.
Methods: Three human induced pluripotent stem cell (iPSC) lines (control, NOTCH1$^{+/-}$, and NOTCH1$^{-/-}$) were generated using CRISPR/Cas9 genome editing and differentiated into VSMCs via second heart field(SHF) lineage. Differentiation of VSMC was performed by adding TGFβ and PDGF-BB to the culture media of SHF progenitor cells for ~12 days. Immunohistochemistry was performed to assess differentiation to SHF progenitors and differentiated VSMCs from each line were characterized for contractile SMC markers by immunofluorescence. Cells were exposed to cyclic stress over 24hrs and RNA collected for Bulk RNA-seq analysis.
Results: Immunohistochemistry staining revealed that expression of SHF cardiac progenitor population markers(ISL1) was not significantly different among control, NOTCH1$^{+/-}$, and NOTCH1$^{-/-}$ mutants cell lines. However, expression of mature SMC-specific contractile proteins(SMA and MYH11) was significantly lower in both NOTCH1$^{+/-}$, and NOTCH1$^{-/-}$ cells compared to control SMCs. Bulk RNA-seq analysis demonstrated a significant impairment of NOTCH1$^{+/-}$ VSMCs to generate a canonical contractile VSMC response to mechanical stress compared to control. RNA expression profiles of NOTCH1$^{+/-}$ VSMCs exposed to hemodynamic stress revealed a more pro-inflammatory/pro- apoptotic response and evidence of VSMC contractile to synthetic phenotype switching.
Conclusions: Our results indicate that loss of NOTCH1 in the human iPSC model did not affect differentiation of iPSCs to SHF progenitor cells but is crucial for the differentiation of contractile VSMCs from SHF progenitor cells. Mutations in NOTCH1 may predispose to the development of AscAA by affecting contractile VSMC differentiation from SHF progenitors and provokes an impaired VSMC response to stress.

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Alarmin S100A8/A9 drives histone methylation-based epigenetic alterations in blood- and bone marrow-derived leukocytes in acute myocardial infarction

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Background. The dysregulation of specific histone methylation-dependent epigenetic mechanisms leading to persistent transcriptional alterations, phenotypic switching, and trained immunity has been implicated in the etiology of cardiovascular disease. The SET7 and MLL1 histone methyltransferases induce histone methylation signatures associated with long-lasting transcriptional responses and monocyte hyperactivity. So far, the triggers of SET7 and MLL1-dependent regulatory mechanisms in myocardial infarction (MI) have not been investigated. S100A8/A9 is a neutrophil mediator that is highly increased in acute MI and drives local and systemic inflammatory responses.
Objective. We aimed to elucidate the potential role of S100A8/A9 in mediating SET7 and MLL1 expression and histone methylation in experimental MI.
Methodology. Male C57BL/6J mice were subjected to permanent left coronary artery ligation to induce MI. Sham-operated and MI mice were treated with 30 mg/kg of the selective S100A8/A9 inhibitor ABR-238901 or its vehicle, administered i.p. for three days. CD45+ cells were isolated from blood and bone marrow with a positive selection kit. Gene and protein expression was assessed by real-time PCR and Western blot.
Results. We found significant increases in SET7 and MLL1 mRNA/protein levels in the ischemic left ventricle and in CD45+ leukocytes from blood and bone marrow of MI mice. Concomitantly, the histone methylation markers reflecting the activation of SET7 (H3K4me1) and MLL1 (H3K4me3) were significantly elevated in the bone morrow-derived CD45+ cells. These epigenetic alterations were efficiently prevented by S100A8/A9 blockade with ABR-238901.
Conclusion. S100A8/A9 induces epigenetic modifications associated with trained immunity in the ischemic myocardium and bone marrow leukocytes of mice with MI. Pharmacological targeting of S100A8/A9 could be a promising therapeutic strategy to prevent long-lasting epigenetic alterations leading to leukocyte hyperactivity and persistent inflammation in acute MI.
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Therapeutic S100A8/A9 inhibition reduces NADPH oxidase expression and counteracts NLRP3 inflammasome priming and activation in the ischemic myocardium

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Background. Enhanced formation of reactive oxygen species (ROS) and alterations in redox signaling have been implicated in the pathophysiology of myocardial infarction (MI). The NADPH oxidase (Nox) complex is an important source of ROS in the infarcted myocardium. The alarmin S100A8/A9, primarily secreted by activated neutrophils, amplifies acute myocardial inflammation in MI and has previously been shown to be a promising therapeutic target to improve cardiac function post-MI.
Objective. We aimed to elucidate the underlying mechanisms linking S100A8/A9 and the inflammatory response in MI.
Methodology. MI was induced by permanent left coronary artery ligation in C57BL/6J mice, followed by treatment with the S100A8/A9 inhibitor ABR-238901 (30 mg/kg) or PBS for 3 days. The *in-vivo* experiments were complemented with mechanistic studies on cultured human macrophages (Mac), important effectors of inflammation and repair in the ischemic myocardium.
Results. Compared to sham-operated animals, we detected significant increases in the Nox1/2/4 catalytic subunits and in the inducible nitric oxide synthase (NOS2) at mRNA and protein level in the left ventricle of MI mice. Concomitantly, elevated levels of phosphorylated p65/NFkB, active caspase-1, and cleaved IL-1β were found in the ischemic left ventricle. S100A8/A9 blockade prevented the up-regulation of Nox and NOS2, suppressed NF-kB activation and prevented the priming and activation of the NLRP3 inflammasome. *In-vitro*, S100A8/A9 treatment induced expression of Nox1/2/4/5 catalytic subtypes and NLRP3 priming in human Mac in a TLR4-dependent manner. These effects were efficiently counteracted by ABR-238901 and by pharmacological inhibition of Nox1/Nox4 activity.
Conclusion. In conclusion, we show that Nox upregulation is an important contributor to S100A8/A9-induced NLRP3 inflammasome priming and activation in acute MI, leading to IL-1β production and myocardial inflammation.
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Manuka Honey and its Phenolic Component, Methyl Syringate, Mediate Neutrophil Inflammatory Behaviors for Potential Use as a Vascular Prosthetic Bioactive

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Neutrophils use reactive oxygen species (ROS) activity and a specialized form of cell death named NETosis to kill and ensnare invading pathogens. A dysregulated response can be deleterious and lead to tissue damage and fibrosis at host-biomaterial interfaces. Manuka honey has demonstrated potent antibacterial properties and recently, anti-inflammatory potential.

It was hypothesized that applying potentially therapeutic compounds found in abundance within Manuka honey to pro-inflammatory neutrophils will reduce intracellular ROS activity and prevent NETosis. Additionally, concentrations of whole Manuka honey were also assayed as a comparison.

Using primary human neutrophils isolated from donor (n=5) peripheral blood, concentrations between 1 nM and 1 mM of each flavonoid, 10μM and 2mM of methyl syringate, and 0.1% v/v and 10% v/v Manuka honey were assayed for reductions in NETosis using Sytox orange extracellular DNA staining and reduction in intracellular ROS activity via standard dichloro-dihydro-fluorescein diacetate (DCFH-DA) oxidation assay.

Results were expressed as relative ROS activity or NET formation (percentage) compared to positive control (stimulated cells) with standard deviation (n=5). Data were pooled, tested for normality, and statistically compared to positive control using Wilcoxon rank sum tests.

Whole Manuka honey reduced NET levels in an increasing pattern across all concentrations up to 91%, but only reduced ROS activity by 36% in a narrow concentration range. Methyl syringate, however, reduced NET levels by up to 68% and ROS activity by 66% within a broad therapeutic window of 10-1700 μM.

In summary, the data from this study demonstrate that methyl syringate and whole Manuka honey are potent modulators of NET formation. In comparison with whole Manuka honey, methyl syringate is much more effective at lowering intracellular ROS activity. The results of the methyl syringate study versus other Manuka honey components indicates that methyl syringate drives the anti-inflammatory capabilities of Manuka honey demonstrated in previous studies.

Atypical melanocytes contribute to murine aortic valve elastogenesis

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The aortic valve (AoV) opens and closes over two billion times in an average lifetime, regulating the blood flow from the left ventricle to the rest of the body. The remarkable ability to withstand the repetitive loading and unloading derives from the extracellular components, such as elastin (Eln) and collagen, that are maintained by specialized valvular interstitial cells (VICs), a heterogenous cellular population within aortic valve leaflets. Pigment producing cells are observed in murine aortic valves and cells with traditional melanocytic markers exist within the interstitium of human aortic valve leaflets; however, the role of melanocytes in AoV development and disease remains largely unexplored. Our recent publications suggest a potential link between pigment production and elastin patterning within the mouse AoV. To further explore the role of melanocytes in patterning the developing AoV, we performed spatial transcriptomic analysis on murine AoV leaflets at postnatal day 3, focusing on genes closely linked to Eln expression. As expected, genes associated with smooth muscle cells (*Acta2*) and those critical for elastic fiber assembly (*Fbln5*, *Lox*) showed strong correlations with Eln. We also observed moderate correlations with melanocytic markers such as *Tyrp1* and a weaker correlation with the endothelial marker VE-cadherin (*Cdh5*). Immunolabeling of common phenotypic markers following *in situ* hybridization to identify Eln expression within AoV leaflets revealed populations of smooth muscle cells, endothelial cells, and melanocytes responsible for Eln production. Lineage tracing experiments using Wnt1-Cre/ZsGreen and Nkx2.5-Cre/YFP mice revealed that AoV melanocytes originate from both neural crest and second heart field lineages, providing the first evidence of non-neural crest-derived melanocytes within the developing heart. Future studies will explore the mechanistic contributions of different melanocytic populations to elastic fiber formation in the AoV.

Enhanced Targeted Repair of Vascular Injury by Apoptotic-cell-Mimicking Nanovesicles Engineered with P-Selectin Binding Peptide

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Background and Objective: Late-stage coronary heart disease can lead to coronary artery occlusion, and treatment options usually involve percutaneous coronary intervention (PCI) with vascular injury as an almost inevitable consequence. Modulating inflammation and promoting re-endothelialization are crucial for repairing vascular injury. Phagocytosis of apoptotic cells at the injury site represents an effective mechanism for attenuating inflammation and improving regeneration during the natural healing process. However, strategies for repairing vascular injury using biomaterials derived from apoptotic cells have not yet been developed.

Method and Results: In this study, apoptotic body-mimetic nanovesicles (ApoNVs) derived from rat adipose-derived mesenchymal stem cells (rASCs) were prepared using a one-step extrusion method. The obtained ApoNVs inherit the unique anti-inflammatory and pro-regenerative properties of the parental apoptotic rASCs (Apo-rASCs). ApoNVs induce polarization of M1 macrophages toward the M2 phenotype. Meanwhile, they promote the proliferation, migration, and tube formation of endothelial cells (ECs). Moreover, ApoNVs-induced repolarized macrophages inhibit the proliferation and migration of vascular smooth muscle cells (VSMCs), and upregulate the functional gene expression of VSMCs. After engineering ApoNVs with P-Selectin Binding Peptide (ApoNVs-PBP), ApoNVs-PBP effectively target activated ECs, suppressing inflammatory gene expression. In a rat wire-mediated femoral artery injury model, ApoNVs-PBP further demonstrates targeted therapeutic effects on vascular injury.

Conclusion: ApoNVs-PBP effectively repairs vascular injuries by modulating macrophage phenotype, enhancing endothelial cell angiogenesis, and reducing inflammation, thereby offering a novel therapeutic strategy based on apoptotic cell.

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Material properties and acute human blood exposure may predict limb graft occlusion in EVAR

Michael F. Wolf, Hannah Jeffers, Job van Helvoirt, and Simon Overeem

Background: Abdominal Aortic Aneurysm (AAA) is the most common cardiovascular disease affecting the aorta and is typically treated with EndoVascular Aortic Repair (EVAR). A prevalent post-EVAR complication is limb graft occlusion (LGO), where thrombi block one or more stent limbs, often leading to rehospitalization. Literature shows different LGO rates between commercial stent grafts, with Zenith Alpha® having significantly higher rates compared to Endurant® and Excluder® [1]. No fundamental research has linked clinical parameters (kinking, reduced diameters, etc.) to the increased risk of LGO with certain devices. Hence, this research investigated the thrombogenicity of the stent fabrics as an explanation.

Methods: The thrombogenicity of Zenith Alpha®, Endurant®, and Excluder® devices was assessed according to ISO 10993-4 and compared using published methods and a dynamic test tube model [2]. Fabrics of the three devices were put in contact with fresh human blood from four donors, at two heparin levels, for one hour. The blood was then quantitatively analyzed (platelet count (Plt), thrombin-anti-thrombin (TAT), beta-thromboglobulin (βTG), and SC5b9), and fabrics were visualized with digital photography and scanning electron microscopy (SEM). ANOVA and Tukey-Kramer HSD analyses were applied.

Results: Analysis showed Zenith Alpha® being the most thrombogenic, with high platelet activation and thrombus formation, followed by Endurant® with significantly lower TAT, and Excluder® being the least thrombogenic with lowest platelet activation and thrombus formation based on significantly lower TAT, βTG release, and higher Plt ($P<0.05$). SEM visualized high clot formation on Zenith Alpha®, mainly red blood cell attachment on Endurant®, and almost no thrombus on Excluder®.

Conclusion: These results show that the thrombogenicity of the materials correlates with the clinical LGO rates. This suggests that the previously uninvestigated thrombogenicity of stent graft materials may be the underlying cause of thrombotic obstructions resulting in Limb Graft Occlusions.

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On the Role and Mechanisms of Notch Signaling in Endothelial-to-Mesenchymal Transition in Cardiovascular Diseases

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Background. Endothelial-to-mesenchymal transition (EndMT) is an important process in cardiac development and tissue maintenance. EndMT is also involved in the development of various cardiovascular diseases such as aortic valve calcification. Notch signaling is suggested to play a key role in EndMT. However, the exact mechanisms are still unclear.
Objectives. 1. To investigate the mechanisms of Notch activation or inhibition of EndMT. 2. To study the mechanisms of Notch activation and inhibition in endothelial cells.
Methods. Notch signaling was activated via lentiviral transduction of either Notch intracellular domain (NICD) or by ligand JAGGED 1 (JAG1). Notch signaling was down-regulated by short-hairpin RNA against either CSL (CBF1, Suppressor of Hairless, Lag-1) or against JAG1. CSL is the transcription factor in the cell nucleus which is activated by NICD. CSL is crucial for most Notch functions. Luciferase reporter assay was applied to investigate the interactions between either NICD or JAG1 with CSL. Immunocytochemistry was applied to verify EndMT.
Results. NICD induced expression of NOTCH2 and NOTCH3 as well as the downstream genes HEY1and SLUG, followed by EndMT. JAG1 alone did not induce EndMT, causing only slight expression of Notch components. Unlike JAG1, NICD dose-dependently increased activity of the CSL luciferase reporter. Downregulation of CSL inhibited Notch as well as EndMT.
Conclusions. It is possible to activate and inactivate EndMT in the endothelial cells by activation and inactivation of Notch pathway components. Different ways of activation and inactivation of Notch signaling in endothelial cells have different effects on downstream signaling.

