macrophage accumulation in aortic sinus and arch, while it did not affect circulating monocyte levels and bone marrow hematopoietic stem cells.
Conclusion: The data suggest physiological microbial condition reduced the accumulation of macrophages and had good effects on the formation of atherosclerosis, although the mechanism should be carefully assessed. Our results increase translational potential how to prevent cardiovascular disease.

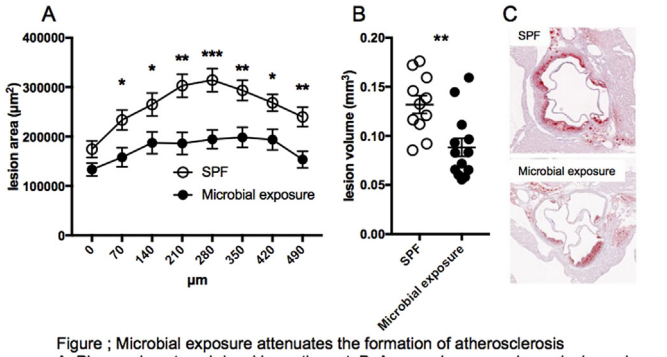

Figure ; Microbial exposure attenuates the formation of atherosclerosis
A, Plaque size at each level in aortic root. B, Area under curve plaque lesion volume. C, Representative pictures stained with Oil red O.

P3.018

DISCOVERING AND DISSECTING THE FUNCTION OF CONSERVED NF-κB BINDING EVENTS IN THE HUMAN GENOME

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Objective: The nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) transcription factor plays a prominent role in inflammation and contributes to the development of atherosclerosis. Genome-wide DNA binding assays of the human NF-κB subunit RELA (p65) have revealed tens of thousands of NF-κB binding sites and hundreds of target genes. However, the function of individual RELA binding sites and the extent to which NF-κB occupancy and function is conserved across mammals are not well understood.
Methods: To better understand the function of NF-κB we characterized the genome-wide binding of RELA in primary vascular endothelial cells (ECs) isolated from the aortas of human, mouse and cow. ECs were stimulated acutely with the pro-inflammatory cytokine tumor necrosis factor alpha (TNFA) and we profiled RELA occupancy, open chromatin, select histone modifications, and RNA expression.
Results: We found ~5000 RELA binding events conserved across all three species and these highly conserved human binding events were enriched for genes controlling vascular development, apoptosis, and pro-inflammatory responses. Approximately 2000 of these highly conserved RELA binding events were also shared across multiple human cell types, revealing a conserved core of robustly bound NF-κB sites. These NF-κB binding sites were also prominent components of ~40 inflammation-induced super-enhancers (SE) common to several tissues. To gain insight into the function of individual conserved NF-κB binding sites we focused on the inflammation-induced SE proximal to the monocyte recruiting chemokine *CCL2*, which we detected as a SE in all three species and across multiple cell types. We tested the functional significance of six conserved RELA binding sites comprising this SE using CRISPR/Cas9 genome editing. We found that only deletion of the most proximal upstream RELA binding site could abolish the induction of *CCL2* upon TNFA treatment. This site also contains a disease associated variant that can modulate *CCL2* induction.
Conclusions: Overall, our comparative genomics assessment of NF-κB binding gives new insight into NF-κB biology and the function of conserved transcription factor binding events within mammalian super-enhancers.

P3.019

EX-VIVO CULTURE SYSTEM AS A MODEL TO STUDY THE MOLECULAR AND CELLULAR MECHANISMS IN INTERNAL MAMMARY ARTERIES

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Objective: The internal mammary arteries (IMA) are grafts of choice for coronary artery bypass because of their superior graft patency vs. other vessels. A better knowledge of cellular and molecular mechanisms at the base of why IMA is not prone to plaque development is important for the prevention of atherosclerosis in native arteries. *Ex-vivo* culture systems (EVCS) investigating graft adaptation have been successfully designed, engineering and applied to saphenous veins, never to IMA due to the small dimension of the latter (external diameter 3-8.5mm for saphenous veins, <3mm for IMA).
Our aim was to design and realize a miniaturized, compact and automated *ex-vivo* EVCS providing a reliable model to study human IMA responses under controlled flow conditions that enable stimulation patterns reproducing those at the grafted artery site.
Methods: Conceptually new EVCS prototype and a tool for IMA mounting in sterile conditions were engineered/ tested in standard incubator at 37C, overnight (Figure A, B). Cryosections from post-experiment frozen IMA (n=4) were analyzed by histology and confocal microscopy.
Results: EVCS was characterized by miniaturized culture chambers hosting native IMA segments of about 15mm length, kept under perfusion at a constant flow rate of about 40ml/min to the IMA luminal side. In this preliminary experimental setting the IMA external side remained in static condition, immersed into sterile medium separated from that flowing inside the vessel. Perfusion of fluoresceinated dextrans demonstrated the absence of leakage in e*x-vivo* experiments with IMA in EVCS. Hematoxylin/eosin and labelling of αSmooth-Muscle Actin, SM22, von Willebrand Factor, CD31 verified preservation of IMA morphology, absence of dissecation signs, and endothelial layer presence (Figure C,D).
Conclusions: Preliminary results indicated the EVCS suitability to comply with IMA size, and the IMA resistance to flow rate analogous to that of the coronary district. EVCS may represent a model for functional and/or local therapy-aimed studies on IMA.

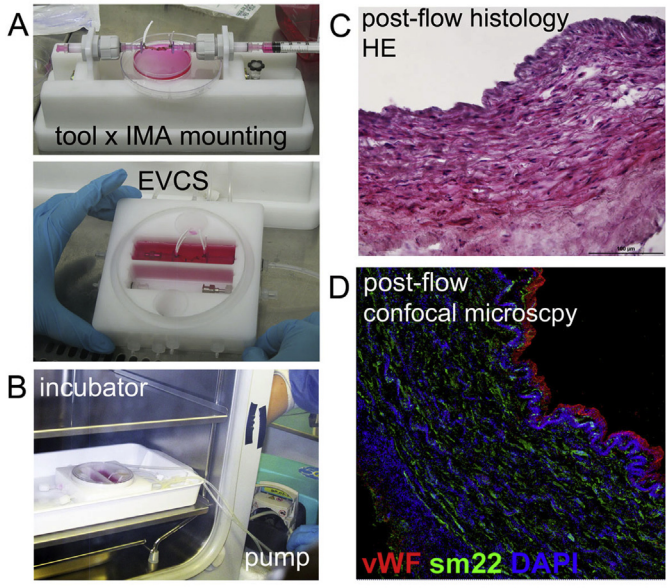

Figure: View of the tool for IMA mounting (A, top), of the IMA mounted inside EVCS (A, bottom), of the EVCS with IMA inside the incubator and connected to pump (B) are shown. Representative histology (C)and confocal microscopy (D) images display the preservation of IMA post-flow