**Mapping platelet response to thrombin using high-sensitivity platelet proteomic analysis**

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**Aim:**Platelets respond to agonists (e.g. thrombin) by the secretion of intracellular proteins and mediators which promote thrombus formation [1]. There are limited studies regarding global protein changes after thrombin stimulation in healthy individuals [2]. Our study aims to establish high-quality characterisation of the healthy platelet proteome, at baseline and after thrombin stimulation.

**Method:**Platelets were isolated from whole blood from healthy volunteers. Baseline PAC-1 and CD62P expressions were determined by flow cytometry. Platelets were stimulated with submaximal (0.025U/mL, n=5) and high dose (0.20U/mL, n=6) thrombin. Proteins from the platelet releasate and lysate were identified and quantified using the Thermo Lumos Tribrid Orbitrap mass spectrometer. Protein secretion was determined using a novel method of protein abundance anti-correlation between the lysate and releasate. Statistical analysis was by R and plotted using Tableau. Significance was determined using a repeated-measures one-way ANOVA for activation (resting vs thrombin) at P<0.05.

**Results:** Platelet activation markers PAC-1 and P-selectin were expressed on <0.5% and <15 % of isolated platelets respectively. Plasma contamination of the platelet preparation was <0.5%. Qualitative changes were seen in platelet proteins secreted after high (**Figure 1**) and submaximal dose thrombin, with 203 and 74 proteins that were significantly increased respectively. There was a significant increase in proteins associated with platelet aggregation (e.g. thrombospondin-1) as expected, but also proteins associated with inflammation (e.g. CXCL3), angiogenesis (e.g. VEGF-C) and yet undetermined platelet functions (e.g. alpha-(1,6)-fucosyltransferase) [3].

Figure 1 (right): Platelet releasate and lysate proteomes after stimulation with thrombin 0.2 U/ml. Dots represent individual proteins. Proteins in orange are significantly increased in the releasate and decreased in the lysate following stimulation.

Platelet releasate (Thrombin = 0.2UmL)

Platelet lysate (Thrombin = 0.2UmL)

**Conclusion:** Our platelet proteomic platform provides a resource to study proteins mobilized by platelets for a spectrum of functions, beyond haemostasis. Further investigations regarding proteomic differences and post-translational modifications may yield novel protein markers and therapeutic targets in disease states.

***References***

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