MICROPATTERNED SURFACE INVESTIGATION TO REDUCE THE RISK OF THROMBUS FORMATION IN CARDIOVASCULAR DEVICES

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Background, hypothesis, and objectives
Implantable cardiovascular devices succeed in treating cardiovascular diseases. However, hemocompatibility-related issues still occur, and anticoagulant treatments are necessary 1–2. The objective of this study is to develop, through innovative technologies, an improved hemocompatible surface modified via specific micropatterns aimed at reducing the adhesion of platelets. Design implementation of the interface between biological tissue and artificial material may be the key point to reduce the related adverse events.

Methods
4 microgeometries were designed based on three criteria: i) hydrophobicity ii) reduction of accessible area for platelets adhesion iii) improvement of the blood Wall Shear Stress (WSS) effects. Micropatterns were printed via 2-photon-polymerization 3d-printer (2PP, UpNano GmbH) and replicated via Nanoimprinting Lithography (NIL, Profactor GmbH). Surface wettability and platelet adhesion were evaluated via Water Contact Angle (WCA) and Static adhesion tests.

Results
Geometries were printed in 4 dimensions: Extra-Small (1-3µm), Small (3-9 µm), Medium (6–18 µm), and Large (12-36 µm). Wettability tests, compared to a flat surface showed higher hydrophobicity for Large and Medium sizes of all structures (WCA 120-130°) compared to Extra-Small and Small structures (WCA 100-110°). All the WCAs resulted in at least hydrophobic behaviour contrary to the flat surface (WCA 69°). Secondly, micropatterned surfaces were incubated with human platelets in physiological conditions, and compared to flat surfaces. All the micropatterned surfaces (16 samples) showed a significant reduction in platelet adhesion (between 20 – 50 %).

Conclusions
Micropatterned surfaces are highly hydrophobic, this feature may play an important role in platelet adhesion reduction through blood repulsion. This result agrees with platelet adhesion tests results. Platelet adhesion is favoured on flat surfaces. Micropatterned surface may be the solution to address this issue identified as first step of the thrombus formation.
Microfluidic experiments, aiming to test microstructure efficiency for a range WSS magnitudes experienced in blood pumps, are currently ongoing.

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Surface modification with Adiponectin improves reendothelialization of synthetic small-diameter vascular grafts

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Over the past decades significant progress have been made in the field of small-diameter vascular graft (SDVG) research. However, clinical issues related to insufficient endothelialization, thrombosis formation and intimal hyperplasia remain unsolved.
The aim of this project was to modify synthetic grafts to improve early reendothelialization. Electrospun thermoplastic polyurethane (TPU) grafts were loaded with Adiponectin via Click Chemistry.
By stimulating the phosphorylation of AMPK, Adiponectin downregulates the expression of pro-inflammatory mediators such as NFkB and TNF-a. It was shown that Adiponectin improves endothelial cell function by upregulating the expression of nitric oxide (NO) to prevent atherosclerosis development, while stimulating COX-2 and thereby reducing apoptosis. Adiponectin further protects intimal hyperplasia by suppressing smooth muscle cell (SMC) proliferation through inhibition of growth factors, making it an interesting therapeutic target in vascular graft research.
The influence of Adiponectin on interactions between cells and the synthetic material was tested *in vitro* by seeding rat aortic endothelial cells (RaOECs) onto the modified surface. Cell viability assessments revealed EC proliferation over time and proved cytocompatibility of Adiponectin-loaded TPU grafts. Immunofluorescence staining demonstrated successful reendothelialization that was further confirmed by electron microscopy. Gene expression analyzed by qPCR revealed no significant changes in cellular expression patterns.
The results of this study demonstrated that Adiponectin promotes the development of an intact endothelium by improving cell-graft interaction. We hypothesize that immobilization of Adiponectin on synthetic SDVGs could have a great impact on early healing processes.

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Salvianolic Acid A (SA) fosters lymphangiogenesis against myocardial ischemic reperfusion injury

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Abstract: All abstracts should include: (1) a brief introduction, (2) relevant information on methods, (3) summarized results, and (4) conclusion drawn from the results.

Ischemic heart disease is one of the leading causes of death globally. The lymphatic system plays a key role in regulating tissue fluid homeostasis, lipid transport, and immune surveillance. In response to tissue damage, lymphangiogenesis could engender favorable microenvironment which highlights its tremendous potential as therapeutic strategies to improve MI prognosis. Salvianolic acid A (SA) is an extraction from traditional Chinese medicinal herb Salvia miltiorrhiza. Previously we developed degradable polyurethane elastomers with SA as a chain extender [1]. Based on it, we employed 3D printing and near-field direct writing technology to designed a novel cardiac patch (PEUU/SA/3D/Near-field, PD-DN), which resembling cardiac extracellular matrix in porosity, orientation and spatial arrangement. Featuring the biomimetic microstructure and persistent release of SA, PD-DN serves as a versatile scaffold for cell adhesion and climbing. In I/R injury SD rat models, we observed that PD-DN patch effectively promoted lymphangiogenesis, alleviates cardiac remodeling, preserved heart strain and remarkably ameliorated heart systolic function. Mechanistically, we predicted the potential targets of SA based on PharmMapper and SwissTargetPrediction databases. Among the top 10 core targets, SA competitively binds to the ATP binding pocket of epithelial growth factor receptor (EGFR), which could directly block the activation of tyrosine kinases and their downstream signaling. Utilizing single-cell RNA sequencing, we identified a unique subgroup of fibroblasts activated by SA in early-stage repair.

Development of a new protective solution for vein grafts in bypass surgery

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Clinical Problem

Bypass operations are often the only treatment option for atherosclerosis [1]. However, saphenous vein grafts (SVGs) frequently fail shortly after surgery. SVGs are stored in preservative solutions, which can lead to functional decline due to the type of preservation solution used [2, 3], surgical trauma, and other factors. Research has shown that saline, the most common solution, is inadequate for storing blood vessels, causing partial detachment of the endothelium [2]. This damage makes the graft more prone to platelet adhesion, macrophage infiltration, plaque development, and intimal hyperplasia, resulting in graft failure [5].

Objective

The aim is to develop a preservation solution that protects endothelial cells (ECs) from cellular stress during bypass grafting. In our previous study, we compared several commonly used solutions. Our goal is to further optimize these solutions to protect endothelial integrity better than standard solutions.

Material and Methods

Remnants of SVGs were used for (immuno-)histological staining to detect endothelial detachment or to isolate ECs for in vitro experiments (e.g., cell viability assays, metabolic activity measurements, migration capability tests, and activity assessments of endothelium-specific genes). Additionally, ex vivo experiments with the vein remnants are planned.

Results

Our findings indicate that full electrolyte solutions with an extracellular composition are more suitable for preserving SVGs compared to saline solution (that showed the overall lowest protection) or solutions with a low sodium content. Additionally, metabolizable buffer substances like lactate show potential in maintaining endothelial integrity by serving as an energy source and increasing migration capabilities.

Conclusions

The research aims to create a perfusion solution that meets the stringent requirements of cardiac and vascular surgery, improving long-term graft patency and patient outcomes. Such a solution could significantly reduce the failure rate of SVGs and enhance the success of bypass operations.

Funding Sources

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In-vivo Evaluation of Biomimetic Vascular Grafts with Artery-Tuned Mechanical Properties

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Native arteries exhibit complex mechanical characteristics, including low stiffness, nonlinearity, and anisotropy, which vary substantially to meet local physiological demands. Synthetic vascular grafts currently lack these mechanical features, which can lead to suboptimal physiology, decreased functional remodeling, and increased failure rates. Despite ongoing efforts to develop mechanically biomimetic synthetic vascular grafts, existing biomaterials and manufacturing methods have not yet succeeded in achieving artery-tuned nonlinear and anisotropic mechanical properties.

This work aimed to develop and refine a manufacturing method that enables tuning stiffness, nonlinearity, and anisotropy of synthetic vascular grafts to specific arteries and evaluate the effects of mechanical biomimicry on graft performance. Electrospun Nanofibrillar Grafts (ENGs) were manufactured from a mixture of biomedical-grade elastomeric polyurethanes. ENG mechanical parameters were controlled by adjusting graft wall thickness, polymer ratio, and fiber alignment. ENG prototypes were then compared to commercial devices via benchtop assessments and preliminary in-vivo feasibility studies in domestic swine models for peripheral arterial and aortic repairs.

The developed synthetic graft manufacturing approach allows the control of ENG stiffness, nonlinearity, and anisotropy in the range of native artery properties. Benchtop studies demonstrate that the ENG prototypes have appropriate surgical properties and cytocompatibility. In-vivo studies reveal the potential benefits of mechanically tuned ENGs over commercial grafts in peripheral arterial and aortic repairs, evidenced by improved endothelialization, smooth muscle cell incorporation, and maintenance of biomimetic mechanical properties for up to 16 weeks.

The developed ENG manufacturing technology enables achieving a wide range of arterial mechanical characteristics that can be independently tuned to specific arteries. While further development and testing is warranted, this technology holds promise in developing synthetic vascular grafts with optimized biomechanics and improved clinical outcomes.

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Reinforced Biotubes as Readily Available and Regenerative Vascular Grafts

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Background and Objective: There is a great clinical demand for vascular substitutes that can achieve vascular regeneration in vascular replacement or bypass for cardiovascular disease, and arteriovenous graft (AVG) for hemodialysis. Decellularized extracellular matrix (dECM) is a promising biomaterial for tissue regeneration. Herein, we sought to develop a decellularized vascular substitute with the capability of recellularization and promoting vascular regeneration.

Method and Results: Decellularized and polymer fiber skeleton (PS) reinforced biotubes (dPB) was constructed by subcutaneously embedding the PS and followed decellularization. The microstructure of dPB was assessed by the scanning electron microscope, which showed that the dECM in dPB was integrated with PS and had a loose and porous microstructure, enabling dPB excellent performance of recellularization after implantation. Proteomics analysis and *in vitro* cell assay showed that dPB could promote the macrophage polarization to anti-inflammatory phenotype, and further promoted vascular regeneration. Long-term *in vivo* evaluation of dPB were performed in different animal model. dPB was demonstrated the capability of promoting vascular endothelial and smooth muscle regeneration, recovering physiological functions, anti-infection and resistance to needle puncture in the models of canine carotid artery replacement, pig coronary artery bypass graft (CABG), and canine AVG. The initial safety and efficacy were validated by using as AVG in a first-in-man clinical trial.

Conclusion: This work offers an off-the-shelf and pro-regenerative dECM vascular graft for future possible wide use in range of vascular applications for patients with no available autologous vascular substitutes.

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Complete Transformation of Bioresorbable Synthetic Vascular Grafts in the Common Carotid Artery

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When arteries occlude, rerouting blood flow with a vascular graft can save limbs and lives. Autologous grafts are the preferred conduit for replacing small-diameter vessels. However, not all patients have suitable donor vessels, and the wound healing complications associated with the harvest can be severe. Synthetic grafts can be used but the foreign material does not degrade or integrate with the host, leading to graft failure and risk of infection. These grafts function best in large caliber arterial reconstruction but perform poorly in smaller arteries such as in the coronary and below the knee arteries. We believe that by improving the materials used in vascular grafts we can overhaul their performance and expand their use in the clinic. We are approaching this challenge by creating acellular, synthetic vascular grafts using a novel elastomer. This material is processed to create highly porous grafts that have been implanted in both rat and sheep common carotid interposition models. We have successfully demonstrated complete transformation of these synthetic grafts to autologous tissue in both animal models – with the presence of elastin in the newly regenerated artery – a feat that to our knowledge has never been achieved in large animals before. Successful translation of this technology stands to benefit millions of patients every year that require below the knee bypass, coronary artery bypass, and arteriovenous graft placement, to name a few. This work is ongoing and is supported by NIH Grant #R01HL159427. This technology is also currently being commercialized through a startup out of Cornell University.

aXess: Progress update on a restorative hemodialysis access vascular conduit

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Current options for hemodialysis access have significant limitations for patients, who often face a cycle of complications and reinterventions. The Xeltis aXess hemodialysis access vascular conduit is composed of an absorbable polymer supported by an intramural nitinol microskeleton. It is designed to enable cell infiltration and new tissue formation to replace absorbed polymer, resulting in functional endogenous tissue restoration (ETR) and efficacious and safe repetitive puncturability. Ongoing preclinical and clinical studies suggest this approach may combine benefits of an off-the-shelf synthetic approach with the potential to harness the body's innate healing capacity.

A GLP preclinical study was performed using a sheep carotid-to-jugular-vein shunt model comparing 16cm long, 6mm inner diameter aXess conduits (1-, 3- and 6-months f-up) with size-matched ePTFE controls (6-months f-up). 24-months f-up on 2 additional aXess sheep is pending and will be available shortly (17 sheep total).

Primary patency at 6 months was 50% (3/6) for aXess grafts compared to 0% (0/3) for ePTFE graft controls. aXess conduits showed expected levels – mild to moderate - of host macrophage-mediated inflammation and polymer resorption starting from the abluminal aspect. Over time the bulk polymer separated into fragments that either replaced or encapsulated by new collagenous tissue, both associated with a reduced inflammatory response once the process locally completes. The remodeling response of grafts is consistent with other ETR applications of this polymer system.

Based on earlier preclinical studies, regulatory approval was obtained in Europe for a First-In-Human clinical study which has enrolled 20 patients, with 12 months follow-up completed for full cohort. Based on favorable safety data and patency rates, an EU pivotal clinical study was started and is now enrolling patients.

These studies demonstrate the potential of restorative polymer-based approaches to hemodialysis in humans, which may offer benefits compared to currently available options.

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Inhibition of mitochondrial complexes I and IV reduces vascular calcification.

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Cardiovascular calcification (CV) is a serious global health issue. It independently increases the risk of CV disease and death with no effective treatments currently available. Factors contributing to vascular calcification include inflammation, endoplasmic reticulum stress, and metabolic imbalances in vascular smooth muscle cells (SMCs). These metabolic imbalances can cause or result from changes in mitochondrial function. Our recent research has demonstrated alterations in mitochondrial function in calcifying SMCs. The five mitochondrial complexes are key to mitochondrial activity. Therefore, we analyzed the different mitochondrial complexes, hypothesizing that their activities contribute differently to vascular calcification.

SMCs were calcified in osteogenic media (OM). We assessed the function of individual mitochondrial complexes using the Seahorse approach to investigate the metabolic impact of the calcifying environment. Through mitochondrial efflux analysis, we observed that calcifying SMCs displayed increased complex I oxygen consumption rate (OCR) (+39%, $p=0.025$) and complex IV OCR (+47%, $p=0.011$). Inhibiting complex I with rotenone and complex IV with potassium azide decreased matrix mineralization in calcifying SMCs dose-dependent (Rotenone -80%, $p=0.002$; Potassium azide -65%, $p<0.001$). Interestingly, complex II activation with succinate increased matrix mineralization (1.56-fold ($p=0.010$), which was prevented by rotenone, suggesting that complex II plays a minor role. Activating complex IV with TMPD did not affect matrix mineralization. Rotenone and potassium azide did not change ALPL mRNA expression and tissue non-specific alkaline phosphatase activity. However, rotenone and potassium azide reduced the extracellular pH without affecting the intracellular pH, suggesting that extracellular acidification may impact matrix mineralization. Subsequent analysis of extracellular lactate showed no changes.

