using CRISPR/Cas9 system, we we obtained one iPS clone with homozygous corrected gene of LDLR (gcHoFH+/+-iPSCs) and two iPS clones with heterozygous corrected gene of LDLR (gcHoFH+/–-iPSCs). Then, we could generate functionally corrected HLCs which exhibited almost recovered LDL uptake abilities in comparison with those before gene correction. Under these conditions, no immunological rejection was observed against patient's serum.
Conclusion: These results demonstrate that the gene corrected iPSCs-derived HLCs can be functionally restored LDL uptake. We suggest that this approach could be a curative treatment of this type of HoFH patients, although further studies are necessary to confirm their function in vivo.
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Developing a new tool for immature platelet analysis

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Introduction: Immature or reticulated platelets (RPs) are young, hyper-reactive pro-platelets that are larger and contain more RNA compared to older platelets. These hyper-reactive thrombocytes are known to be independent predictors of antiplatelet response to thienopyridine treatment. Moreover, the RPs are strong predictor of cardiovascular death in coronary artery disease. However, the reason of this correlation as well as their intrinsic hyper reactivity is unknown. Furthermore, mRNA and miRNA of RPs have not been characterised so far and they could influence RPs reactivity. All data published so far consist in mere observational studies and did not provide any pathophysiological information concerning the RPs hyper-reactivity. The lack of specific antibodies and the hyper-reactive nature of these cells have been a strong limitation to further biological investigation.
Purpose: We aimed to develop a staining and sorting protocol that allows to isolate RPs from peripheral blood.
Methods and results: We performed a double staining of platelet reach plasma from healthy donors with CD41, a well-established platelets marker, and Thiazole orange (TO), a fluorescent dye that forms high-intensity bonds with nucleotides. TO is a non-selective, concentration- and time-dependent dye. Thus, we could not expect to find two distinct cell populations at the FACS analysis. For this reason, in order to separate the RPs, we had to set a gating threshold to select only the highly TO positive cells (TOhigh) as shown in Panel A and B. At the FACS analysis, the CD41+TOhigh cell population represent the larger platelets, as it is expected for RPs, Panel C. In order to further characterize the sorted cells, we performed a P-Selectin/CD62 Staining. In fact, P-Selectin promotes platelet aggregation through platelet-fibrin and platelet-platelet binding and correlate with platelet reactivity. As shown in Panel E the CD41+TOhigh (TO+) population express more P-Selectin compared to mature platelets CD41+TOlow (TO-) even without an activation stimulus ($p<0.05$). As proof of principle, we activated CD41+TOhigh and CD41+TOlow cells with 5 μM/ml ADP after sorting and we stained for P-Selectin after activation. After ADP stimulation the CD41+TOhigh group showed a higher P-Selectin expression compared to mature platelets, confirming the high reactivity of RPs even after sorting (Panel F, $p<0.05$).

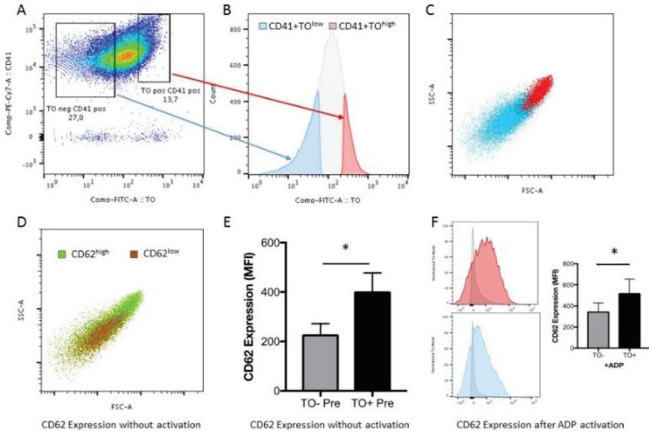

Figure 1, A: FACS analysis of CD41 (Y-axis) and TO (X-axis). B: TO-staining of the gated cells. C: FSC-A and SSC-A after gating for CD41+TOhigh (red) and CD41+TOlow (blue). D: FSC-A and SSC-A for CD62high and CD62low. E: P-Selectin expression before sorting. F: P-Selectin expression after sorting and ADP activation. N=4.

Conclusion: With our protocol we can successfully stain and sort the young and hyper-reactive cell population corresponding to immature platelets. We believe to have developed for the first time an effective protocol that can allow wide biological investigation of RPs.

