of physiological aortic stiffness to investigate the impact of HDAC6 inhibition on the contractile response of angiotensin II stimulated quiescent VSMC function. In this study, we utilise HDAC6 inhibitor BRD 9757 and Tubastatin a HDAC6 inhibitor inducing tubulin hyperacetylation.

**Results**

Our data shows that HDAC6 inhibition resulted in increased alpha-tubulin acetylation and decreased VSMC area. Further analysis revealed that although VSMC volume was unaltered, nuclear volume was decreased. Immunofluorescence microscopy revealed that HDAC6 inhibitor treatment resulted in DNA damage accumulation in VSMCs. We hypothesised that altered microtubule stability participated in this phenotype. To test this possibility, we performed a cold-stable microtubule stability assay, which revealed that HDAC6 inhibitor treated VSMCs possessed decreased microtubule stability. To test whether changes in microtubule stability induced DNA damage accumulation, we used the microtubule destabilising agents colchicine and demecholcine, and the microtubule stabilising agent paclitaxel. Importantly, either colchicine or demecholcine treatment increased DNA damage accumulation in VSMCs. In contrast, paclitaxel treatment had no effect on DNA damage levels.

**Conclusion**

These results indicate that microtubules play a role in protecting against DNA damage accumulation in VSMCs. Potentially, deacetylation of microtubules leads to a greater actomyosin force that mechanically squashes the nucleus and physically induces DNA damage. Further work is ongoing to test this possibility and to confirm this response in isolated aortic rings. Our data also highlights the potential vascular side effects of clinically using HDAC6 inhibitors.

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**BS20 DEXAMETHASONE INHIBITS OPN-ACTIVATION ASSOCIATED WITH INTIMAL HYPERPLASIA IN VEIN GRAFTS**

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**Background**

The long saphenous vein (LSV) is commonly utilised in CABG surgery to facilitate revascularisation. However, over time these grafts develop intimal hyperplasia (IH) and accelerated atherosclerosis, leading to stenosis and occlusion. A common feature of IH is vascular calcification (VC) within the affected vessel. Recently, the matricellular protein osteopontin (OPN) has been implicated in this process at endothelial injury sites in porcine models, but this has not been expanded to humans. Consequently, studies have implicated the arterial haemodynamic environment as a major driver of the pro-inflammatory conditions facilitating VC and IH. As such, treatment with a synthetic glucocorticoid, dexamethasone, which has proven beneficial in inhibiting IH in murine models, may beneficially modulate this process in humans. This work aims to assess the role of OPN on VC and IH in an ex vivo model, whether dexamethasone can modulate this process, and whether detection of VC in situ can act as a novel clinical monitoring approach to graft patency.

**Methods**

LSV segments were obtained from consented patients undergoing CABG surgery. LSV tissue was (1) cultured for up to 10 days, with samples untreated or pre-treated with dexamethasone (10ng/mL) for 1 hour, or (2) subjected to arterial flow conditions using an ex vivo perfusion bioreactor to mimic haemodynamic conditions for 4 hours. Gene and protein expression changes were quantified using qRT-PCR, RNA-Scope and immunofluorescence (IF). Accumulation of calcium was identified using Alizarin Red and McNeal’s Tetrachrome stain.
Abstract BS20 Figure 1  IF and RNAScope imaging of OPN expression in LSV sections under static, flow, and culture conditions. Dixa = Dexamethasone; *** = p< 0.0001.

Abstract BS20 Figure 2  Figure 2: Calcification detection in LSV sections, using McNeal’s Tetrachrome, Alizarin Red and 18F autoradiography.
staining, visualised using light microscopy, as well as 18F autoradiography.

Results Significant OPN expression was identified over time and in response to flow in untreated samples, whilst dexamethasone pre-treated samples showed significant reduction in expression by day 10, validated using qRT-PCR, IF and RNA-Scope, in both the tissue culture and perfusion model (p<0.0001) (Figure 1). Calcium staining methods identified significant calcification in day 10 culture samples, predominantly in the intimal and medial layers. Dexamethasone pre-treatment significantly attenuates calcification, with day 10 calcium expression comparable to day 0 samples. This was further validated in the 18F autoradiography data, revealing significant reductions in detectible calcium (Figure 2).

Conclusions Dexamethasone pre-treatment inhibits OPN RNA and protein expression, as well as calcium deposition, following culture and ex vivo perfusion, suggesting that LSV pre-treatment prior to surgical implantation may directly mitigate early IH development in patients. Detection of VC using 18F autoradiography may also facilitate a novel approach to clinical monitoring of vein graft patency in situ.

BS21 ENDOTHELIAL CELL PROFILE IN RESPONSES TO HIGH SHEAR STRESS IS DIFFERENT IN HEALTHY ARTERIES AND PLAQUES

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Introduction Shear stress is a parallel force generated by blood flow on the endothelial surface of blood vessel. It controls endothelial physiology and plaque biology. It is well established that low shear stress promotes the atherogenesis by increasing endothelial apoptosis, inflammation and vascular permeability. However, the role of shear stress in plaque growth and rupture is still controversial. It is hypothesized that endothelial cell (EC) responses to shear stress may be different in plaques compared to EC responses in healthy arteries. One interesting possibility is that plaque endothelium may have altered mechanosensing compared to healthy ECs. To test this hypothesis, we perform a transcriptome analysis of high shear stress endothelium in health versus diseased tissue.

Methods ApoE-/- mice were exposed to a high-fat diet for 16 weeks to induce atherosclerotic plaques or were given normal chow as a control. Wild-type (WT) mice were also studied. Aortic arches were optically cleared using the CUBIC protocol and eNOS was analysed in 3D by immunofluorescent staining coupled to light-sheet imaging (Zeiss Light-sheet Z.1). Shear stress maps were generated by OPT (optical projection tomography) imaging and computational fluid dynamics, registered against maps of eNOS expression using ITK snap software. Aortic ECs were analysed by single-cell RNA sequencing (scRNAseq) by enzymatic digestion, sorting of CD31+ CD45- cells and analysed using Bioturing software.

Results We established that eNOS is a high shear stress marker both in healthy and diseased aorta and used this to compare the transcriptional profiles of EC exposed to high shear stress in health and disease. We performed scRNAseq analysis of aorta from ApoE-/- normal diet (ND; intermediate cholesterol) and ApoE-/- high fat diet (HFD; high cholesterol) mice. eNOShigh cells were selected for transcriptome analysis (Figure 1A). We observed in t-distributed stochastic neighbour embedding (t-SNE) plot that eNOShigh cells in ApoE-/- HFD had a strikingly different transcriptional profile compared to eNOShigh cells from WT and ApoE-/- ND (Figure 1A). Some shear stress related genes were differently expressed between healthy arteries and plaques, including Klk10 which was