In conclusion, complexes I and IV may contribute to vascular calcification, as blocking these complexes lowers SMC mineralization. This decrease in mineralization does not follow the traditional pathway associated with reduced ALPL mRNA expression and activity. The role of extracellular acidification in this mechanism requires further investigation.

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Title: Exploring the Role of Caveolin-1 in the Calcification Paradox: Cellular Insights into Divergent Mineralization Pathways in Vascular and Bone Tissues

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Though vascular smooth muscle cells (VSMCs) adopt an osteogenic phenotype during pathological vascular calcification, clinical studies note an inverse correlation between bone mineral density and arterial mineral— a phenomenon known as the calcification paradox. Both mineralization processes are mediated by extracellular vesicles (EVs) that sequester calcium and phosphate. Previous studies associate calcification paradox with an imbalanced phosphate/calcium content due to bone loss and/or increased systemic inflammation. However, modulation of caveolin-1 (CAV1), a membrane scaffolding protein essential to calcifying EV formation in the vasculature, also results in inverse effects on mineralization in bone and vascular tissues. Moreover, across two independent epidemiological cohorts, CAV1 genetic variation demonstrates inverse relationships between bone mineral density (BMD) and coronary artery calcification (CAC). These findings indicate that the calcification paradox may not be fully attributed to the systemic distribution of mineral components or inflammation. To further investigate this divergent role of CAV1 in mineralization, CAV1 expression was knocked down by siRNA in primary human coronary artery VSMC and human osteoblast (HOB) cell cultures, grown in both control and osteogenic media. Methyl β-cyclodextrin (MβCD) and a calpain inhibitor were used to disrupt and stabilize the caveolar domains, respectively, in VSMCs and HOBs. At the study endpoint, calcification was measured via Alizarin Red S staining. *In vitro* siRNA knockdown of CAV1 abrogated calcification production in VSMCs ($p < 0.05$), while having no effect on osteoblast mineralization. MβCD-mediated caveolae disruption led to a 3-fold increase of calcification in VSMCs treated with osteogenic media ($p < 0.05$) but hindered osteoblast mineralization ($p < 0.01$). Conversely, stabilizing caveolae by calpain inhibition prevented VSMC calcification ($p < 0.05$) but had no significant effect on osteoblast mineralization. Our data indicate fundamental cellular-level differences in physiological and pathophysiological mineralization mediated by CAV1 dynamics in VSMC and HOB cultures.

Discovering the Effects of Cancer Cells on Lymphatic/Blood Vessel Mispatterning

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The tumor microenvironment in known to induce microvascular remodeling, including both angiogenesis and lymphangiogenesis. Motivated by the challenge to recreate the multi-cell/system complexity of the tumor microenvironment, our laboratory recently developed an *ex vivo* biomimetic model that integrates tumor spheroids and cultured rat mesentery tissues. The model enables real-time observation of cancer cells, blood vessels, and lymphatic vessels and its application led to the discovery of tumor spheroid-associated lymphatic/blood vessel mispatterning. To validate the patho-physiological relevance of our model, the objective of this study was to determine the effects of tumor spheroids on microvascular remodeling post transplantation in analogous *in vivo* studies. H1299 human lung carcinoma cell lines were labeled with DiI and suspended in a hanging drop culture system consisting of MEM supplemented with 10% serum for 4 days to form fluorescent spheroids. These spheroids were then seeded onto exposed rat mesentery tissues. Tissues were then harvested 3- and 5-days post seeding to match *ex vivo* time points and labeled with BSI-lectin, PECAM, and/or LYVE-1. The spheroid group was compared to sham (no spheroids) and unstimulated tissues. On day 3 and day 5, clusters of DiI cancer cells were observed. A distribution of individual cells across the tissues suggested migration from their initial spheroid configurations. Evidence of cancer cell effects on angiogenesis and lymphangiogenesis was supported by increased blood capillary sprouting and density at both 3 and 5 days. 5-day spheroid tissues also displayed examples remodeled lymphatic vessels characterized by dramatic changes in morphology, apparent blebbing, segment specific increases in diameter, and examples of segment specific decreased LYVE-1 labeling. The results support the relevance of using our *ex vivo* model to guide *in vivo* investigation and offer a new view for understanding altered microvascular structure and function in a tumor microenvironment. Funding source: American Heart Association Grant 23TPA1142184.

Algorithm-Driven Optimization of Helical Vascular Grafts to Suppress Neointimal Hyperplasia in Arteriovenous Grafts

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Background and Objective: There is a high clinical demand for arteriovenous grafts (AVGs) that can inhibit intimal hyperplasia and maintain long-term patency in hemodialysis. Helical flow can enhance the hemodynamic performance of grafts, thereby suppressing intimal hyperplasia and thrombosis at the anastomotic site. However, the optimal helical structure design remains unachieved. Therefore, we aim to develop an efficient helical artificial blood vessel capable of inhibiting intimal hyperplasia and maintaining long-term patency by combining finite element methods and mathematical statistical algorithm.

Methods and Results: We constructed computational models of AVGs with various helical structures (including anastomotic angle, pitch, and ridge height). Using finite element analysis, we extracted key hemodynamic parameters from the anastomotic region of these helical AVGs. Mathematical statistical methods were then employed to analyze and optimize the hemodynamic parameters of each model, leading to the design of a helical blood vessel structure that provides optimal hemodynamic conditions. The optimized helical blood vessels were fabricated according to the design parameters using a combination of melt spinning and electrospinning. Mechanical characterization showed that the fabricated blood vessels met the requirements for artificial vascular graft transplantation, including burst pressure, suture strength, and mechanical strength. *In vivo* evaluation was conducted using a pig AVG model, which demonstrated that the optimized helical artificial blood vessels could induce helical flow *in vivo* and exhibit excellent capability to inhibit intimal hyperplasia, thereby maintaining long-term patency.

Conclusion: This study provides a new method and approach for the structural optimization of artificial blood vessels. The optimized helical artificial blood vessels, which effectively inhibit intimal hyperplasia and support long-term patency, hold promise for applications in various cardiovascular diseases requiring artificial vascular graft transplantation.

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Creating order out of chaos – Modelling the influence of chronic inflammation on restoring cardiac structural anisotropy

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Heart attacks affect one in five men and one in six women, posing a significant health concern.[1] After myocardial infarction (MI), cardiac function declines due to the loss of cardiomyocytes, which is later replaced by disorganized scar tissue. Recent studies from our group suggest that restoring tissue to its natural, anisotropic organization significantly improves cardiac function. However, it remains unclear whether tissue anisotropy can be restored in the presence of chronic inflammation - typical for infarcted myocardium. This study will be devoted to unravelling the impact of chronic inflammation on restoring anisotropy. We hypothesize that chronic inflammation will hamper tissue anisotropy restoration due to adverse tissue remodelling, as it dysregulates the ECM turnover and cellular activation.

To adequately mimic tissue organization, we utilize a mini-tissue model in which cells are seeded in between two Velcro constraints for anisotropic organization, and four constraints for chaotic organization. Cardiac fibroblasts and cardiomyocytes are suspended in a 30:70 ratio within a gel composed of collagen type I and Matrigel. Tissue contractility is enhanced through electrical pacing. To study remodeling, the four-constraint tissue is cut from two opposing Velcro constraints and observed for one week. During this period, tissues are exposed to various cocktails of pro- and anti-inflammatory cytokines to mimic chronic post-MI inflammation. At different time points, cellular and collagen alignment will be analyzed using our in-house developed CNA-OG488 probe and cell-specific markers. Additionally, cell proliferation and activation will be assessed using qPCR, immunofluorescence, and DNA content measurement with the dsDNA BR assay kit. Hydroxyproline will be quantified with a Chloramin-T assay. Mechanical properties and rupture behavior will be evaluated through uniaxial tensile testing. Finally, cardiac contractility will be assessed using a calcium transient assay.

This model allows for systematic dissection of the influence of inflammatory cytokines on structural remodeling and, consequently, cardiac function.

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[1]American Heart Association, Women vs Men Heart Attack

Lymphatic and Tenascin C crosstalk in Left Ventricular Hypertrophy-Induced Cardiac Remodeling

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Introduction: Boosting lymphangiogenesis has proven to alleviate inflammation, fibrosis and myocardial edema after myocardial infarction (MI) and induce overexpression of Tenascin-C (TNC). TNC is a matricellular protein that negatively regulates lymphangiogenesis post-MI, contributing to impaired repair and remodeling process. Nevertheless, the dynamic changes in lymphangiogenesis in pressure-overload induced left ventricular hypertrophy (LVH) and its crosstalk with TNC are poorly understood.

Methods: LVH was induced by transverse aortic constriction (TAC) in adult male C57BL/6 mice, allocated to groups of sham, 1-week and 6-weeks post-TAC. Hearts were processed for histology of Masson's trichrome and immunostainings for lymphatic markers (Podoplanin and LYVE1) and TNC. Alterations of lymphatic vessels were quantified in perivascular areas and total heart tissue. In addition, lymphatic endothelial cells (LEC) were subjected to 48h low (1-3%) or high (18-21%) elongation using FlexCell to mimic stretch overload. The expression of NF-κB and fibrotic markers (Collagen I and III) were assessed by western blot and RT-qPCR. Scratch-wound healing and XTT cell viability assays were performed on LEC after treatment with recombinant human TNC for 24h (1µg/ml or 5µg/ml).

Results: Region-specific alterations of lymphatic vessels in heart tissue after pressure overload were detected. In addition, 1-week post-TAC, a regression of lymphatic structures was observed exclusively in perivascular areas, along with increased perivascular fibrosis and TNC expression. High stretch conditions in LEC showed increased expression of inflammatory and fibrotic markers, suggesting lymphatic remodelling. Scratch-wound healing and XTT assays revealed prolonged wound healing and a tendency towards reduced viability in LEC after TNC treatment.

Conclusion: Our study demonstrates dynamic spatio-temporal changes of lymphatic vessels in LVH due to pressure overload. Moreover, TNC appears to be a crucial player in modulating lymphatic vessel growth in LVH. Thus, inhibiting TNC and/or boosting lymphangiogenesis may be a potential novel therapeutic strategy to alleviate fibrosis and remodeling in LVH.

Carnosine-copper chelator-modified small-diameter vascular grafts for the promotion of anticoagulation and endothelial regeneration

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Abstract: Thrombosis and poor endothelialization of small-diameter vascular grafts (SDVGs) remain the leading cause of frequent failure, despite current tissue-engineered vascular grafts providing a feasible strategy in clinical treatment strategies for the small-diameter (<6 mm) blood vessels in management of cardiovascular disease. Copper ions are normally used to achieve the purpose of antithrombotic because they can produce nitric oxide (NO) with the catalysis of NO donors. However, copper ions also participate in the Fenton reaction to produce reactive oxygen species (ROS), which would adversely affect vascular graft remodeling. Here carnosine, an ion-chelating agent with anti-inflammatory and antioxidant properties, was used to chelate with copper ions to synthesize carnosine-Cu (II) chelate which showed effective activity of antithrombotic, anti-inflammatory, and antioxidant. Besides, our research further demonstrated that copper ions still retain the catalytic activities for producing NO after chelating with carnosine accompanied by the production of lower levels of ROS (the average fluorescence intensity of ROS decreased from 79.69 to 7.13). Furthermore, the grafts achieved excellent patency with satisfactory endothelialization (the endothelial coverage reached 80.64%) and significantly improved deposition of collagen and glycosaminoglycan for up to 12 weeks. In general, Car-Cu (II) modified SDVGs provided a facile approach to addressing the problems of thrombus and poor endothelialization in SDVGs for the clinic, which is significant in cardiovascular regenerative medicine.

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Simulated microvasculature in thick 3D bioprinted collagen-based matrices promoted in active perfusion bioreactor

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The 3D bioprinting of high-concentrated collagen bioinks is a promising technology for tissue engineering and regenerative medicine involving creating new tissue models or replacements. Due to its natural abundance in the extracellular matrix of many tissues and its biocompatibility, collagen is a widely used biomaterial for bioprinting. High-concentrated collagen hydrogels have shown great potential due to their favorable structural and biomechanical properties. However, achieving high cell proliferation rates within these hydrogels remains challenging, especially for thick (> 1.5 mm) samples, due to reduced diffusion and exchange of nutrients and metabolites. In this study, we aimed to prepare 2 mm thick collagen matrices with incorporated stromal/stem cells with maximum viability and their ability to differentiate into smooth muscle cells and endothelial-like cells. The collagen bioinks were based on porcine collagen isolated from porcine skins. We have optimized the procedure for printing at densities of 20, 30, and 50 mg/mL with incorporated porcine adipose-derived stromal cells and Wharton's jelly stem cells. We then used several approaches in the cultivation and creation of microvasculature. Static cultivation of the samples showed minimal viability only in border layers, even for samples 1 mm thick. We then utilized controlled active perfusion with freely floating samples, maintaining whole-volume wetting and culture media exchange. This resulted in viable cells in 1 to 1.5 mm thick samples. Punctured and print-patterned microchannels were then incorporated. This approach increased the overall diffusion area, promoting cell viability in thick samples. Downsizing reduces mechanical stability and requires precise preparation of the microchannels because they are prone to collapse. However, combining microchannels in collagen matrices with active perfusion in a bioreactor showed promising results in thick sample preparation. In addition, cells were able to remodel the substrate by differentiating into SMC-like structures.

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Supporting Autologous Internal Jugular Vein Grafts with FRAME Mesh in a Porcine Carotid Artery Model

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Background: Autologous vein grafts are commonly utilized in cardiovascular bypass surgeries. Despite their frequent use, these grafts often fail due to vein graft disease.

Objective: This study was designed to assess the effects of the latest-generation external FRAME (VGS, Tel Aviv, Israel) support on the remodeling of vein grafts using a porcine preclinical model [1].

Methods: Autologous internal jugular vein interposition grafting was conducted in the carotid arteries of pigs over a one-month period. Out of the total grafts, four were reinforced with FRAME mesh, and seven served as unsupported controls. These grafts were evaluated using flowmetry, angiography, macroscopy, and microscopy.

