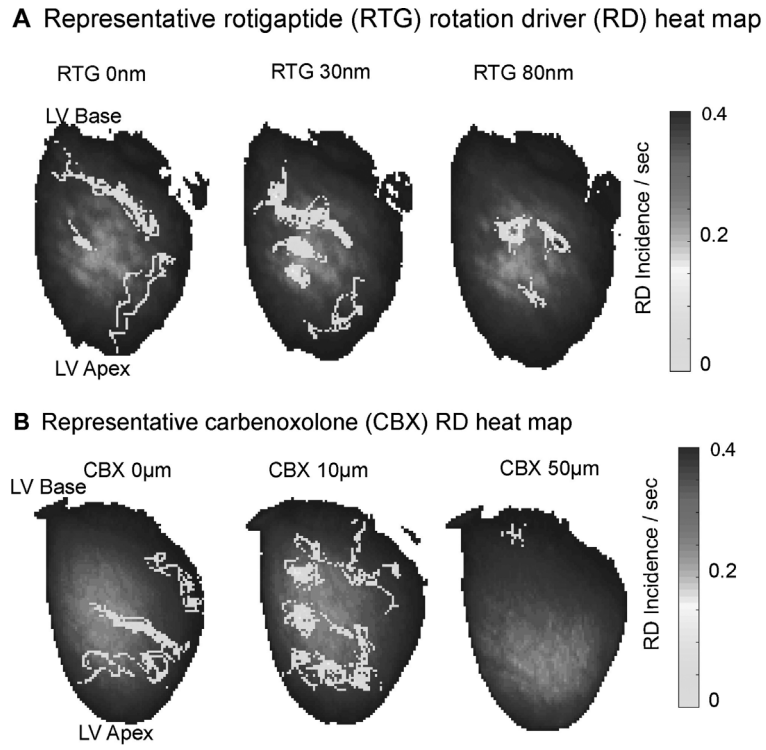

Abstract BS62 Figure 2

Results In the fibrosis group, VF was driven predominantly by disorganised activity in CF, RDs were detected 26±7% of time comparative to 51.2±4% in DF and 69.5±8% in PF group ($p=0.001$). PF stabilised RDs, average maximum rotations for a single RD in PF were 31.6±7.1 comparative to 12.5±1.7 in DF and 6.4±1.1 in CF, $p<0.001$. VF organisation measured by FDI was higher in PF (PF: 0.61±0.07, DF: 0.47±0.04, CF: 0.33±0.03, $p=0.004$) (figure 1). In the GJ modulation group, maximum rotations for a RD increased with RTG (0nm: 5.4±0.45 vs 80nM: 48.20±12.32, $p<0.001$) and decreased with CBX (0µM: 8.0±1.3 vs 50µM: 0.3±0.3, $p<0.001$). Proportion of time RDs were detected in VF increased with RTG (0nM: 44±6 vs 80nM: 93±2, $p<0.001$) and decreased with CBX (0µM: 61±9% vs 50µM: 3±2%, $p<0.001$). FDI increased with RTG (0nM: 0.53±0.04 vs 80nM: 0.78±0.3, $p<0.001$) and decreased with CBX (0µM: 0.60±0.05 vs 50µM: 0.17 ±0.03, $p<0.001$) (figure 2).

Conclusion VF mechanisms occur along a spectrum between organised activity with discrete drivers and disorganised myocardial activation. The degree of GJ coupling and ventricular fibrosis are key determinants of the underlying mechanism of VF. Enhanced GJ coupling and patchy fibrosis organised fibrillation and stabilised RDs, whilst GJ uncoupling and compact fibrosis disorganised VF. This study presents a unifying explanation for the numerous mechanisms reported for sustaining fibrillation. Characterising the degree and pattern of fibrosis in patient groups vulnerable to VF might be beneficial in identifying patients with targetable substrate, and GJ modulation might be a potential therapeutic target.

Conflict of interest Nil

BS63 DEVELOPMENT OF E-SENSE: A FLEXIBLE IN VITRO PLATFORM TO DETERMINE CADRIOVASCULAR RISK

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Introduction Coronary heart disease (CHD) is the leading cause of mortality and morbidity globally. Atherosclerosis, a key underlying mechanism in the potentiation of CHD, is often defined as a chronic inflammatory disease provoked by the oxidation of lipids retained in arterial walls resulting in plaque formation. Plaque formations have been demonstrated to preferentially form at bifurcations and arcs exposed to disturbed blood flow patterns (athero-prone), whereas uniform sections exposed to laminar flow (athero-resistant) promote an athero-protective response, implicating the endothelium in the initiation of the disease. Various genes are known to regulate atherosclerosis development; NFκB and AP-1 (inflammatory cytokine response), IRF3 (toll-like receptor signalling), XBP1 (unfolded protein response/ER stress) and KLF2 and NRF2 (protective signalling pathways).