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The endothelial-derived miR-181 regulates tissue factor-dependent extrinsic clotting

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Background: Tissue Factor (TF) is the primary initiator of the extrinsic clotting cascade. When expressed in the vasculature, TF leads to a pro-thrombogenic state promoting FXa generation and cardiovascular complications. The short non-coding microRNA (miR)s have been revealed as important regulators of vascular homeostasis. We and others have shown that miRs bind to the TF transcript and reduce thrombogenicity. Therefore, increased vascular TF expression can also be the consequence of insufficient post-transcriptional control. The endothelial miR-181 controls vascular homeostasis by buffering pro-inflammatory signals. Its role in coagulation, particularly in the clinical setting of thrombosis remains elusive. Here, we investigate its impact on TF-dependent thrombogenicity in vitro and in vivo.
Methods: Human microvascular endothelial cells (HMEC-1) and human monocytic cells (THP1) were transfected with miR-181 or anti-miR-181 under basal conditions and following stimulation with TNFα or LPS. Expression of TF, VCAM1, and FXa generation was assessed. In addition, aortas, primary endothelial cells, and bone marrow-derived macrophages (BMDMs) from miR-181 –/– mice were probed for TF expression and FXa generation. Finally, miR-181 and TF expression were assessed in the plasma of cardiovascular patients.
Results: Transfection of miR-181 abrogated TNFα-induced TF mRNA, protein and activity in HMEC-1. In contrast, anti-miR-181 increased TF expression. When transfected with miR-181, also THP1 cells showed lower levels of TF mRNA and reduced FXa generation. In miR-181–/– mice, higher TF expression on mRNA and protein level could be seen in the heart and aortic tissue. miR-181 –/– BMDMs showed higher levels of FXa following stimulation with LPS as compared to the wildtype cells. When we analyzed plasma in patients with diabetes, miR-181 strongly correlated with reduced TF activity and reduced markers of vascular inflammation.
Conclusion: miR-181 reduces the expression and clotting activity of TF in the vascular wall and blood cells in vitro and in vivo. Reduced levels of circulating miR-181 may contribute to increased thrombogenicity in patients with diabetes and may help to identify patients at risk for thrombosis.
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APAC, a dual antiplatelet and anticoagulant, attenuates platelet procoagulant activity and localizes to extravascular matrix during vascular injury

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Background: APAC, heparin proteoglycan mimic, inhibits both collagen-induced platelet aggregation and coagulation in vitro and in vivo (STH 2014). In two arterial thrombosis baboon models: collagen-coated shunt and 30–90% stenosis model of injured femoral artery (FA), APAC reduced platelet thrombus formation maintaining vessel patency. In PET labeling APAC retained at rat FA anastomosis site for 48 -120 h.
Purpose: We further characterized APAC efficacy 1) in vitro on aggregation in human blood and platelet-rich plasma (PRP); 2) in global coagulation; 3) and in vivo APAC targeting of injured porcine vessels, specifically von Willebrand factor (VWF), laminin, podocalyxin, and PECAM.
Methods: APAC-spiked citrated blood of healthy volunteers was challenged to collagen (3.2 μg/ml), ristocetin (0.77 mg/ml) and ADP (6.4 μM), and to collagen (0.5 μg/ml) in PRP, and in rotational thromboelastometry (ROTEM) for intrinsic and extrinsic activation, with and without platelet inhibition by cytochalasin D or heparinase treatment. We used porcine models of iliac artery balloon angioplasty and created an arterio-venous fistula (AVF) on FA and vein. Biotinylated APAC (0.5 mg/ml) was exposed to vessel injury site for 2 min prior to releasing blood flow. Binding was detected by fluorescence in confocal microscope.
Results: APAC dose-dependently prolonged lag time and decreased the slope of aggregation. In blood APAC (150 μg/ml) inhibited collagen- and ristocetin (but not ADP) -induced aggregation by 58±15% (mean±SD) and by 25±2%, respectively, unlike unfractionated heparin (UFH). In PRP APAC specifically inhibited collagen-induced aggregation (n=9) already at 1 μg/ml by 55±31%, 3 μg/ml by 75±15%, 10 μg/ml by 79±21% and 30 μg/ml by 85±11%. Inhibition of maximal aggregation varied, but improved in all donors by increased APAC doses. In ROTEM APAC (3 μg/ml) broadly attenuated fibrin elasticity and prolonged clotting times (CT) 4.5–8-fold, in both intrinsic and extrinsic pathways, while UFH (3 μg/ml) prolonged