Results: After one month, the patency rate for the FRAME-supported grafts was 100% (4/4), while it was 43% for the unsupported controls (3/7, Log-rank $p = 0.071$). Angiographic examination at explant revealed that FRAME-supported grafts significantly exhibited more areas with no or mild stenosis (9/12 segments) compared to the controls (3/21 segments, $p = 0.0009$). The blood flow in the FRAME-supported grafts was higher (145 ± 51 ml/min) than in the controls (46 ± 85 ml/min, $p = 0.066$). The area and thickness of neo-intimal hyperplasia (NIH) at the proximal anastomoses were comparable between the FRAME and control groups: 5.79 ± 1.38 mm² vs. 6.94 ± 1.10 mm² ($p = 0.558$), and 480 ± 95 μm²/μm vs. 587 ± 52 μm²/μm ($p = 0.401$), respectively. However, the NIH area and thickness in the midgraft portions were significantly lower in the FRAME group compared to the control group: 3.73 ± 0.64 mm² vs. 6.27 ± 0.64 mm² ($p = 0.022$) and 258 ± 49 μm²/μm vs. 518 ± 36 μm²/μm ($p = 0.0002$), respectively.

Conclusions: The perivascular FRAME mesh enhanced the patency and reduced the incidence of stenosis in vein-to-carotid artery grafts within our porcine model, particularly in the midgraft regions. Midgraft neo-intimal hyperplasia was substantially less pronounced in the FRAME-supported grafts compared to the controls, demonstrating a two-fold difference in thickness.

[1] Chlupac J., Frank J, Sedmera D., et al. *Biomedicines.* 2024; accepted, manuscript ID 3016779.

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Relaxin Receptor Agonist ML290 Inhibits Calcification in Vascular Smooth Muscle Cells

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Vascular calcification contributes to the rupture of atherosclerotic plaques—the leading cause of heart attacks. No therapeutics exist to treat vascular calcification. We hypothesized that relaxin, a vasoprotective and anti-fibrotic small peptide hormone of the insulin/relaxin family, may provide a therapeutic option for vascular calcification. However, recombinant relaxin has short stability *in vivo*, poor bioavailability, and is expensive to synthesize, limiting its clinical utility for chronic conditions such as vascular calcification. ML290 is a biased allosteric agonist of the human relaxin receptor (hRXFP1), which was previously shown to reduce alkaline phosphatase activity in human aortic vascular smooth muscle after 7 days. This study aimed to determine if ML290 may stop the progression of vascular calcification in vitro after chronic exposure to pro-calcific media. HCAVSMCs were cultured for 21 days. The first group was cultured with standard media (DMEM, FBS, Pen/Strep). The second group was cultured in pro-calcific media (LAA, BGP, Dexa). The final groups were cultured with pro-calcific media and 1, 5, and 10 uM of ML290. Media was collected every week. The extracellular vesicles were then collected from the media, and a commercially available colorimetric alkaline phosphatase assay was used to determine differences in alkaline phosphatase activity. After 21 days, the cells were fixed and stained with alizarin red to visualize calcification deposition in the wells. The stain was also dissolved and quantified. Data suggest that relaxin agonism reduces alkaline phosphatase activity and calcium deposition, as shown by decreased alizarin red stain, dose-dependently in HCAVSMCs. This study will elucidate the dose-dependent effects of ML290 on human vascular smooth muscle cells.

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A PORCINE MODEL OF CONTROLLED PERIPHERAL ARTERY CALCIFICATION

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Peripheral artery disease (PAD) in the femoropopliteal artery (FPA) leads to significant morbidity and mortality. FPA calcification contributes to poor clinical outcomes and greatly increases the risk of amputation. Current treatments for calcific lesions are limited and yield suboptimal results. A large animal model that closely mimics calcific PAD and accommodates human-sized devices could enhance the development of safer and more effective therapies. Our objective was to create a swine model of late-stage arterial calcification to test the efficacy and side effects of intravascular lithotripsy (IVL). To induce lesions, swine received injections of $CaCl_2$ directly into the media and periadventitial spaces of the abdominal aorta, iliac, femoral, and popliteal arteries using a micro-needle catheter. The injection sites were varied to create eccentric and concentric lesions of differing lengths and patterns, with dosage adjusted to prevent aneurysmal expansion. Adjacent non-calcified arterial segments served as intersubject controls. The lesions were allowed to mature over 30 days. Computed Tomography Angiography (CTA) and Intravascular Ultrasound imaging demonstrated ring-like calcification patterns and no pulsatility. Mechanical testing of excised arteries mirrored the mechanical properties of calcified human FPAs, including characteristic circumferential stiffening. Histological examination revealed calcified arteries that closely resembled human calcified FPAs, exhibiting inflammation, thickened and inflamed adventitia with extensive vasa vasorum, degenerated and collagen-rich media with round-shaped smooth muscle cells, severely degraded elastin in the external elastic lamina, and substantial neointimal hyperplasia composed of dense collagen, cellular debris, and glycosaminoglycans. The acute effects of IVL on calcific lesions included periadventitial hematoma, extensive arterial wall damage with complete separation of the tunica media, exposure of the adventitial collagen to flowing blood, and acute thrombosis in smaller arterial segments. This porcine model effectively replicates key aspects of calcific human PAD and provides a rigorously controllable environment to evaluate calcium-modifying treatment impacts and mechanisms.

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Biomimetic Electrospun Tri-layer Tissue Engineered Heart Valve with Low Calcification and Good Regenerative Ability

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Background of the clinical problem: Transcatheter aortic valve replacement (TAVR) technique is rapidly advancing in clinic, however, as it expands to low-risk populations and younger patients (age < 65 years), device durability is becoming a weakness.

Objective: Developing tissue-engineered heart valves (TEHVs) as a potential alternative with low calcification and good regenerative ability.

Methods: A biomimetic Tri-layer tissue-engineered heart valve was constructed with poly (L-lactate-co-ε-caprolactone) (PLCL), gelatin (GEL), hyaluronic acid (HA) and silk fibroin (SF), to simulate the fibrosa, spongiosa and ventricular layer of natural heart valves, respectively. To obtain a scaffold with proper strength and regenerative capacity, we optimized the component ratio of each layer. The physical, mechanical and cytocompatibility properties were tested, calcification deposition and regeneration potential were further evaluated by subcutaneous implantation in rat model. Finally, the hydrodynamic function of the new TAVR device was verified.

Results: The strength of the Tri-layer valve could reach up to 10 MPa, significantly higher than that of the PLCL and Mono-layer groups. Most importantly, *in vitro* calcification related gene expression of valvular interstitial cells (VICs) was down-regulated in TEHVs group compared to calcification-inducing group, and calcification levels of TEHVs in *in vivo* assay were below 0.5 μg/mg. Besides, we found HA in the middle layer was very conducive to rapid cell infiltration and good angiogenesis, which ultimately promoted host tissue regeneration at 8 weeks after implantation. **Conclusions:** In this study, we provide a biomimetic Tri-layer electrospun leaflets with appropriate mechanical strength, low calcification and good regenerative capacity, which has great potential as a TEHV leaflet.

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Bilayer small diameter vascular graft with dual anti-calcification property by loading with baicalin and cathepsin S inhibitor

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Abstract

Long-term transplantation of small diameter vascular grafts (SDVGs) is associated with calcification risk, a key factor limiting SDVG development in the clinic. Hence, there is an urgent clinical need to develop new SDVGs with anti-calcification properties. Here, we used the decellularized extracellular matrix (dECM) and poly (L-lactide-co-ε-caprolactone) (PLCL) as base materials, baicalin was crosslinked, and cathepsin S (Cat S) inhibitor was loaded to prepare PBC-SDVGs by electrospinning. The vascular grafts crosslinked with baicalin reduced the calcium deposition in simulated body fluid experiments and inhibited vascular smooth muscle cells (VSMCs) switch to synthetic phenotype, downregulating the expression level of osteogenic genes. Vascular grafts containing both baicalin and Cat S inhibitor can effectively promote macrophage polarization toward an anti-inflammatory M2 phenotype. Subcutaneous implantation of the rat for 12 weeks demonstrated that the vascular graft reduced the occurrence of calcification. In summary, vascular grafts crosslinked with baicalin and loaded with Cat S inhibitor effectively reduced the osteogenic phenotype and synthetic phenotype transition of VSMCs, inflammation occurrence, and exhibited dual anti-calcification effects. The dual anti-calcification vascular graft provides a promising strategy for the design and construction of anti-calcification SDVGs with significant clinical application potential.

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Conflict of interest

The authors declare no conflict of interest.

Self-EmPOWERment - Road to a Self-Powered, Intelligent Vascular Graft

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Even after 70 years, vascular grafts (VG) have not seen any of the significant improvements desperately needed to improve patient survival rate[1]. The unexpectedness of graft failure raises the need for continuous monitoring of VG performance, as there are rarely symptoms prior to a life-threatening event. However, this is not yet clinically implemented, since the necessary electronics rely on batteries that are bulky, toxic, and have a short lifespan, requiring replacement surgeries. Recently, it was described that fluids flowing through tubular structures can generate electrical energy, by so-called solid-liquid triboelectric nanogenerators (SL-TENG) [2].

Our main aim is to evaluate if blood flowing through commercially VGs is able to generate electric energy to supply the electronics needed for intelligent-VG.

SL-TENG based on commercially available ePTFE VGs (SL-TENG-ePTFE) were assembled by painting a conductive ink on the outer surface. An endovascular simulator mimicked physiological blood flow through SL-TENG-ePTFE. Open circuit voltage and energy storage potential were evaluated using an electrometer. The best VGs were evaluated *in vivo* by establishing an AV-shunt in pig to promote the blood flow through the SL-TENG-ePTFE.

By testing different SL-TENG-ePTFE *in vitro*, it was possible to generate 1.68 V and charge 200 µF capacitors up to 2.6 V. The best SL-TENG-ePTFE was able to supply the emission of a Bluetooth signals via a commercially available circuit every 23 min. The capacity to generate energy by blood flowing through vascular grafts was also proved *in vivo*, being possible to obtain 0.50 V in open circuit.

Our straightforward SL-TENG systems show the ability to be used as an endless source of power towards a smart VG that can monitor its performance 24/7 which will, in the future, prevent their unexpected failure that culminates in patient death.

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Electrospun Vascular Grafts with Artery-Tuned Anisotropy

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Electrospun elastomeric biomaterials hold high promise in developing novel mechanically biomimetic vascular grafts with improved clinical performance. Controlling fiber alignment in such materials can potentially achieve mechanical anisotropy similar to that of human arteries, further advancing graft performance. However, the level of achievable anisotropy and its relation to microstructure parameters has not been sufficiently studied to allow the development of biomimetic grafts with artery-tuned anisotropy. This work aimed to develop a theoretical model for anisotropy control in electrospun vascular grafts and quantify the level of achievable anisotropy values through a parametric study.

Electrospun elastomeric fabrics with varying levels of fiber alignment were produced through the modulation of rotational collector speed. Fiber alignment was quantified by calculating Chebyshev order parameters from fiber orientation histograms. Experimental anisotropy (A_e) was quantified as the ratio of circumferential and axial stiffness values and theoretical anisotropy (A_t) was obtained by calculating Krenchel's fiber efficiency factor from the orientation histogram in both principal directions.

Our results demonstrate that fiber alignment through modulation of rotational collector speed allows mimicry of a wide range of physiological anisotropy values seen in human arteries. A_t values show good agreement with A_e at varying levels of fiber orientation, validating this theoretical framework. Furthermore, we developed a mathematical relationship, showing that A_t can be calculated directly from Chebyshev order parameters. These results provide new insight into structure-property relationships in aligned fibrous biomaterials and provide a novel theoretical framework for optimizing the anisotropy in electrospun elastomeric vascular grafts. Such grafts may provide better mimicry of healthy artery biomechanics, ultimately leading to improved clinical outcomes.

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High-resolution auxetic cardiac patches as a potential tool to support myocardium after infarct

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Introduction: Cardiac patches aid heart function after myocardial infarction (MI), adding strength, supplying growth factors and bridging impaired conduction. Auxetic microstructures, stretching perpendicular to the applied force, can add mechanical properties favorable for a demanding, anisotropic implantation site like the myocardium. Herein we aim to fabricate auxetic cardiac patches of synthetic polymers to be functionalized with composite hydrogels for implantation after MI.
Methods: Micropatterned PCL scaffolds were fabricated on a 3D bioplotter by a custom printing protocol for precise control over printhead movement. Mechanical properties were studied in unidirectional, quasistatic tensile tests. Biocompatibility was assessed via XTT, live/dead assays and SEM. Hemocompatibility was investigated via hemolysis and clot formation assays. Inflammatory response was studied via expression of macrophage and cytokine markers. In a pilot *ex-vivo* study in a Langendorff isolated heart system, patch applicability and effects on cardiac function were evaluated on rabbit and rat heart models.
Results: Optimizing the fabrication process for different polymers resulted in uniform scaffolds with defined pore sizes and tunable mechanical properties. Tensile tests confirmed an anisotropic stiffness-ratio. Ultimate strain (7-20%) was close to species-specific myocardium (15-22%). Biocompatibility was proved in cell viability assays and SEM. Patches showed low hemolysis rate. Immunomodulatory effects of the patches were observed. Pro-inflammatory marker expression was significantly reduced after 1 week (CD80, CCR7, IL-1a & TNF-a). For *ex-vivo* testing, auxetic patch parameters were adjusted to the anisotropic ratio of effective stiffness of the model organ. Patches were adhered by fibrin sealant and sutures, without compromising epicardial tissue or patch movement, resulting in modulation of cardiac function.
Conclusion: 3D printing of auxetic, mechanically tunable and biocompatible cardiac patches showed promising results, offering a therapeutic platform for supporting cardiac regeneration, which could be scalable and translatable clinics.

The study was partly funded by the Ludwig Boltzmann Institute for Cardiovascular Research.

Radiation-Modulated Decellularized Pericardium Vascular Patches with Wharton's Jelly Stem Cells in a Porcine Carotid Artery Model

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Background: There is a demand for various types of vascular substitutes in cardiovascular and oncological surgery, particularly when autologous grafts are unavailable due to previous use.

Objective: Our aim was to explore preclinically the in vivo performance of tissue-engineered vascular patches.

Methods: The patches were fabricated from the porcine pericardium by decellularization using sodium dodecyl sulfate and DNase. We created four groups: non-irradiated or electron beam (50 kGy) irradiated samples, with or without static recellularization with human Wharton's jelly stem cells (WJCs) attached through fibrin/heparin/VEGF assemblies: 1. Non-irradiated + WJC, 2. Irradiated + WJC, 3. Non-irradiated, and 4. Irradiated. The patches (three per group) were implanted in twelve carotid arteries in six pigs for one month and evaluated through flowmetry, angiography, macroscopy, and microscopy. Data presented as mean ± SE were compared using one-way analysis of variance (ANOVA).

Results: All patched carotids remained patent and were endothelialized without stenosis signs. No significant differences in blood flow were observed across the groups and time points ($p = 0.8178$). Three clinically silent aneurysms developed, each one in a different group (Non-irradiated + WJC, Irradiated + WJC, and Irradiated) and were excluded from further analysis. Microscopical examinations revealed significantly thicker neo-intimal hyperplasia (NIH) in the Non-irradiated + WJC group (867 ± 90 μm²/μm) compared to the Non-irradiated group (615 ± 26 μm²/μm, $p < 0.05$). Though significant, this negative impact was not clinically apparent. NIH thickness in the Irradiated + WJC group and the Irradiated group measured 665 ± 76 and 688 ± 76 μm²/μm, respectively. There were no other differences among the groups.

