were isolated at six, 24 weeks and two years of age. Immunohistochemical stainings were performed to examine age related structural changes. We observed loss of elastin organisation (RF staining), enlargement of the media and cell loss in both 24 week Ercc1$^{d/-}$ and two year old WT aortas (HE staining). The VSMC markers SMA, vimentin and smoothelin were decreased in old compared with young Ercc1$^{d/-}$ aortas. Stress marker p21 was increased in Ercc1$^{d/-}$ aortic VSMCs. As literature suggests that ageing VSMCs undergo a phenotypic change from contractile to synthetic, we isolated VSMCs from young (WT) and ageing (Ercc1$^{d/-}$) aortas, to study these phenotypes further. To mimic ageing we are using ERCC1 deficient patient fibroblasts, which are being reprogrammed into iPSCs. As these patients have different genetic backgrounds, therefore lacking an isogenic control, we are also mutating the ERCC1 gene in control iPSCs with CRISPR/Cas9. We have now performed first CRISPR/Cas experiments in cells and are screening clones for proper mutation introduction. We have successfully differentiated control iPSCs to VSMCs and ECs. Once the ERCC1 mutant iPSCs are available, we will perform differentiation experiments together with isogenic controls. To set up the 3D vascular ageing model *in vitro*, first VSMCs are coated in a microchannel of the microfluidic system, with the ECs coated on top of them. This forms microvessels, which we can subject to clinically relevant flow levels.

Conclusion: Progeroid Ercc1d/- aortas and cells show an age related increase in vascular ageing markers, which can be used as readouts for the human 3D *in vitro* vascular ageing system. Once Ercc1 mutated iPSCs are generated, we can test their differentiation potential to VSMCs and ECs, and optimise our 3D *in vitro* vascular model for ageing studies. With this model we will investigate the interaction between the ageing vasculature and circulating factors in plasma from healthy subjects and patients with age related cardiovascular disease and screen for intervention compounds.

Disclosure: Nothing to disclose

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Silk Fibroin Vascular Graft: From Design to In Vitro and In Vivo Test

Peripheral Arterial Disease

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Introduction: A biomaterial scaffold simulating the three layered structure of blood vessels has been proven to be an effective way to mimic the native architecture, the mechanical behaviour, and functional features of native arteries. In this study, production and characterisation of an innovative multilayered Silk Fibroin Vascular Graft (SF-VG) is reported. Aim of our paper is to demonstrate the *in vitro* cell interaction studies performed to investigate the biological response to the device and *in vivo* preliminary pilot trials on animals to evaluate short and medium term behaviour of the graft in terms of handling, risk of infection, tissue integration and patency.

Methods: The SF-VGs were manufactured according to patented technology (WO 2016/067189 A1). Adult Human Coronary Artery Endothelial Cells (HCAECs), Human Aortic Smooth Muscle Cells (HASMCs), and Human Aortic Adventitial Fibroblasts (HAAFs) were used for the *in vitro* tests. In the *in vivo* studies, 7-8 cm SF-VGs were implanted as carotid artery substitutes in minipigs and sheep. Four weeks after the surgery the SF-VGs were harvested, and a complete histopathological investigation was performed. After in vitro tests a pilot *in vivo* study was performed by replacing resected portions of the common carotid arteries of two minipigs and one sheep with SF-VGs, sacrificing the animals four weeks later and examining their histopathologic patterns. The results of the pilot study led us to perform a medium term *in vivo* test operating on 12 carotid arteries in six sheep followed up for 12 months.

Results: The SF-VG device was manufactured by combining electrospinning and knitting/braiding technologies. The results of *in vitro* cell interaction studies demonstrated the biocompatibility of SG-VG. The high basal secretion of Monocyte Chemo-attractant Protein-1 and Protein-2 was indicative of a cell proliferation capacity. Interferon gamma induced protein 10 (known for its anti-fibrotic and angiostatic properties) and Regulated on Activation, Normal T Cell Expressed and Secreted (known for its ability to regulate leucocyte diapedesis, angiogenesis, and some scarring processes) were expressed at significant levels. The pilot animal study showed the feasibility of using SF-VG as *in vivo* arterial substitute and assessed the sheep as the best animal model for in depth investigations on the behaviour of the SF-VG *in vivo*. A medium term study (follow up at 1, 6, 12 months) was initiated on six sheep with bilateral common carotid artery resection and replacement by SF-VGs. The study is still ongoing and after 12 months no lumen stenosis and hence a persistent good blood flow was observed in the SF-VGs implanted.

Conclusion: SF-VG is proposed as a new vascular graft entirely made of pure silk fibroin. *In vitro* cell interaction studies showed that the device favours cell adhesion and their survival and growth. Short term *in vivo* studies to assess the patency and wall remodelling of SF-VGs seem to be satisfying in term of patency rate.

Disclosure: Nothing to disclose

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Formation of Vascular Wall De Novo in Tissue Engineered Vascular Graft Based on a Biodegradable Poly(L-Lactide) Scaffold

Miscellaneous

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Introduction: Cardiovascular morbidity and mortality are still rising in the world [World health statistics, 2019]. That is why surgery needs a great amount of plastic material. The ideal one could be the tissue engineered vascular graft (TEVG). The aim was to access