29.9. Platelet-activating Factor: A Critical Link Between the Inflammation and Coagulation Systems. H. B. Moore,[1] M. Wohlauer,[1] E. Gonzalez,[1] A. Banerje,[1] E. E. Moore[1,2]; [1]University of Colorado Denver, Aurora, CO; [2]Denver Health Medical Center, Aurora, CO

Introduction: Platelet-activating factor (PAF) is a tightly regulated inflammatory mediator in a variety of settings; including shock, trauma, and sepsis. A variety of cell types, many of which are central to the inflammatory and haemostatic systems, are capable of synthesizing PAF. the expression of PAF is tightly regulated involving crosstalk between endothelial, inflammatory and vascular cells. PAF, as its name implies, also induces platelet aggregation, however traditional clinical assays that monitor platelet activity commonly employ the agonists ADP and arachidonic acid and not PAF. We therefore hypothesized that PAF would have a potent, independent effect on platelet aggregation using TEG(c) Platelet Mapping, a modern platelet function assay. **Methods:** Whole blood was collected from healthy volunteers in heparin (68 U/4 ml). Platelet mapping was performed within 2 hrs of sample collection. Platelet function was determined using the TEG platelet mappingassay. A heparinized blood sample was collected, with 360 ml of the blood sample placed into the pre-warmed cup of the TEG analyzer, followed by 10 ml of the prepared activator solution, comprised of reptilase, factor XIIIa, and phospholipids. Reptilase cleaves fibrinogen to generate fibrin and is used to replace thrombin. Next, either ADP (2 μM final concentration, MAADP), or arachidonic acid (1 mM final concentration, MAAA) orPAF (12μM), was added as a platelet agonist. to determine the independent fibrin contribution to the clot, the activator solution was added to 360 ml of heparinized blood (MAFibrin). the maximum hemostatic activity (MAThrombin) was measured using a kaolin activated whole blood sample collected in citrate, rather than heparin. the percent platelet inhibition in response to either the ADP,AA, or TPA agonist was calculated using the following equation: (100 - [(MAADP(AA) - MAFibrin)/(MAThrombin- MAFibrin)$\times$ 100] **Results:** PAF (12μM) induced platelet aggregation was as effective as AA (1mM) and ADP (2μM) in promoting clot formation (29.6 $\pm$ 4.1 vs. 44 $\pm$ 5.0 MA (mm), p=0.3 PAF vs. AA; 29.6 $\pm$ 4.1 vs. 18.9 $\pm$ 2.6 MA (mm), p=0.1 PAF vs. ADP) [Table 1]. **Conclusions:** PAF signaling has a pivotal role linking inflammation and coagulation. Augmenting the current role of PAF in the laboratory as an inflammatory agonist, PAF-mediated platelet aggregation using TEG ® Platelet Mapping has potential to measure the bioactivity of blood and blood products.

TABLE 1

Agonist	Clot Strength MA (mm)
PAF	29.6 $\pm$ 4.1
AA	44 $\pm$ 5.0
ADP	18.9 $\pm$ 2.6
Reptilase (control)	2.7 $\pm$ 2.1

29.10. Gene Networks Involved With Mechanoregulation of Cell Population in the Fibroblast-Populated 3D Collagen Matrix. M. A. Carlson,[1,2] J. D. Eudy,[1] L. M. Smith,[3] M. A. Carlson[1,2]; [1]University of Nebraska Medical Center, Omaha, NE; [2]University of Nebraska Medical Center, Omaha, NE; [3]University of Nebraska Medical Center, Omaha, NE

Introduction: The 3D collagen matrix has been used to study mechanical states in wound healing and tissue engineering. Human foreskin fibroblasts (HFFs) in a mechanically-stressed matrix (MS; attached to culture plate) increase their population, while HFFs in a stress-released matrix (SR; detached, floating in medium) decrease their population. Our intention was to use gene array data to uncover relevant gene networks which might mediate the decrease in matrix cell number associated with the MS-SR transition. **Methods:** HFFs (10^6/mL) were cultured in bovine collagen (1.5mg/mL; 0.2mL matrix vol; medium = 5% FBS in DMEM). Differentially-expressed (DE) genes in MS vs. SR matrices were identified at 6 and 24hr after SR with gene arrays (3 HFF strains; nonpooled mRNA; 6 chips). DE was corroborated with immunoblotting, ELISA, and qPCR. DE genes were analyzed with third-party software for putative gene networks associated with the MS-SR transition. Relevance of putative networks was tested by augmenting/inhibiting network participants and then measuring matrix cell number 48 and 72hr after SR. **Results:** There were 187 and 425 DE genes identified at 6 and 24 hr, respectively, including a 10-fold induction of IL6 and IL8 24hr after SR; NF-kB was not DE, but many of its targets were. the software analysis of the DE genes identified >20 putative networks as highly significant in the MS-SR transition; central participants included IL6, IL8, NF-kB, TGF-B1, MAP kinases, Akt, p53, and cyclins. With this data, we initially hypothesized that gene networks with IL6, IL8, or NF-kB helped mediate the decrease in matrix cell number after SR, and then began testing. Matrix treatment with IL6 or IL8 did not affect cell number, but treatment with neutralizing antibodies to either cytokine decreased matrix cell number (Table). As expected, matrix cell number decreased in SR with respect to MS matrices using medium only. Control IgG had minimal effect. Treatment of matrices with TNF-α (stimulant of canonical NF-kB signaling) produced a large decrease in cell number in MS matrices, with minimal effect in SR matrices. **Conclusions:** It can be speculated that IL6 and/or IL8 release was a counter-regulatory mechanism that mitigated the decrease in matrix cell population occurring after SR, as suggested by (1) the late induction, (2) networks generated from the DE genes, and (3) cell number downregulation after antibody neutralization. NF-kB signaling may have helped drive the population decrease after SR, as suggested by (1) modulation of multiple NF-kB targets, (2) generated networks, and (3) cell number downregulation after TNF-α treatment in the MS matrix. Future studies will focus on dissection of these and other gene networks active in the MS-SR transition.

TABLE

Cell/Matrix (x 105; Mean±SD of ≥3 Experiments); *p < 0.05 compared to medium (ANOVA)

treatment	MS, 48 hr	MS, 72 hr	SR, 48 hr	SR, 72 hr
medium only	2.21±0.36	2.77±0.40	1.52±0.22	1.25±0.29
IL6, 5 ng/mL	2.45±0.30	2.67±0.31	1.44±0.27	1.48±0.21
IL8, 5 ng/mL	2.30±0.28	2.58±0.45	1.73±0.36	1.40±0.25
anti-IL6, 10 μg/mL	*1.41±0.27	*1.99±0.13	*1.08±0.20	1.04±0.26
anti-IL8, 10 μg/mL	*1.55±0.35	*1.84±0.23	*1.11±0.09	*0.93±0.12
IgG, 10 μg/mL	2.03±0.40	2.75±0.43	1.36±0.28	1.09±0.17
TNF-α, 10 ng/mL	*1.36±0.24	*1.40±0.21	1.42±0.27	1.19±0.22