Conclusions/interpretations: Modifications such as irradiation or seeding with WJCs did not enhance the in vivo performance of our decellularized porcine pericardial vascular patches compared to unmodified samples. Moreover, aneurysmal degeneration occurred in some cases. Possible explanations include the small size of the patches, insufficient to reveal clinically significant benefits, a small sample size, and challenges associated with decellularization, recellularization, and the use of xenogeneic cells.

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Modulating *in vivo* Long Term Remodeling Outcomes of a Polyurethane Based, Antithrombogenic Tissue Engineering Vascular Graft via Compliance Matching

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Background: Tissue engineered vascular grafts (TEVGs) are a promising solution to many of the challenges faced in small diameter vascular grafting procedures. Compliance matched TEVGs have demonstrated increased vascular smooth muscle cell presence and decreased proinflammatory markers but demonstrate concerns with long term graft viability due to rapid gelatin degradation.

Objective/hypothesis: This study aims to understand the long-term effects of compliance matching a hybrid gelatin/polyurethane TEVG *in vivo*.

Brief methodological details: CM TEVGs were fabricated via electrospinning pure PESBUU and PEUU:gelatin solutions. Grafts were crosslinked in genipin and mechanically tested on a custom microbiaxial device. TEVGs were then implanted as abdominal aortic grafts in Sprague Dawley rats and were monitored for patency, compliance, and hemodynamics using ultrasound for 6 months (n = 8).

Results: Both CM TEVGs and Hypo TEVGs were successfully matched to their target compliances *in vitro*. In *vivo*, the compliance of CM TEVGs and Hypo TEVGs were found to be 0.0012 ± 0.0004 $mmHg^{-1}$ (within native range) and 0.0002 ± 0.0005 $mmHg^{-1}$, respectively. CM TEVGs experienced significant drops in compliance loss over the first two months of remodeling Hypo TEVGs experienced no shifts. CM TEVGs experience a significant drop in PSV at month 6. Preliminary 28 day explant histology shows complete degradation of the highest gelatin content layer in all CM TEVG samples with abluminal cellularization. Additionally 2 female rats experienced graft dilation (diameter increase) and increased collagen deposition abluminal.

Conclusions/interpretations: Compliance can be successfully modulated with gelatin inclusion. Upon implant, CM TEVGs and Hypo TEVGs fall within their compliance target which can be measured *in vivo* via ultrasound. Neither of the gelatin containing grafts alter vascular hemodynamics. The remodeling profile of CM TEVGs is more dynamic than those of Hypo TEVGs as noted by shifts in compliance.

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Harnessing programmed cell death to guide in situ remodeling of human tissue-engineered matrices

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In situ tissue-engineered heart valves (TEHVs) using human cell-derived tissue-engineered matrices (hTEMs) offer a promising solution to the limitations of current heart valve prostheses. hTEM is a hybrid biomaterial consisting of an extracellular matrix (ECM) and a polymer, which provides initial mechanical stability and functionality and allows for tissue engraftment, repopulation, and remodeling once implanted into a host. Decellularization is a crucial step in the production of hTEMs, aimed at removing cellular components while minimally impacting the ECM. Currently available detergent-based decellularization protocols are insufficient to effectively reduce all cellular residues. We hypothesize that a programmed cell death-assisted decellularization approach, combined with hTEM functionalization with the secretome from dying cells can enhance the adaptive remodeling capacity of hTEM.
TEHVs derived from hTEMs were decellularized using a programmed cell death-assisted method and characterized by histology, biochemical assays, and mass spectrometry. Additionally, TEHVs were evaluated in an *in vitro* pulse duplicator under pulmonary conditions. The secretome from dying human dermal fibroblasts (hDFBs) was characterized and used to functionalize hTEMs.
Our results demonstrate that the programmed cell death-assisted decellularization method meets the decellularization standards in hTEMs. TEHVs decellularized by this method exhibited good leaflet functionality in the *in vitro* tester for up to one hour under pulmonary conditions. Furthermore, the secretome contains high levels of cytokines that influence cell proliferation and trigger a tissue repair response in THP-1 cells.
In conclusion, using programmed cell death to decellularize and functionalize hTEMs is a promising strategy to reduce potential immunogenic residues and introducing biochemical cues that could enhance and guide remodeling *in situ*.

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Tissue-engineered vascular grafts utilizing automated decellularization of allo- and xenogeneic pericardial and vascular tissues modified with collagen and stem cells 3D bioprinting

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Cardiovascular surgery still lacks suitable biomaterials for whole vessel bypass surgery and patch repair. These currently possess many shortcomings – limited patency for small calibers, limited remodeling, and risk of thrombosis. We aim to prepare tissue-engineered vascular grafts and patches based on decellularized and lyophilized matrices (pericardium and vessels) followed by recolonization with adipose-derived stromal or Wharton's jelly stem cells. Acellular cadaveric donors or animal tissues are an abundant source of promising biomaterials. However, these tissues must be decellularized to minimize the immunogenic response and then processed to reduce thrombogenicity and improve the healing and remodelation process. Decellularization was performed using our custom-built semi-automated system. This system allows the cyclic change of decellularization agents (SDS, Na-Deoxycholate, DNAse) and rinsing water, maintaining a reproducible process. Concentrations, cycle times, and their repetitions were optimized based on residual DNA in the tissue and structural and biomechanical properties. Special chambers were created depending on the tissue (tubular and planar) to maintain homogeneous contact and complete decellularization. Recellularization with stromal/stem cells attracts the ingrowing host cells through a paracrine mechanism, improving vascular remodeling in vivo and tending to suppress the formation of intimal hyperplasia, facilitating remodelation. To ensure homogeneous recolonization, we used the 3D bioprinting and microextrusion. Planar tissues were recolonized using the 3D bioprinting method using porcine collagen with incorporated cells. For coating the inner lumen of tubular tissues, we used microextrusion utilizing custom microcannulas and bioprinting for the outer lumen. These microcannulas contain evenly spaced microchannels in which collagen with cells leaving the inner lumen is homogeneously coated. The prepared substrates were then cultivated in perfusion bioreactors with pressure stimulation, promoting cell proliferation and ingrowing into decellularized tissues. Prepared planar patches and tubular grafts were then implanted into pigs for one month of observation. Cell recolonized replacements accelerated remodelation and substrate resorption; WJCs also have immunomodulatory properties.

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Go with the flow: on the way of advancing native blood vessels as energy harvesters

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Implantable electronic devices are currently limited by the use of batteries to power them. Such batteries have a finite life, are large (hindering their application in smaller devices), and may heat or leak their content (damaging the surrounding tissues). This raises a need for continuous, sustainable and biofriendly sources of energy to supply these devices.

This work proposes an innovative approach based on a fluid-solid triboelectric nanogenerator (Flu-TENG) that harvests energy from blood flowing through the native vessels, an unexplored energy source so far. To develop the Flu-TENG, different electrodes, including conductive silver inks and a conductive polymer, were assembled by brush painting on human umbilical cord arteries, and evaluated for (i) *in vitro* stability; (ii) impact on the intrinsic biomechanical properties of the native arteries, and (iii) efficiency in generating electrical outputs, using PBS as fluid-phase and pumped in a similar way as the pulsatile flow of blood circulation.

Electrodes were successfully assembled and remained stable after washing. Indeed, no particles were detected from the leachables of coated native arteries, nor significant differences were observed when comparing the optical density of uncoated and coated native arteries leachables. Furthermore, electrodes were confined to the outer surface of arteries, with no infiltration into the lumen, as observed by optical and scanning electron microscopy. Interestingly, silver inks did not significantly affect the arteries biomechanics, while the conductive polymer resulted in an increase of the compliance and the maximal burst pressure. Additionally, an electrical output was generated with PBS flowing in a pulsatile rate similar to blood circulation (60 bpm). Collectively, our findings highlight the potential of exploring blood flowing through the native vessels to power implantable electronic devices.

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Fabrication and Mechanical Characterization of Near-Field Electrospun Bioresorbable Vascular Grafts Mimicking the Arterial Extracellular Matrix

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Cardiovascular disease is a global health problem, characterized by the thickening of arterial walls restricting blood flow. One treatment option is a surgical procedure utilizing an autologous or synthetic vascular graft to bypass or replace the diseased arterial segment. It was hypothesized that by mimicking the arterial extracellular matrix in bioresorbable vascular grafts via near-field electrospinning (NFES), vascular templates could be produced with mechanical properties that mimic the native vasculature more accurately than the current synthetic vascular grafts while maintaining sufficient pore sizes to allow transmural ingrowth of capillaries and the reendothelialization of the graft lumen via sprouting endothelial cells. Polydioxanone vascular constructs with circumferential fiber alignment angles of 15°/75° and 30°/60° (0° representing circumferential fiber alignment) were fabricated using a custom built NFES system. The vascular construct mechanical properties were compared to the saphenous vein (SV) and internal mammary artery (IMA) through longitudinal and circumferential uniaxial mechanical testing, suture retention, and burst pressure evaluations. The results demonstrated that the 15°/75° templates were closest to mimicking the native vessel target properties as compared to the 30°; however, neither of the vascular template designs achieved or exceeded all the target values. For the ultimate tensile strength, both the constructs met the SV value on the circumferential axis (2.61 MPa) and the IMA value on the longitudinal axis (4.3 MPa). In terms of suture retention, the 15°/75° template was the only construct that was in the IMA and SV targeted range of 138-200 gf. Finally, the burst pressure testing results indicated that neither of the vascular constructs achieved the level of the SV or IMA (1600-3196 mmHg), however, the 15° constructs were within the lower error of the SV. In conclusion, with further design modifications, NFES constructs have demonstrated promise as small-diameter vascular grafts by mimicking native arterial architecture and mechanical properties.

Promoting cell adhesion and regeneration in a 3D-printed cardiac patch via the human placenta chorion extracellular matrix

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Introduction:
Cardiac patches provide mechanical support and a platform for tissue regeneration after myocardial infarction. However, the hydrophobic surface of 3D-printed polymeric patches can hinder cellular attachment and regeneration. Integration of human placenta chorion extracellular matrix (hpcECM) could induce higher cell affinity and cell adhesion ligand for cardiac related cells resulting an excellent regeneration.

Methods:
Polycaprolactone (PCL) was used to print porous patchs, which were then coated with various concentrations of hpcECM. The coating was assessed using an antibody against fibronectin and surface nano-mechanics measurements. Hydrophilicity was measured. Human umbilical vein endothelial cells (HUVEC), human foreskin fibroblasts (HFF), H9c2 and endothelial progenitor cells (EPCs) were utilized to assess the cell viability, attachment, activation via XTT and live-dead-assays and electron microscopy. An initial cell attachment assay was performed. Immunological behavior was analyzed using PCR to investigate macrophage pro- and anti-inflammatory gene expression. Hemocompatibility was evaluated via hemolysis and blood clot formation assays. The in vitro assays spanned from initial time points up to one week.

Results:
With an increase of the hpcECM concentration on PCL a significant increase of the initial cell attachment has been shown. An increase of cell viability was observed in HUVEC, HFF and differentiated H9c2 cells using XTT and live-dead-assays. Coated constructs showed a higher attachment and cell activation of EPCs compared to uncoated PCL constructs. PCR results revealed a slight upregulation of pro-inflammatory markers after 72 hours followed by high expression of anti-inflammatory IL-10 cytokine after one week confirming immunomodulatory behavior of hpcECM. Hemocompatibility was observed in all analyzed groups.

Conclusion:
HpcECM showed a high potential of as a matrix supporting attachment and activation of cardiac related cells compared to its present competitors such as fibronectin. This improvement could lead to better functionality of cardiac patches, supporting cardiomyocyte regeneration in vivo.

Abstract for the Biennial Meeting of the ISACB in Vienna 2024

The Tissue-Engineered, Pre-Vascularized Blood Vessel Wall Construct

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Background: The clinically used vascular prostheses are based only on materials, whether synthetic polymeric (e.g. ePTFE, PET) or biological (e.g. decellularized matrices), and lack the cellular component arranged in the physiological layers of the vascular wall.

Objective: We therefore attempted to reconstruct the *tunica media* and *intima* simultaneously on a polymeric scaffold modified with a collagen hydrogel.

Methods: Electrospun nanofibrous membranes made of degradable polyesters (PLA, PCL) were seeded with human adipose tissue-derived stem cells (ASCs). After reaching subconfluence, ASCs were overlaid with a collagen hydrogel mixed with human umbilical vein endothelial cells (HUVECs). These cell-laden and control cell-free constructs were implanted onto the chicken chorioallantoic membrane (CAM) in an *ex ovo* model.

Results: The ASCs gradually migrated into the collagen hydrogel and, together with the HUVECs, spontaneously formed pre-capillaries. The ASCs that remained in the space between the pre-capillaries were then able to differentiate towards smooth muscle cells. When the pre-capillaries reached the surface of the collagen hydrogel, they exited onto it, and the HUVECs formed a confluent layer on top. When implanted on CAM, the chicken blood vessels showed a slightly higher affinity for the cell-free constructs.

Conclusions/interpretations: A pre-vascularized tissue construct mimicking the *tunica media* and *tunica intima* of the blood vessel wall has been created. This construct can be used as a vascular patch in planar form and as a vascular graft in tubular form. After implantation *in vivo*, it is expected that the pre-capillaries in the construct will connect to the capillaries of the surrounding tissue, which will also grow into the construct and help to endothelialize its luminal surface.

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Biomimetic tri-layered small-diameter vascular grafts with decellularized extracellular matrix promoting vascular regeneration and inhibiting thrombosis with the salidroside

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Abstract

Small-diameter vascular grafts (SDVGs) are urgently required for clinical applications. Constructing vascular grafts mimicking the defining features of native arteries is a promising strategy. To realize biofunctional SDVGs, anti-thrombotic and pro-regenerative activities are necessary. Here, we constructed a tri-layered vascular graft with a native artery decellularized extracellular matrix (dECM) mimicking the component of arteries. Natural blood vessels dECM maintain bioactive factor contents conducive to vascular tissue regeneration. The porcine thoracic aorta was decellularized, frozen, and milled to retrieve dECM powders from the differential arterial layers. The intima and media dECM powders were blended with poly (L-lactide-co-caprolactone) (PLCL) and utilized as the inner and middle layers of electrospun vascular grafts, respectively. Pure PLCL was electrospun and used as a strengthening sheath for the outer layer. Salidroside (Sal), an anti-thrombotic agent derived from Rhodiola rosea plants, was loaded into the inner layer of vascular grafts to inhibit thrombus formation. The incorporation of dECM significantly promoted human umbilical vein endothelial cell (HUVEC) extension adhesion, proliferation, migration, and tube-forming *in vitro*. *In vivo*, studies demonstrated that PLCL-dECM-Sal SDVGs improved extracellular matrix formation, lumen endothelialization, and smooth muscle layer regeneration, compared to PLCL SDVGs lacking dECM. Incorporating Sal promoted macrophage polarization to the M2 phenotype and attenuated calcification, thereby promoting tissue regeneration. Adding Sal and dECM inhibited thrombosis and promoted vascular tissue regeneration successfully, providing a promising rationale for their implementation in the construction of SDVGs.