Methods Human coronary artery endothelial cells (HCAECs) were immortalised via lentiviral overexpression of anti-senescence polycomb protein, BMI1, which allowed for the extended proliferative lifespan of the primary cells, without affecting their morphology. Immortalised human coronary

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artery endothelial cells (iHCAECs) were then integrated within our parallel-plate flow apparatus, in which the response to varying flow conditions was observed.

Results From extensive published and unpublished data, the overexpression of BMI1 enables prolonged cell proliferation whilst inhibiting cell senescence. Utilising lentiviral overexpression, we were able to immortalise HCAEC with BMI1. Isolated iHCAEC clones were placed under varying hemodynamic conditions in order to screen their response. These iHCAEC clones lined up in conjunction with HCAEC counterparts demonstrating a continued mechanosensitivity. Current work investigating gene expression related to the differing hemodynamic environments is underway with full benchmarking against HCAEC controls in progress. TFAR gene constructs have been developed known to regulate atherosclerosis. The TFARs; NFκB, AP-1, IRF3, XBP1, KLF2 and NRF2 have been developed along with lentiviral vectors with secretable luciferase reporters (VLuc and NLuc) allowing for incorporation into validated iHCAECs. Validating these reporter constructs in response to ox-LDL, TNFα and cigarette smoke extract via luciferase activity measurement is currently underway, with selected constructs already cloned.

Conclusion Our initial results indicate the overexpression of BMI1 in HCAEC results in an extended lifespan and inhibition in cell senescence, with morphology unaffected. The resulting iHCAECs exhibit mechanosensitivity to a changing hemodynamic environment comparable to primary HCAECs. This highlights the advantages of this cell line for future CHD modelling, with its integration with our E-Sense system allowing for the development of a novel research tool and the potential to replace pre-existing methods.

Conflict of interest no

Young Investigators Award

A ENDOTHELIAL CELL DERIVED EXTRACELLULAR VESICLES MEDIATE NEUTROPHIL DEPLOYMENT FROM THE SPLEEN FOLLOWING ACUTE MYOCARDIAL INFARCTION

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Background Acute myocardial infarction (AMI) induces transcriptional activation of monocytes *en route* to the injured myocardium, in part driven by endothelial cell derived extracellular vesicles (EC-EV), which contain proteins and microRNA (miRNA) cargo. However, neutrophils are the first immune cells to arrive at sites of injury and mediate further damage to the ischemic myocardium. Here, we describe for the first time how neutrophils are released from the spleen in AMI and show that this is driven by EC-EV signalling.

Methods and results Experimental AMI in wild-type mice caused a significant increase in peripheral blood neutrophils and a simultaneous reduction in splenic-neutrophil number ($P<0.01$), suggesting splenic-neutrophil deployment, which is a previously unknown neutrophil reserve in AMI. Patients have elevated peripheral blood neutrophil (1.6-fold, $P<0.01$) and plasma EV numbers (2.2-fold, $P<0.01$) at the time of AMI presentation, which significantly correlate ($R=0.29$, $P=0.037$) and suggests plasma EV-neutrophil interactions. EC-EV can alter immune cell motility from the spleen (Akbar *et al*, 2017). Patient plasma EV (isolated by differential ultracentrifugation, EV confirmed by protein markers TSG101, ALIX, CD9, HSP70 and morphology by transmission electron microscopy) show enrichment for EC-vascular cell adhesion molecule-1 (VCAM-1) and EC-miRNA-126-3p. AMI induces EC activation; EC activation with pro-inflammatory TNF-α models this *in vitro*, causing increased EC-EV release ($P<0.001$) and enrichment for miRNA-126-3p ($P<0.01$). EC-miRNA-126 is a negative regulator of EC activation and may dually control EC-EV release. CRISPR-edited-miRNA-126 knock-out EC display a pro-inflammatory phenotype, as evidenced by increased VCAM-1 ($P<0.001$) expression and show enhanced EC-EV release ($P<0.001$). To better understand the potential role of miRNA-126 on neutrophil biology we analysed miRNA-126-putative-mRNA targets and compared these to neutrophil Gene Ontology (GO) pathway terms. miRNA-126-mRNA targets are significantly over represented when compared to neutrophil GO terms for: degranulation ($P<0.001$), activation ($P<0.001$), chemotaxis ($P=0.008$) and migration ($P=0.008$). EC-EV exposure to primary human neutrophils alters inflammatory IL-6 ($P<0.01$) and chemokine gene expression (CCL7 ($P<0.01$) and CCL18 ($P<0.05$)), substantiating bioinformatic findings. EC-EV tail vein injected into wild-type, naive mice mobilise splenic-neutrophils to peripheral blood ($P<0.001$), confirming splenic neutrophil mobilisation by EC-EV.

Conclusions (I) Neutrophil deployment from the spleen is a novel finding in acute injury and interactions with (II) EC-EV may mediate their splenic liberation and (III) activation following AMI, *en route* to the injured myocardium. The splenic neutrophil reserve may be a novel therapeutic target in AMI to modulate the inflammatory response before recruitment of cells to sites of injury.

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