PLAQUETEC

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**INTRODUCTION**

Natural history studies of atherosclerosis (eg. PROSPECT, VIVA) using anatomical imaging modalities including grayscale and virtual histology intravascular ultrasound have identified features of plaque vulnerability but have largely failed to predict future acute coronary events. Similarly, physiological indices of lesion severity may identify ischemia-inducing lesions but cannot predict lesion progression. There is a growing understanding that addition of biological data to anatomical imaging information may be synergistic in enhancing our prognostic and risk-stratifying ability for both patients and coronary lesions. Approaches under investigation include measurement of endothelial shear stress and plaque strain. However, whilst peripheral circulating biomarkers/biomolecules (cytokines, growth factors, inflammatory mediators) have demonstrated some limited value in patient risk discrimination (eg. hsCRP), plaque-specific biomarker expression at the endothelial level is the key to determining lesion progression and vulnerability. Conventional means for assessing such biomarkers are hampered by dilutional effects (the coronary blood makes up less than 1/1000 of circulating volume), 'noise' from other vascular beds (eg. carotid, peripheral arteries) and especially inadequate sampling methods: in any vessel or tube, the boundary layer (a layer of laminar bloodflow adjacent to the vessel wall) does not mix with the bulk flow within the vessel, and hence using a non-dedicated microcatheter risks missing vital information held within this layer.

PlaqueTec has designed and developed the Liquid Biopsy System (LBS), the first dedicated intracoronary blood sampling device, designed to mix the boundary layer and harvest up to 4 discrete spatially-separated blood sample simultaneously from within the coronary artery. The device is 6F- and Rx-compatible, and has achieved CE mark following successful preclinical and first-in-human studies. We now present the findings of the first proof-of-concept study in diseased/stenosed human coronary arteries and the future regulatory and commercialization strategy for the device.

## Abstract

\Of the biomolecules showing significant gradients before balloon angioplasty (Table 1A), six are known to be components of blood platelet α-granules [8], namely IL8, DKK1, CXCL1, CD40LG and PDGFB. It is therefore biologically plausible that these metabolites are derived from adhered platelet thrombi. However, IL8 is produced by activated monocytes, macrophages and endothelial cells (ECs) [9], DKK1 mRNA is found within atherosclerotic plaques, in ECs and leukocytes [10], CXCL1 is secreted by macrophages [11] and CD40LG is prevalent in lymphocytes as well as other plaque cells [12]. Hence, non-platelet sources of these metabolites cannot be ruled out. DKK1, CXCL1, CD40LG and PDGFB can all cause activation of ECs [10-12], which are likely sources of ORL1, IL8, PLAUR, CXCL5/6, HBEGF, and PDGFB. ECs are overwhelmingly the most abundant source of ORL1 in the vasculature [13] and thus the concentration gradient of ORL1 may arise from ECs overlying culprit coronary plaques before balloon dilation. Furthermore, production of CXCL5/6 is upregulated approximately 100-fold in ECs treated with the inflammatory mediator TNFα [14] and disturbed flow increases the release of HBEGF from ECs [15]. However, CXCL5/6 and HBEGF could also be released from underlying smooth muscle cells

(SMCs). All of the remaining mediators are known to be secreted by blood leukocytes or mature macrophages, which are present in plaques but also potentially trapped within thrombi.

The nine analytes released from plaques *after* balloon injury (Table 1B) include three of the known platelet products released before predilatation of plaques (IL8, DKK1, and PDGFB) plus CXCL5/6 and HBEGF that could be derived from ECs or SMCs. Most interestingly, new gradients of CCL2, HSPB1 and MMP12 were detected after balloon dilatation. CCL2 is a secreted chemokine that is localised to macrophages and smooth muscle cells in plaques [16]. On the other hand, HSPB1 is primarily an intracellular chaperonin with anti-inflammatory activity that is more highly expressed in SMC of normal vessel walls than in plaques [17]. Given its localization, the gradient of HSPB1 is most likely to be derived from cells injured during plaque disruption by angioplasty. Consistent with this increased plasma levels of HSP27 have been reported in patients after acute coronary syndromes [17]. Release of MMP12 after angioplasty may be particularly significant because it is normally undetectable by histology in ECs or SMCs and expressed only in plaque macrophages surrounding the necrotic core of rupture-prone plaques [18, 19]. Moreover, the abundance of MMP12 expressing cells in carotid artery plaques predicts the subsequent occurrence of strokes and major adverse events [19]. It is highly likely therefore that the post-angioplasty gradient of MMP12 is derived from the exposure of the plaque core to the circulation, and the size of gradient may potentially possess diagnostic or prognostic information.

In conclusion, our results demonstrate that gradients of bioactive molecules exist across intact and disrupted atherosclerotic plaques, and these may be sampled and assessed using this novel technology. Until now, the existence of such gradients has been postulated but never directly demonstrated. Hence, we have already shown that the LBS can yield biological insights impossible to deliver by any other current *in vivo* methodology. Furthermore, our results provide proof of concept for even more ambitious studies, to test whether LBS sampling can provide a surrogate marker for activity of plaques that are vulnerable to rupture. In this context, ORL1 and CXCL5/6 seem particularly promising since they are likely to be endothelium-derived before angioplasty. In addition, levels of MMP12 may be especially informative after balloon dilatation, for the reasons detailed above. Future studies should address whether gradients of released biomolecules have the potential to monitor the impact of existing and new therapeutics, and when fully validated and coupled to high content analyses, LBS-detected gradients could be used as biomarkers to identify patients with high risk plaques. The potential combination of LBS-derived biological information could be used to complement conventional anatomical imaging methods in order to augment and refine plaque/patient risk-stratification and prognostic power, enabling therapeutic changes that may improve patient outcomes.