Funding sources

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Conflict of interest

The authors declare no conflict of interest.

Differential neutrophil deposition on synthetic versus biological scaffolds for in situ cardiovascular tissue engineering

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Background
Neutrophils are the most prevalent cell type in blood, yet their role in tissue regeneration is not well understood. Recent studies by us and other groups reveal neutrophils are the first cells to colonize cardiovascular scaffolds upon implantation into the bloodstream. In addition, neutrophils have garnered significant attention recently, including extensive research investigating their interactions with biomaterials, such as the formation of neutrophil extracellular traps (NETs), on various surfaces. However the response of neutrophils to different scaffold materials and its implication on the subsequent course of the in situ regenerative response remain important open questions.

Objective
To investigate the relevance of neutrophil deposition on various tissue-engineered cardiovascular (TECV)scaffolds and their significance for subsequent tissue regeneration.

Methods
Primary human neutrophils were seeded onto electrospun: polycarbonate polyurethane (PCBU), poly(4-hydroxybutyrate (P4HB), polycaprolactone (PCL) and decellularized porcine valves (dPHVs). Neutrophil characterization, activation and NET quantification was performed after 4 and 24 hours. Donor-matched peripheral blood mononuclear cells were seeded onto the neutrophil-conditioned scaffolds to assess their influence on macrophage polarization via ELISA, PCR and IHC.

Results
The data reveal neutrophil deposition on all examined electrospun synthetic scaffolds. The NET formation seems to occur in a binary manner and shows local heterogeneity. In contrast, on dPHV, no NET formation was detected. Additionally, preliminary data shows neutrophil deposition on electrospun biomaterials enhances the pro- or anti-inflammatory macrophage response. PCR and ELISA analyses are currently being conducted to provide a deeper understanding of the genetic and protein secretion profiles.

Conclusions
Our findings indicate that neutrophils deposition occurs on all electrospun scaffolds, with notable heterogeneity among replicate groups. Interestingly, this phenomenon was not observed with dPHVs. We are currently analyzing whether this difference is due to the synthetic versus biological nature of the scaffold or its shape. Further analysis is ongoing to investigate the impact on the subsequent macrophage response.

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Characterization of the inflammatory potential of different vascular graft materials

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Clinically available synthetic vascular grafts are well suited for the replacement of large diameter vessels, but fail in small diameter applications due to an insufficient long-term healing process. In particular, excessive inflammation and immune cell activation during the first days of implantation can lead to early stenosis and reduced biomechanical properties caused by fast scaffold degradation. The aim of the current study was to investigate inflammatory reactions to different graft materials in the early healing phase.
The interaction of immune cells with decellularized human umbilical cord arteries (dUCAs), thermoplastic polyurethane (TPU)/TPU-urea (TPU/TPUU) and expanded polytetrafluoroethylene (ePTFE) was investigated by seeding monocytes and neutrophils onto the materials in vitro. The expression of pro- and anti-inflammatory proteins and genes was determined by immunofluorescence staining and RT-qPCR. Autologous, TPU/TPUU and ePTFE small diameter vascular grafts (SDVGs) were implanted into the abdominal aorta of male Sprague-Dawley rats for 24 h or one week (n=5 per time point). Immunohistochemistry and cytokine arrays of the anastomotic areas were performed.
TPU/TPUU and ePTFE grafts induced higher proinflammatory protein and gene expression of immune cells in vitro. Qualitative analysis of immunohistochemical staining of one-week-old implants showed higher anti-inflammatory CD163 expression in the graft wall of autologous implants. Cytokine arrays showed a significant upregulation of proinflammatory chemokines CCL2, CCL3, CCL5, CCL11, CXCL2, CXCL5 and the peptide hormone resistin in anastomotic regions of ePTFE implants after 24 hours, which partially persisted after one week.
In summary, ePTFE induced a significantly higher proinflammatory response than dUCAs and TPU/TPUU in vitro and in vivo. Seeding of immune cells on TPU/TPUU resulted in higher proinflammatory gene expression in vitro, which was not confirmed in vivo.

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Abstract for the Biennial Meeting of the ISACB in Vienna 2024

Development of Vascular Patches with Reconstructed *Tunica Media* and *Tunica Intima*

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Background: Coronary and peripheral artery bypass grafting (CABG and PABG) is a standard treatment for coronary and peripheral artery disease (CAD and PAD), utilizing autologous or synthetic vascular grafts. While autologous grafts possess regenerative and anti-thrombogenic properties, they are limited by availability and donor site morbidity. Synthetic grafts face challenges in small-diameter applications due to thrombogenicity and intimal hyperplasia.

Objective: This study aims to develop planar vascular wall grafts with reconstructed *tunica media* and *tunica intima*, leveraging decellularized tissues, synthetic polymers, and various cell types.

Methods: Decellularized human saphenous veins and horse umbilical blood vessels, along with electrospun polymers (PCL and chitosan) are used to construct vascular patches. The scaffolds were modified (plasma treatment, fibrin network coating, gelatin coating) and seeded with adipose-derived stem cells (ASCs) or Wharton's jelly-derived mesenchymal stem cells (WJSCs), which were differentiated into smooth muscle cells (SMCs). The constructs were then seeded with human umbilical vein endothelial cells (HUVECs). Mechanical stimulation (laminar shear stress and pulsatile pressure) will simulate the *in vivo* environment and support differentiation. *In vitro* assessments will include histology, immunocytochemistry and marker expression analysis.

Results: Initial results are expected to demonstrate successful differentiation of ASCs and WJSCs into SMCs, with appropriate marker expression. Histological and immunocytochemical analysis is anticipated to show a bi-layered structure mimicking the vascular wall components, namely the *tunica media* and *tunica intima*.

Conclusions: This research aims to advance vascular tissue engineering by developing planar vascular patches with reconstructed vessel wall components, with potential future development into (small-diameter) tubular structures.

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Engineering bioadhesive polymer scaffolds to enhance extracellular matrix hydrogel vascular graft mechanics

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Background: Lack of recellularization, tissue regeneration, and integration with host tissues are predominant instigators of small diameter vascular graft (SDVG) shortcomings and inevitable failure. Thus, there is a high demand for bioactive 'off-the-shelf' vascular graft alternatives to currently available synthetic grafts. Decellularised extracellular matrix (dECM) from vascular tissues reconstituted into hydrogels provide ideal bioactive niches to support recellularization and tissue regeneration. However, construction of vascular substitutes with these materials suffers from lack of mechanical performance.

Hypothesis: Engineering supporting scaffolds may enhance dECM mechanics and allow the construction of suitable pro-regenerative vascular graft alternatives.

Methods: Wet spinning was used for the fabrication of uniform and aligned poly(ε-caprolactone) (PCL) fibers. Various methods such as heat treatment and functionalisation with bioadhesive polydopamine (PDA) were used to assess the enhancement of the scaffold properties. Mold-poured bovine aorta dECM hydrogel encapsulated the fiber scaffold and cross-linking methods were used to optimise mechanical properties.

Results: The wet-spun fibers yielded a uniform and criss-cross polymer scaffold structure with high elasticity and strain resistance. Heat treatment further bolstered strain resistance and coating with PDA facilitated strong adhesion to the surrounding dECM hydrogel, which filled the pores between the scaffold fibers. Photo cross-linking ensured the resultant graft had appropriate mechanical properties to be employed as vascular graft.

Conclusions: This work provides a proof-of-concept strategy of employing polymer scaffolds to support and reinforce bioactive hydrogels, offering a promising solution for the manufacture of 'off-the-shelf' tissue engineered vascular grafts.

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Additively manufactured multimaterials with the elastic properties of human tissue

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To create the best possible conditions for practicing surgery, the training material should closely resemble real tissue. Despite the rapid growth in the variety of 3D printing materials in recent years, finding the right material to replicate a specific structure is still challenging. Particularly, replicating gradients in elasticity, color, or surface characteristics is not possible with pure materials. Material jetting enables the simultaneous 3D printing of multiple materials, leading to composite materials with new properties. Our studies have investigated how to combine multimaterials to achieve specific elastic properties and how to predict these properties using finite element method (FEM) analysis to obtain the right mixture for mimicking human tissue.

Using material jetting (Connex3, Stratasys Ltd., Minnesota, USA), commonly used, commercially available materials were 3D printed as composites and mechanically tested. These materials were simulated in silico, with particular emphasis on the interfaces between different material phases.

As a result, various multimaterials have been created with Young's moduli ranging from 100 kPa to 2.5 GPa. This includes the successful replication of the haptic properties of periosteum, which also has a similar feeling when sutured with a needle. However, the typical strain to failure is less than 50%, which limits the ability to replicate the high stretchability of materials such as skin or large vessels. In terms of predictive models, it has been shown that it is critical to account for the non-ideal interfaces between materials, as well as the deviations from the planned geometry that occur during 3D printing. However, the employed FEM models can consistently simulate previously tested materials and accurately predict the properties of modified multimaterials.

The authors believe that this approach will enable the rapid and cost-effective production of a variety of training objects for various surgical procedures, such as suturing blood vessels, using 3D printing.

ISACB 19th Biennial Meeting

Analysis of life quality parameters and circulating biomarkers in a prospective surgical aortic valve replacement study.

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Introduction:
Surgical aortic valve replacement (SAVR) is followed by reverse remodeling of the myocardium and comprises complex physiological and structural adaptation of the heart after cessation of pressure overload. Before operation patients often experience symptoms such as reduced activity, dyspnea, angina, and dizziness. We aimed to reveal the impact of SAVR on life quality and circulating biomarkers reflecting cardiac damage, myocardial stretch, inflammatory status, kidney, and liver function up to one year after SAVR.

Methods:
This prospective single center study was performed on patients older than 18 years of both sexes undergoing elective SAVR with either bioprosthetic or mechanical aortic valves alone or in combination with Bentall-de Bono procedure. Inclusion was based on standard criteria of aortic stenosis. Patients with documented prior myocardial infarction, endocarditis, states necessitating cardiac bypass surgery, electrophysiological device implantation or resuscitation, glomerular filtration rate (GFR) below 60 mL/min/1.73 m^2, or evidenced oncologic malignancies were excluded. Heart failure functional scoring was performed according to the recommendations of the New York Heart Association (NYHA) and angina according to the Canadian Cardiovascular Society (CCS) scoring. Kansas City (KCCF) and SF-12 life quality questionnaires were collected.
Apart of relevant echocardiographic data, we conducted extensive blood sampling with differential cell count and evaluated ~~the~~ biomarkers reflecting the cardiomyocyte damage (high sensitivity troponin T, HsTnT), general muscular damage (creatine kinase, CK) myocardial stretch (N-terminal B-type natriuretic peptide, NT-proBNP), systemic inflammation (C-reactive protein, CRP), kidney function (creatinine; BUN; GFR) and liver metabolism (alanine aminotransferase – AST; aspartate aminotransferase - ALT, gamma-glutamyl transferase GGT) prior to surgery, 4-6 days, 6 month and 1 year after the SAVR.

Results:
We included 95 patients, 15 were lost to the follow up and the observed cohort of 80 comprised 14 females and 66 males (mean age: 69±6 and 63±8 years). We evaluated 71 patients at 6 months and 37 after 1 year. SAVR significantly reduced the maximal ascending aortic flow both in male and female patients (from 4.3±0.9 to 2.3±0.4 m/s and from 4.6±1.2 to 2.3±0.2 m/s, respectively).
After SAVR, NYHA and CCS scores decreased significantly. In the NYHA-I their ratio increased from 28 % prior SAVR to 86% and to 91% after 6 months and 1 year, respectively. At 1 year neither of the patients belonged to the most severe NYHA-III or IV classes (Fig1). The CCS classification developed analogically. KCCF revealed time-dependent increase of life quality 1 year after SAVR. Concomitantly, the SF-12 questionnaire reported an improvement of mental and physical health in all patients compared to the 6 months.
Serum NT-proBNP levels were initially higher and elevated to the greater extent in females early after operation with return to the baseline in both sexes after 1 year (Fig 2). HsTnT and CK were elevated only during the early post-operative phase. AST, ALT, and GGT were higher in men at all timepoints and normalized during the follow up. GFR was higher in men and in some women reached the critically low level at 6 months. Creatinine and BUN were increasing gradually in time-dependently. While leucocytes were higher in men at all timepoints, CRP increased more in women early after SAVR.

Conclusions:

Development of Autologous Tissue-Engineered Artificial Heart Valve for Transcatheter Pulmonary Valve Implantation

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Background: Current mechanical valves and xenogenic bioprosthetic valves have limitations in anti-thrombogenicity and long-term durability, especially for young patients. Our research focuses on developing autologous tissue-engineered artificial heart valves (Biovalves) using an "in body tissue architecture" technique. In this method, tissue-forming molds are embedded subcutaneously, and only the connective tissue that forms around the molds becomes the grafts for implantation. Biovalves show promise for patients with congenital heart disease (CHD) who require multiple open-heart surgeries, offering potential tissue regeneration and long-term valve function. The aim of this study was to develop Biovalves with self-expandable stents, implant them in large animals using a transcatheter technique, and evaluate valve function and tissue structure.

Methods: 3D-printed plastic molds with an internalized shape memory alloy stent were embedded subcutaneously in adult goats. After 2-3 months, the molds were removed with the surrounding tissue, isolating heart valve-like tissue (Biovalves) consisting of autologous connective tissue integrating the stent (stent Biovalves). The stent Biovalves were then implanted into goat pulmonary valves. After 6 months of follow-up, the Biovalves were harvested for histological analysis.

Results: The stent and Biovalve tissue exhibited strong adhesion and remained firmly fixed under crimping conditions. Biovalves were successfully implanted with minimal complications, and no significant stenosis or regurgitation was observed. There was no thrombotic adherence, even without anticoagulation. The implanted Biovalve bound smoothly to the native tissue, showing cellular migration and ECM reconstruction.

Conclusion: The stent Biovalve can be implanted with minimal invasiveness, offering the possibility of long-term functionality through tissue regeneration. This innovation is a promising treatment for patients with CHD and offers an attractive alternative to conventional surgical intervention.

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Small Biohybrid Valves and Scaffolds for Implant Applications

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Background

Small valve replacements are essential for pediatric patients with cardiac diseases and elderly patients with conditions like chronic venous insufficiency. These replacements must meet high standards of biocompatibility, mechanical robustness, and haemodynamic performance. However, current options often fall short to meet such demanding requirements.

Objective

In this study, we address such medical need by developing biohybrid miniature valves. We fabricated small valves made of thermoplastic polyurethane (TPU) and elastin-like recombinamers (ELRs). We evaluated biocompatibility, mechanical properties, and haemodynamic performance in vitro to determine their suitability as new prosthetic valves.

