An ex-vivo cardiac model able to accurately predict the benefits and side-effects of new treatments could reduce the large number of flawed treatments entering in vivo testing. We have previously shown that cardiac tissue slices are a promising experimental platform as they retain structural and functional properties of the native heart, while allowing control over interventions and straightforward observations. Porcine hearts share anatomical, physiological and electrical characteristics with human hearts and are readily available from veterinary laboratories and abattoirs. The aim of this study was therefore to establish an ex-vivo model of porcine myocardium using organotypic slices from easily accessible porcine hearts and assess their electrophysiological and calcium handling properties.

Vibratome-cut myocardial slices (300–350 μm thick) were prepared from the sub-epicardial region of the left ventricle of porcine hearts (by-product from porcine carcasses provided by the Pirbright Institute). Slices were point-stimulated at cycle lengths (CL) ranging from 500 ms to 4000 ms, and analysed using a multi-electrode array system. Conduction velocity (CV) and field potential duration (FPD), an index of action potential duration, were measured. Ca2+ cycling was assessed using Fluo-4 AM and optical mapping, during field-stimulation at 500–4000 ms CL. Ca2+ +transient duration at 50% recovery (CaTD50) and 75% recovery (CaTD75) were measured at each pacing CL. Data was analysed using one-way ANOVA and presented as mean ± SEM.

CV was 46 ± 10 cm/s along muscle fibre orientation and 14 ± 1 cm/s perpendiccular to fibre orientation, with an anisotropic ratio of 3:1 (n=4 slices). Mean FPD was 316 ± 11 ms at 1000 ms CL (n=5 slices), similar to previously reported data using ventricular wedges. All slices displayed FPD restitution, with FPD prolongation at longer pacing cycle lengths (CL 500 ms: 239 ± 6 ms; CL 1500 ms: 351 ± 21 ms; CL 2000 ms: 367 ± 14 ms; CL 4000 ms: 412 ± 13 ms; n=4–5, ANOVA p<0.0001). CaTD50 and CaTD75 were significantly prolonged at longer pacing cycle lengths, indicating Ca2+ +transient duration restitution properties (figure 1; n=5, ANOVA p=0.0004 and p<0.0001 for CaTD50 and CaTD75, respectively).

Our results show that myocardial slices prepared from porcine hearts maintain electrical and calcium cycling properties of the native myocardium and provide a novel ex-vivo multicellular preparation with potential applications in cardiac preclinical research.

**Background**

Emerging evidence has suggested that adventitia stem/progenitor cells (Ad-SPCs) migrate into intima in response to injury, where they differentiate toward smooth muscle cells (SMCs) and participate in neointima hyperplasia. Matrix metalloproteinase-8 (MMP-8) has potent proteolytic activity on various matrix proteins, and has been shown to be able to cleave many important chemokines/cytokines/growth factors. We have previously identified MMP-8 as a key determinant in atherosclerosis formation and progression, stem cell mobilisation to atherosclerotic plaques, atherosclerotic angiogenesis, angioplasty restenosis as well as macrophage differentiation/polarisation. However, little is known about the functional involvements of macrophage-derived MMP-8 in SMC differentiation from Ad-SPCs and its contribution to neointima hyperplasia.

**Purpose**

In this study, we aimed to investigate the functional roles of macrophage-derived MMP-8 in Ad-SPC differentiation and injury-induced arterial remodelling.

**Methods and results**

By using Ad-SPCs isolated from MMP-8 knockout (MMP_KO) and control wild-type (WT) mice, we observed no significant difference in terms of SMC differentiation induced by TGF-β between WT and MMP-8_KO Ad-SPCs. Further analysis showed that Ad-SPCs expressed a very low level of MMP-8. However, macrophages and Ad-SPCs coculture studies showed that while WT macrophage could significantly promote MMP-8 KO Ad-SPC differentiation towards SMCs, such differentiation processes was dramatically impaired when the Ad-SPCs were co-cultured with MMP-8 KO macrophages. Moreover, we found that the MMP-8 protein secreted from macrophages was responsible for SMC differentiation from Ad-SPCs. Mechanistically, our data revealed that MMP-8 promoted TGF-β secretion from macrophages and increased its bioactivity, triggering SMC differentiation. Additionally, we showed that macrophages-derived MMP-8 activated Notch1 Signalling through increasing the cleavage of a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) in Ad-SPCs, and the activated Notch1 signalling promoted SMC differentiation from Ad-SPCs. We further demonstrated that the binding site for CBF1, Suppressor of Hairless, and Lag-1 (CSL) within SMC gene promoters is responsible for Notch1 mediated SMC differentiation from Ad-SPCs. Finally, by using a well-established arterial remodelling model and macrophage transplantation we demonstrated that macrophage-derived MMP-8 increased neointima SMC hyperplasia by promoting Ad-SPC differentiation towards SMCs in response to vascular injury.