Methods

Tubular TPU scaffolds were manufactured by electrospinning. The material properties were determined with a uniaxial stretcher (UniVert, CellScale), SEM, and a burst pressure testing device. For biocompatibility testing of the material, platelet adhesion and activation, and cell viability using endothelial cells were performed (ISO 10993). The results were evaluated with confocal microscopy, SEM, and with a cell viability kit (CellTiter-Blue, Promega). The TPU scaffold was coated with ELRs by dip-coating and miniature valves were created by suturing the tube into an adapted and modified stent scaffold (optimed). The valve was tested in bioreactors for haemodynamic performance (ISO 5840).

Results

The electrospun TPU showed excellent elastic properties with strain values over 300% and maximum stresses exceeding 4 MPa, surpassing the values of native valves. The ELR-coated TPU scaffold withstood burst pressures over 1000 mmHg. Blood compatibility tests revealed lower platelet activation for ELR-coated TPU compared to clinically used ePTFE, and good cell viability in contact with (ELR-coated) TPU. Haemodynamic testing of the assembled valve showed regurgitation fractions below 15% and complete valve closure.

Small-diameter Vascular Grafts with Tunable Compliance via Melt Electrowriting

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Background

Synthetic small-diameter vascular grafts (SDVGs) show high failure rates *inter alia* due to intimal hyperplasia. A mismatch in compliance between graft and native vessel has been identified as major contributing factor to intimal hyperplasia.

Objective

Following the paradigm of *in situ* tissue engineering, we propose a multiscale polymeric graft architecture to obtain off-the-shelf SDVGs with a tunable compliance covering the full range of native vessel characteristics and the capability to transform into living tissue.

Methods

Tubular scaffolds (Ø 4 mm) with controlled patterns of various fiber winding angles were additively manufactured from polycaprolactone microfibers via melt electrowriting (MEW) and subsequently dip coated in gelatine. The scaffold architecture was based on a luminal microporous spiral pattern complemented by a macroporous mechanically-defining outer spiral pattern. All grafts were assessed for compliance, suture retention, burst pressure, and kinking behavior following ISO 7198. Grafts were anastomosed to explanted saphenous veins and porcine coronary arteries.

Results

Controlling the fiber winding angle allowed to tune the compliance from 0.6 ± 0.4 %(100 mmHg)$^{-1}$ to 12.7 ± 2.0 %(100 mmHg)$^{-1}$ (n = 3, i = 4), which covers the full physiological range of human veins and arteries. All grafts showed adequate burst pressure (1411 ± 280 mmHg, n = 5) and suture retention (0.7 ± 0.3 N, n = 7). No kinking was observed for bending radii down to 1.5 mm (n = 3). Grafts with venous compliance were successfully anastomosed to human saphenous veins and pulsated with the same amplitude when subjected to pulsatile flow. Arterial compliance was evaluated by coronary artery bypass grafting on porcine hearts.

Conclusion

Our biofabrication strategy leverages microscale additive manufacturing to form physiologically relevant SDVGs featuring compliance tailored to that of the native target vessels. Based on a degradable polymeric scaffold architecture this holds the promise for remodeling into a healthy living vessel.

Thrombus Characterization in Abdominal Aortic Aneurysm and Aortic Dissection Models Using Histology and Scanning Electron Microscopy

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Aortic dissections (ADs) and abdominal aortic aneurysms (AAAs) are common aortic pathologies. AD is characterized as a tear in the intima, causing separation of the layers within the vessel wall, forming a false lumen. AAA is the localized-dilation and weakening of the aortic wall, resulting in expansion and complex flow patterns. While distinct, both frequently develop thrombus within the lumen (AAA) or within the wall (AD). Due to the differences in hemodynamics, we hypothesize that thrombus structure and composition will vary between these two conditions. To examine this, we utilized murine experimental models, including 1) topical elastase-β-Aminopropionitrile for AAA and 2) angiotensin II-infused apolipoprotein E-deficient mice for AD. Using serial ultrasound measurements, we detected vessel expansion in both models and monitored 1) diameter changes and 2) thrombus deposition. Using histology, we quantified the degree and composition of intramural (IMT) and intraluminal thrombus (ILT) in ADs and AAAs respectively. Color segmentation of Movat's pentachrome slides revealed a significantly higher percent area of fibrin ($p<0.001$) in ILT, while red blood cells (RBCs) were significantly higher ($p<0.001$) in IMT. Collagen, elastin, and proteoglycans were present in small amounts in both models. Semi-quantitative examination of histology and scanning electron microscopy images showed layers of fibrin in the ILT structure, while the IMT formed a compact network of fibrin and RBCs. Higher amounts of fibrin appear to correlate with a greater degree of organization in the ILT, which may be caused by the active blood flow present in AAAs. This pattern is mostly absent in the IMT due to the recirculation of blood and low wall shear stress in the false lumen of ADs. These data represent an initial effort to understanding how thrombi formation correlates with vessel growth rate and rupture risk to improve patient outcomes for those suffering from AD and AAA.

Vasoreactivity Testing of Tissue-Engineered Vascular Grafts *In Vivo* in an Ovine Model

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Background: Tissue-engineered vascular grafts (TEVGs) may represent a better alternative to the prosthetic biomaterials that are currently used in cardiovascular reconstruction. Composed of a biodegradable scaffold onto which autologous cells may be seeded, TEVGs provide sites for the formation of neotissue when implanted *in vivo*. The resultant neovessel is theorized to have several advantages over prosthetic grafts, including enhanced compliance, potential for growth, and vasoactive functionality. Using an ovine model, we investigated the vasoreactivity of TEVGs implanted as interposition grafts in the inferior vena cava (IVC).

Methods: Three sheep implanted with TEVGs and one implanted with a polytetrafluoroethylene (PTFE) graft were compared to an age-matched control with native IVC. Animals underwent catheterization procedures during which norepinephrine (NE) was injected into the abdominal IVC at doses of 0.25 mcg/kg and 0.75 mcg/kg. Intravenous ultrasound was used to evaluate changes in the cross-sectional area of the mid-graft or mid-thoracic IVC at maximum constriction in response to drug injection.

Results: The average cross-sectional area of TEVGs decreased by 19.0% and 17.6% from baseline following injections of 0.25 mcg/kg and 0.75 mcg/kg of NE, respectively, compared to 23.5% and 67.3% in the native IVC, while the PTFE graft showed no vasoconstriction and minimal change in diameter. Changes in cardiac output (CO) were inversely related to the degree of graft or IVC constriction.

Conclusion: TEVGs more closely resembled native IVC in their response to localized injections of norepinephrine, unlike PTFE grafts, which had no vasoactive function. Vasoconstriction of the IVC or interposition grafts had a direct and immediate effect on cardiac output. The vasoactive function of TEVGs may allow these grafts an enhanced ability to adapt to the body's dynamic physiologic needs.

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2024 ISACB ABSTRACT

Title

Sex-dependent Differences in the Progression of Chronic Kidney Disease Induced Cardiac Dysfunction

Authors

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Abstract

Cardiovascular disease (CVD) in chronic kidney disease (CKD) patients exhibits notable sex-dependent differences. Women with CKD have lower incidences of atherosclerotic events and heart failure but higher rates of CVD events in end-stage CKD compared to men. These variations highlight the need for sex-specific CVD diagnosis and management strategies in CKD patients. Electrocardiograms (ECG) can quickly detect cardiac abnormalities, but common ECG markers are often unreliable in CKD patients. In this study, we assess cardiac structural and functional changes during the progression of CKD-induced CVD in mice using electrocardiography and echocardiography to identify how these changes differ due to disease progression and sex.

Adult C57B/6J mice of both sexes were assigned to either a Control group or an Adenine group, which developed CKD and cardiac dysfunction via a high-adenine diet. Mice were sacrificed at 3, 6, 9, and 12 weeks post-CKD induction, and their cardiac structure and function were analyzed using echocardiography and ECG. Differences in cardiac parameters considering sex and regimen type were statistically assessed.

At week 12, left ventricular thickness in Adenine males was 120.18% greater than in Control males and 135.64% greater than in Adenine females, indicating hypertrophy. They also showed higher ejection fraction and prolonged isovolumetric contraction and relaxation times, indicating systolic and diastolic dysfunction. Adenine males had increased QTc interval duration (63.4±10.3 ms) compared to Control males (49.5±1.7 ms) and Adenine females (47.4±4.6 ms), a marker of abnormal repolarization and cardiovascular event risk. We identified that Speak-J interval also differed by sex, with significant increases in Adenine males at week 6 and Adenine females at week 12.

This study demonstrates the feasibility of this mouse model for investigating sex-dependent differences in CKD-induced cardiac dysfunction, which could aid in the development of tailored diagnostic and management strategies for CVD in CKD patients.

Characterization of the immune response and in vivo remodeling of a new generation of allogeneic Cell-Assembled extracellular Matrix after subcutaneous implantation in sheep.

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The number of bypasses and vascular access procedures for hemodialysis grafts in Europe is increasing. Due to the limited availability of autologous vessels, surgeons often rely on synthetic grafts, which are prone to infection and chronic inflammation. Moreover, synthetic grafts perform poorly for small-diameter applications (< 6mm). A promising therapeutic is tissue-engineered vascular grafts (TEVGs).

We developed a biological TEVG using a cell-assembled extracellular matrix (CAM) and a textile-based approach. Fibroblast cells synthesize CAM sheets in vitro, which are then processed into yarns and woven into vascular substitutes.

To better simulate clinical conditions, we used a sheep model to produce CAM sheets comparable to human-origin sheets. These ovine CAM yarns were implanted in sheep to study the host immune response in an allogeneic context. We compared the host response to various CAM yarns (devitalized vs. decellularized, laser vs. blade cutting, the addition of an anti-inflammatory molecule, and xenogenic vs. allogeneic CAM yarns) to select the best-performing yarns for weaving our TEVG.

The CAM yarns were implanted subcutaneously in sheep and explanted after 2, 4, 12, and 24 weeks to assess changes in tensile strength, inflammatory response, and remodeling through histological analyses and immunofluorescence.

After 24 weeks, all CAM yarns were retrieved and found to be surrounded by fibrous neo-tissue. The tensile strength of the CAM threads increased over this period, indicating an absence of a degrading inflammatory response.

Differences in immune response and remodeling are currently being analyzed to evaluate the impact of decellularization and laser processes. Human CAM yarns showed a similar increase in strength, suggesting low immunogenicity of our CAM material. Based on these results, CAM yarns are compatible with weaving a TEVG that will maintain its structural integrity and mechanical properties in vivo.

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Non-invasive electrophysiological evaluation of new-onset atrial fibrillation after cardiac surgery: Preliminary results from the BigMap Study

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Objective
New-onset atrial fibrillation after cardiac surgery (NOAF) is a frequent complication and is associated with postoperative stroke, increased mortality, prolonged hospital length of stay, and higher treatment costs. The structures maintaining NOAF are mainly unknown. Thus, the present study aims to describe electrophysiological patterns of NOAF that might be future therapy targets.
Design and method
Consecutive patients undergoing cardiac surgery have been included and monitored for the first seven postoperative days for the development of NOAF. Patients with NOAF underwent non-invasive electrophysiological mapping using a 252-electrocardiogram vest to identify focal and rotational potential drivers of NOAF (PDs). After mapping, a computed tomography scan of the chest was performed to generate a 3-dimensional model of the atria, thus allowing an identification of NOAF maintaining structures. The primary outcome was the electrophysiological description of potential drivers (PD) of NOAF and NOAF-maintaining structures within the atria.
Results
Of 205 enrolled patients, 62 (30%) developed NOAF. Electrophysiological mapping was performed in 23 NOAF patients (37%). A median of 29 (21-48; IQR) rotational PDs and a median of 26 (20-35[IQR]) focal PDs were identified per patient. The most frequent localizations of rotational drivers were the upper half of the right atrium (23 patients, 100%), the inferior/posterior left atrium (22 patients, 96%), and the left pulmonary veins (22 patients, 96%). The most frequent localizations of focal drivers were the left (22 patients, 96%) and right pulmonary veins (20 patients, 87%) and the upper half of the right atrium (22 patients, 96%).
Conclusions
Based on these preliminary results, structures such as the upper right atrium might play a role in maintaining NOAF and might be targets for future preventive or therapeutic strategies. As the study is ongoing, more precise results with identifying risk factors for specific locations and associations with outcomes are expected.

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The Solution is Dilution: Parabiosis of WT to LYST Mutant Improves TEVG Performance

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Tissue-engineered vascular grafts (TEVGs) for use in the repair of single ventricle disease are limited due to stenosis. We discovered that LYST gene mutation protects against graft stenosis. Global LYST mutation is therapeutically inviable due to numerous cellular dysfunctions. Efforts to design a therapeutic led us to investigate whether 100% of cells need the LYST mutation to have a therapeutic benefit or if partial LYST dysfunction will be beneficial without completely impairing cellular functions. Our hypothesis is that LYST will protect against stenosis at a lower ratio of mutated cells. We used a surgical parabiotic model to determine whether a 50:50 ratio of WT to LYST mutant cells could prevent stenosis. Two mice are surgically attached at the elbow, knee, and skin, allowing formation of shared microvasculature and circulating cells. We investigated 11 parabiotic pairs of WT-GFP and LYST KO mice. Flow cytometry showed that 50% of all live circulating cells were GFP positive and 50% were GFP negative by 6-weeks, indicating equilibration of the pair's cells. Additionally, we injected fluorescent nanoparticles (0.05 um and 2 um) into the WT mouse. All assessed nanoparticles crossed over from one mouse to the other within 10 minutes of injection. We assessed graft patency via explantation at 2-weeks. Of the six assessed pairs, 100% of the WT-GFP mice were patent. This suggests that sharing 50% of the circulating cells was sufficient to protect against TEVG stenosis. Understanding this principle of LYST functionality will be essential when developing therapeutics.

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Powering the Future: Sputtering Technique for Superior Flu-TENG Performance

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Abstract:

Cardiovascular diseases (CVD) are the leading cause of death, claiming 17.9 million lives each year. To address this critical issue, Cardiovascular Electronic Devices (CEDs) have become essential in both treatment (e.g. pacemakers) and diagnosis (e.g. implantable sensors – Digital Health). However, many CEDs face challenges related to their batteries, which often have short lifespans, large sizes, and the potential for toxic leakage, creating a huge demand for battery-free CEDs. Indeed, increasing the lifespan of batteries by 50% would reduce total costs by 22%, representing savings of 12500€ per patient. Recently, our group discovered that the application of liquid-solid triboelectric nanogenerators (Flu-TENGs) to the biomedical field has great potential to revolutionize the way of harvesting electricity from human body. This method involves the movement of a liquid along a solid surface (e.g., synthetic vascular grafts), which generates opposite charges in both materials and results in charge transfer across the interface. Typically, an electrode is placed on top of the solid-phase to collect the electrons generated on its surface. This work aims to correlate the effect of different electrodes deposited by sputtering with the electric output generated with our Flu-TENG system. Different coatings with different thicknesses (25nm to 300nm) were deposited using a diagonal coating method with a rotation target by sputtering. The results suggest that optimizing the electrode materials and deposition processes can significantly enhance the triboelectric performance of Flu-TENGs, achieving maximal peak-to-peak voltages of 2.93V with well-defined peaks and noise reduction of up to 8%, compared with brush painting. By improving the efficiency of charge transfer, we can achieve higher energy output from Flu-TENG. This advancement will be key towards the development of battery-free CEDs, reducing the frequency of surgical interventions and the associated healthcare costs, powering the future of the biomedical field.

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Durable immunomodulatory nanofiber niche for the functional remodeling of cardiovascular tissue

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Functional remodeling and prolonged anti-inflammatory responses are both vital for repairing damage in the cardiovascular system. Although these aspects have each been studied extensively alone, attempts to fabricate scaffolds that combine these effects have seen limited success. In this study, we synthesized salvianic acid A (SA, danshensu) blocked biodegradable polyurethane (PCHU-D) and enclosed it within electrospun nanofibers to synthesize a durable immunomodulatory nanofiber niche (DINN), which provided sustained SA release during inflammation. Given its excellent processability, mechanical properties, and shape memory function, we developed two variants of the DINN as vascular scaffolds and heart patches. Both these variants exhibited outstanding therapeutic effects in in vivo experiments. The DINN was expertly designed such that it gradually decomposes along with SA release, substantially facilitating cellular infiltration and tissue remodeling. Therefore, the DINN effectively inhibited the migration and chemotaxis of inflammatory cells, while also increasing the expression of angiogenic genes. As a result, it promoted the recovery of myocardial function after myocardial infarction and induced rapid reendothelialization following arterial orthotopic transplantation repair. These excellent characteristics indicate that the DINN holds great potential as a multifunctional agent for repairing cardiovascular tissue.

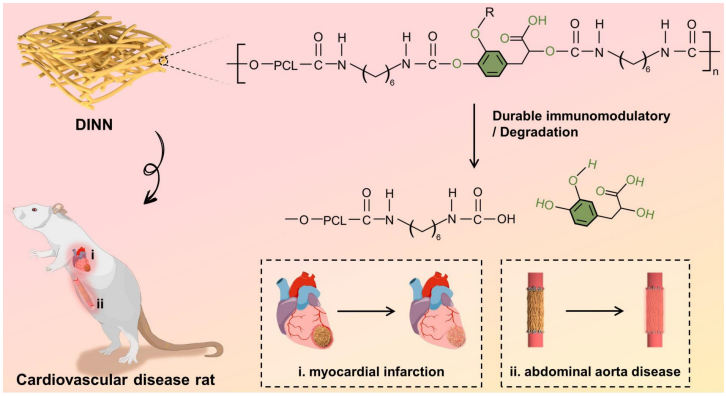

Fig. 1 The scheme of synthetic durable immunomodulatory nanofiber for the functional remodeling of cardiovascular tissue.

Key Words: inflammation; nanofiber niche; cardiovascular tissue; matrix remodeling

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Development of a simple same-day implantation technique for allogeneic *in vivo* tissue-engineered vascular grafts to be completed in the operating room

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Background of the clinical problem
We have developed the autologous connective tissue vascular grafts for children constructed in subcutaneous spaces of the patients. In 2014, we clinically applied this technology in pediatric pulmonary artery patch augmentation. However, the formation of reliable connective tissue membranes is an issue in high-risk pediatric patients due to limited subcutaneous areas and insufficient regeneration activities.
Objective/hypothesis
In this study, we explored the option of graft creation in healthy parents for allogeneic transplantation to their children. Furthermore, simplification and shortening of the decellularization process are indispensable to achieve same-day transplantation in the operating room. This study focuses on optimizing decellularization process and conducting preliminary animal implantation experiments.
Brief methodological details
Silicone rod molds were implanted subcutaneously in beagle dogs for four weeks, after which the formed connective tissue tubes were excised. These tissue tubes were decellularized using a 1% sodium lauryl ether sulfate (SLES) solution with horizontal shaking (2h/1h/30min). Following decellularization, DNA quantification and tensile strength measurements in the short-axis direction were performed. The tissues were then trimmed into sheets and transplanted as allogeneic patches into another beagle dog's carotid artery. Post-implantation assessments were conducted using ultrasound, and the grafts were excised after three months.
Results
For one hour or more decellularization was required to ensure the complete removal of cellular components from the connective tissue membranes. Tensile strength measurements indicated no significant differences before and after decellularization. During three month-implantation, the grafts did not develop aneurysmal dilation. Morphological examination exhibited no thrombus formation on the luminal surface, which was covered with smooth neointima.
Conclusions/interpretations
Shaking methods successfully simplified and shortened the decellularization process compared with the previous perfusion methods. The decellularized connective tissue membranes maintained mechanical properties and excellent regenerative performance comparable to previous autografts, which suggests their potential as substitutes for allogeneic vascular grafts.
Funding sources
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Nothing to disclose.

TISSUE ANALYSIS CORE (TAC) CAPABILITIES AT THE CENTER FOR CARDIOVASCULAR RESEARCH IN BIOMECHANICS (CRIB)

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Supported by the NIH's Center of Biomedical Research Excellence (COBRE) program, the University of Nebraska Omaha has launched the Center for Cardiovascular Research in Biomechanics (CRiB). This center focuses on translational research to develop innovative materials and devices for treating cardiovascular diseases. A key facility, the Tissue Analysis Core (TAC), leverages Nebraska's biomechanics expertise of comprehensive mechanical, structural, and biological evaluations of arteries from >1,000 human subjects aged 12-99 years. TAC has developed protocols and established partnerships with organ procurement organizations to access scarce human tissues, enhancing the scope of research possibilities. TAC supports CRiB's initiatives and offers its specialized services to the broader scientific community. These services include the mechanical assessment of soft tissues and vascular repair materials through multiaxial tensile testing and pulsatile flow circuits. These tests are critical for assessing tissue compliance, performing constitutive modeling, and studying the impact of various risk factors on tissue elasticity. Equipped with a large micro-CT imaging device, TAC provides high-resolution, non-destructive 3D imaging to examine the structural integrity of biological tissues, engineered materials, and medical devices. Additionally, TAC is equipped for Scanning Electron Microscopy, Duplex and Intravascular Ultrasounds, histological evaluations, and medical device prototyping and design, including implantable devices. Collaborating closely with the neighboring University of Nebraska Medical Center, TAC plays a vital role in the preclinical evaluation of devices and materials using a swine model. This collaboration extends to assisting with regulatory compliance, instrument preparation, animal surgeries, and result evaluations. Current projects at CRiB include developing peripheral stents, aortic stent-grafts, peripheral bypass grafts, and wound dressings, all utilizing large animal models. We invite the scientific community and the biomedical device industry interested in human artery analysis and preclinical evaluations to engage with CRiB and TAC. For more information or to discuss potential projects, please contact us at crib.center@unomaha.edu and crib.tac@unomaha.edu.

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Guidelines:

Abstracts should include:

Title
All author names (underline the presenting author)
All author affiliations, denoted by number
Main text

Abstract main text should include:

Background of the clinical problem
Objective/hypothesis
Brief methodological details
Results
Conclusions/interpretations
Funding sources
Financial disclosures relevant to the abstract, if any

Demographics and clinical relevance of animal models in haemodialysis research: a systematic review

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Background & Aims: With the dialysis population growing, improving haemodialysis (HD) is crucial. While innovations undergo extensive preclinical testing, we lack consensus on which animal models or methods of inducing kidney failure are appropriate and on how we should validate dialysis efficiency. We systematically reviewed the literature for kidney failure animal models investigating the effect of dialysis [HD, haemofiltration (HF), HdiaF and ultrafiltration, excluding peritoneal dialysis] innovations on kidney function.

Methods: The methodology was registered on PROSPERO-CRD42022307144. PubMed and Embase were searched for studies detailing a dialysis intervention in any animal model with acute (AKI) or chronic kidney failure. Primary outcomes included reduced kidney function and dialysis-related urea clearance. Other parameters related to species, kidney injury induction and vascular access. We aimed to identify influential factors by meta-regression analysis.

Results: We included n=51 publications reporting on n=72 experimental comparisons. Dialysis was most frequently performed on dogs (61%), rats (20%) or on goats, pigs, cats or sheep. Characteristics such as sex and age were poorly reported (38% and 19%). Studies primarily surgically induced AKI (91%), validated by plasma urea and creatinine increases. Vascular access in dogs and goats was mostly arterio-venous, while veno-venous in pigs and rats. Most studies investigated HD or HF (>95%), with animals undergoing a single dialysis session (median, [IQR 1,0 – 2,0]). Randomization and blinding was poorly reported (<25%). Moreover, there was limited monitoring of key quality indicators including blood pressure and body temperature (<40%) during dialysis. Data synthesis is ongoing

Conclusion: Our systematic review shows low internal validity of studies due to poor reporting and unclear risk of bias. The infrequent use of sepsis or chronic disease models to test dialysis moreover contributes to low external validity. We recommend a better alignment of experimental set-ups with the patient-population in need of dialysis to propel innovations.

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Advancements and Challenges in Cardiovascular Health and Translational Innovation in Bosnia and Herzegovina

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Background: Cardiovascular diseases (CVD) remain a leading cause of mortality in Bosnia and Herzegovina, driven by high prevalence rates of risk factors such as hypertension, obesity, and smoking. This clinical problem is compounded by the evolving healthcare landscape and the need for innovative technological interventions and innovations. **Objective:** This study aims to analyze the latest data on cardiovascular health and translational innovation in Bosnia and Herzegovina, evaluate the impact of innovative health technologies, and identify ongoing research efforts and public health initiatives aimed at improving cardiovascular outcomes. **Methodology:** A comprehensive review of recent studies, healthcare reports, and public health data was conducted. Sources included national health statistics, research publications, and reports from international health organizations. The focus was on the prevalence of CVD, risk factors, and the implementation of telemedicine and other digital health solutions. **Results:** Recent statistics indicate that diseases of the circulatory system account for approximately 52.8% of all deaths in Bosnia and Herzegovina, with ischemic heart diseases and cerebrovascular diseases being predominant. Innovative technologies, such as telemedicine and electronic health records (EHR), are gradually being integrated into the healthcare system, improving access to care and patient management. Research efforts are increasingly focusing on local risk factors and the development of tailored interventions. Public health initiatives are promoting lifestyle changes and early detection of CVD. **Conclusions:** Despite the high burden of cardiovascular diseases, the adoption of innovative health technologies and targeted research initiatives are promising steps towards improved management and prevention. Continued investment in healthcare infrastructure and public health campaigns is crucial for mitigating the impact of CVD in Bosnia and Herzegovina. Collaboration with international institutes and adherence to global health standards will further enhance these efforts.

GO-Graft: From Hydrogel to Anti-Adhesive Vascular Graft for Bypass Surgery

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Heart and limbs small-arteries blockage can lead to death or limb amputation, respectively. Autologous grafts (patient's vessels) are the gold-standard to restore blood flow, but are limited and, while superior than ePTFE-commercial grafts, present thrombosis risk. Thus, we created the GO-Graft, a synthetic, anti-adhesive, small-diameter vascular graft made of FDA-approved hydrogel poly(2-hydroxylethilmetacrylate)(pHEMA) reinforced with graphene oxide (GO), one of Earth's strongest materials. GO-graft prototype overperforms ePTFE in acute thrombogenicity studies in pigs, but cannot be sutured to host arteries.

This work aims to increase its suture retention (SR) from 55 to ≥140g, making GO-Graft viable for patients.

GO-graft 2D films (pHEMA/GO) were produced by *in-situ* polymerization of HEMA monomers with GO and tetraethylene-glycol-dimethacrylate (molding). To improve SR, we explored: incorporation of co-polymers (alginate); fibers (polypropylene(PP) or GO); films(GO); and meshes(polycaprolactone melt-electrowritting (PCL-MEW) or polyethylene terephthalate(PET) textile) before pHEMA/GO polymerization. GO properties were assessed by XPS and TEM; ELR methacrylation was verified by NMR and FTIR. Hydrogels' surface roughness was evaluated through SEM; SR was assessed by tensile tests (ISO-7198).

Among the tested conditions of co-polymer incorporation, only 0.03%(w/v) alginate ionically-crosslinked with 0.5–1MCa^{2+}/Ba^{2+}/Sr^{2+} incorporation increased SR (up to 157g), but the presence of divalent-ions induced thrombus formation, excluding its suitability for GO-graft. PP fibers improved SR (~170g), yet, poor interaction with pHEMA/GO's matrix led to fiber detachment, resulting in unstable films. To improve hydrogel's matrix interaction, GO fibers or films were incorporated, but the slight increase in SR(~80g) was still substandard. The suture retention strength of pHEMA/GO with polycaprolactone melt-electrowriting (PCL MEW) meshes was 137 g, whereas Polyethylene terephthalate (PET) textiles incorporation resulted in an unrupturable hydrogel under the tested conditions. PET meshed incorporated pHEMA/GO is a suturable hydrogel, paving the way for GO-Graft's future application as a vascular graft.

Graphene Oxide: A Masterpiece for Enhancing Decellularized Tissue Applications in Cardiac Regeneration

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The development of small-diameter vascular grafts (diameter <5 mm) and effective cardiac regeneration biomaterials is crucial for cardiovascular diseases, the leading cause of death worldwide. Decellularized tissues and gels have promising features for tissue regeneration but weak mechanical properties, limiting their use in load-bearing applications. We propose incorporating graphene oxide (GO) into decellularized extracellular matrix gels (dECM-gels) and decellularized arteries to overcome these limitations and enable their broader application.

Umbilical cord arteries were decellularized and perfused with a GO suspension (C/O ratio 2:1) with ~1.5 µm lateral size to coat the lumen [1]. The dECM gels were obtained from placental tissue and digested with pepsin to obtain pre-gels, which were mixed with different amounts of GO (1-4% v/v) [2]. Gelation was promoted overnight at 37°C.

For decellularized umbilical cord arteries GO-coatings improved their mechanical properties, increasing the maximum force by 27%, burst pressure by 29%, strain by 25%, and compliance by 10%, comparable to human saphenous veins and mammary arteries, used in bypass surgery. Importantly, GO coatings did not compromise endothelial cell adhesion while reducing platelet and bacteria adhesion, potentially preventing thrombosis and infection until re-endothelialization occurs.

In dECM-gels, SEM images showed that GO intercalated within collagen fibers, and rheological tests revealed a remarkable increase in the complex modulus by 21768% at the highest GO concentration. This enables the production of a stable filament for 3D-printing of scaffolds. Although GO did not decrease bacterial adhesion due to its non-exposure on the surface, it reduced clotting time, indicating increased pro-coagulant properties

Overall, GO significantly enhances the mechanical properties of decellularized arteries and dECM-gels and biocompatibility, representing a major advancement in the application of decellularized tissues for cardiac regeneration and as small-diameter vascular grafts for bypass surgery.

References

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