**Conclusions**

We have identified macrophage-derived MMP-8 as a critical regulator in SMC differentiation from Ad-SPCs and neointima SMC hyperplasia in response to injury. Our data provide new insight into the roles of MMP-8 in Ad-SPC differentiation and the pathogenesis of neointima formation and/or angiographic restenosis, and aid the development of novel therapeutic agents for the prevention of these diseases.
Methods and results NE genetic deficient mice (ApoE-/-;NE/-; mice), bone marrow transplantation, and a specific NE inhibitor (GW311616A) were employed in this study to establish the causal role of NE in atherosclerosis. Aortic expression of NE mRNA and NE plasma activity was significantly increased in high-fat diet (HFD)-fed wild-type (WT) (ApoE-/-) mice, but as expected not in NE-deficient mice. Selective NE-knockout markedly reduced HFD-induced atherosclerosis and significantly increased indicators of atherosclerotic plaque stability. Whilst plasma lipid profiles were not affected by NE-deficiency, decreased levels of circulating pro-inflammatory cytokines and inflammatory monocytes (Ly6Chi/CD11b+) were observed in NE-deficient mice fed with HFD for 12 weeks as compared to WT. Bone marrow reconstitution of WT mice with NE/-/- bone marrow cells significantly reduced HFD-induced atherosclerosis, while bone marrow reconstitution of NE/-/- mice with WT bone marrow cells restored the pathological features of atherosclerotic plaques induced by HFD in NE-deficient mice. In line with these findings, pharmacological inhibition of NE in WT mice through oral administration of NE inhibitor GW311616A also significantly reduced atherosclerosis. Mechanistically, we demonstrated that NE promotes foam cell formation, by increasing ATP-binding cassette transporter ABCA1 protein degradation and inhibiting macrophage cholesterol efflux.

Conclusions We have outlined a pathogenic role for NE in foam cell formation and atherosclerosis development. Consequently, inhibition of NE may represent a potential therapeutic approach to treating cardiovascular disease.

SIRT1 levels within diabetic patients was confirmed via ELISA of diabetic serum and correlated with tissue histology. Results hCASMCs, cultured in high glucose osteogenic conditions in the presence of SRT1720, showed a significant decrease in ALP activity (p<0.05) at day 4, which was sustained until day 7. A 3-fold reduction in alizarin red staining was observed (p<0.05), alongside a decline in the osteogenic transcription factor RUNX2 mRNA expression to a tenth of its control levels (p<0.05), and a reduction in RUNX2 protein by a half. Additionally, calcification associated senescence within hCASMCs was shown to significantly decline with the addition of SRT1720 (p<0.05) with P16 expression also shown to be decreased by tenfold (p<0.05). Conversely, SIRT1 inhibition via Sirinol significantly increased ALP activity (p<0.05) with an increase in alizarin red staining by day 21 in both osteogenic and control treatments (p<0.05). RUNX2 activity was significantly increased at mRNA level (p<0.001) and at protein level, with downstream osteocalcin significantly increased (p<0.01). Sirinol increased X-Gal staining by 20% (p<0.01) alongside P16 expression (p<0.05), the results of which are confirmed with corresponding SIRT1 siRNA knockdown.

Conclusions SIRT1 activation via small molecule activator attenuates deposition of a calcified matrix in hCASMCs grown in diabetic conditions, via the downregulation of the RUNX2 transcription factor and subsequent downstream calcifying markers. These data suggest an essential role of SIRT1 in the protection against vascular calcification, which may be compromised within diabetic patients.

Abstracts

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Introduction Vascular calcification is highly correlated with cardiovascular disease mortality, often seen in type II diabetes and attributed to over 422 million cases worldwide. It is now widely accepted that VC is an active process with similarities to bone ossification, occurring within the smooth muscle cell (SMC) layer, involving the transition of SMCs from the cell cycle into a senescent phenotype, activation of osteogenic transcription factors and the loss of mineralisation inhibitors. Recent evidence demonstrates the beneficial role of Sirtuin 1 (SIRT1), an NAD+ -dependant deacetylase, in insulin sensitivity and glucose homeostasis, and suggests a link between smooth muscle cell senescence, hyperglycaemia and SIRT1 downregulation. The current study aims to investigate the therapeutic role of SIRT1 activation for the prevention of vascular calcification.

Methods Human coronary artery SMCs (hCASMCs) were incubated in high (25 mM) glucose to reflect a diabetic milieu and compared to low glucose (5 mM) and osteogenic media containing β-glycerophosphate and calcium chloride. SIRT1 was inhibited by Sirinol and activated by SRT1720. Cellular calcification was confirmed via alizarin red staining, alkaline phosphatase activity, qPCR and western blot analysis. β-galactosidase staining and qPCR confirmed cellular senescence.