Human platelet concentrates treated with microbicidal 405 nm light retain hemostasis activity

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Abstract

Chemical and UV light-based pathogen reduction technologies are currently in use for human platelet concentrates (PCs) to enhance safety from transfusion-transmitted infections. Relative to UV light, 405 nm violet-blue light in the visible spectrum is known to be less harmful. Hence, in this report for the first time, we have assessed the global hemostasis activity of PCs stored in plasma and the activities of six plasma coagulation factors (CFs) as a measure of in vitro hemostatic activity following exposure to the microbicidal 405 nm light. Apheresis PC samples collected from each screened human donor (n =22) were used for testing of PCs and platelet poor plasma (PPP). Both PCs and PPPs were treated for 5hrs with 405 nm light to achieve a previously established microbicidal light dose of 270 J/cm^2. Activated partial thromboplastin time and prothrombin time-based potency assays using a coagulation analyzer and hemostatic capacity via Thromboelastography were analyzed. Thromboelastography analysis of the light-treated PCs and plasma present in the PCs showed little difference between the treated and untreated samples. Further, plasma present in the PCs during the light treatment demonstrated a better stability in potency assays for several coagulation factors compared to the plasma alone prepared from PCs first and subjected to the light treatment separately. Overall, PCs stored in plasma treated with 405 nm violet-blue light retain activity for hemostasis.

Keywords: 405 nm Light, Plasma, Platelets, Coagulation factors, Hemostasis

Introduction

Human platelet concentrates (PCs) stored in plasma in blood banks are life-saving transfusion products to manage bleeding in various clinical settings [1]. Platelet aggregation along with activated coagulation factors (CFs) and a few other proteins present in plasma participate in a cascade of events that ultimately facilitate hemostasis and eventual clotting of blood, coagulation at the site of vascular injury [2]. This sequential activation of CFs (Fig. 1), coupled with positive feedback loops is a highly regulated process, where upon dysfunction can result in suboptimal performance of both platelets and plasma CFs [3].

In order to protect the critical hemostasis functions of PCs and CFs, it is imperative that any treatments performed during PC manufacturing, prior to storage for transfusion, should be gentle to both platelets and plasma. While treatment of blood components with existing chemical and UV light-based pathogen reduction technologies (PRTs) effectively inactivate pathogens, these PRTs are also known to cause measurable, but somewhat tolerable, impediments to both platelets and plasma CFs [4]. Consequently, while the transfusion medicine field recognizes the benefits of PRTs, it acknowledges a measurable reduction in quality of cellular and protein components following PRT treatment [5]. Thus, there is a need for improving PRTs by identification and evaluation of better approaches while being cognizant of the fact that there will be a measurable reduction in the quality of cell and protein components following a PRT treatment [5]. Therefore, improvements to PRT refer to identifying and choosing a technology that demonstrates the least effect on the product by maintaining product quality.

Within this context, there has been a surge of research initiated by our group and others in evaluating alternatives to UV light-based technologies, such as 405 nm violet-blue light of the visible spectrum that does not require use of chemicals or photosensitizers [6, 7]. We have previously demonstrated that a 405 nm light dose of up to 270 J/cm^2 is effective in reducing (by inactivation)

several bacteria, HIV-1, and parasites in plasma alone or in platelets suspended in plasma [8, 9]. Further, we have shown that microbicidal 405 nm light does not affect the recovery and survival of the light-treated platelets in a SCID mouse model [10], demonstrating the safety of the treatment for *ex vivo* platelets. In another study, we evaluated the effect of 405 nm light on the integrity of plasma proteins, higher doses of 405 nm light up to <720 J/cm^2 did not impart any visible changes to plasma protein integrity based on SDS-PAGE analysis [11]. This report implies that the 405 nm light dose of 270 J/cm^2 that has been used for pathogen inactivation in our studies is well below the threshold light dose (720 J/cm^2) at which plasma protein integrity is affected. However, to date, experimental verification of the impact of 270 J/cm^2 dose of 405 nm light on functions of individual CFs and global hemostasis potential of PCs is lacking.

In this report, we evaluated the impact of 405 nm light on global hemostasis potential of PCs and the coagulation profile of several individual CFs of platelet poor plasma obtained from PC treated with 405 nm light (denoted as PPP-1) and platelet poor plasma treated directly with 405 nm light (denoted as PPP-2), by using three well established *in vitro* standard assays (i) activated partial thromboplastin time (aPTT) -based potency assay and (ii) prothrombin time (PT) -based potency assay, as well as (iii) thromboelastography (TEG) for global hemostasis potential of PCs. The results demonstrated that global hemostasis potential of PCs was retained and has a milder effect on coagulation protein activity following treatment with 270 J/cm^2 dose of 405 nm violet-blue light.

Materials and Methods

Sample preparation and 405 nm light treatment

The study involving human subjects' protocol was approved by FDA Research Involving Human Subjects Committee (RIHSC. Exemption Approval #11-036B). Human apheresis platelet concentrates

(PCs) from 22 individual donors, were received from the National Institutes of Health (NIH) Blood Bank, Department of Transfusion Medicine (Bethesda, MD, USA) and stored at 22°C under gentle agitation. Sample preparation and experimental procedures for specific experiments (i.e., PC treated samples and PPP treated samples) are described in Fig. 2. Platelet-poor-plasma was prepared from each unit of PC by centrifugation at 4,000 *x g* for 30 mins using horizontal swing buckets and supernatant was carefully collected and pooled to generate a master stock termed as controlled pooled plasma (CPP). CPP was used as a control sample for all coagulation factor assays described in this report. Commercially available normal reference plasma, Characterized Human Plasma (CHP) was purchased from Precision Biologic (Nova Scotia, Canada) was used to generate calibration curves in aPTT- and PT-based potency assays.

For experiments with PCs, each PC unit was split evenly into two transfer bags (Terumo Transfer Bag T-150; Terumo BCT, Lakewood, CO, USA). Briefly, in each experiment, one PC containing transfer bag was designated as treated and exposed to 405 nm light for 5 hrs to achieve a light dose of 270 J/cm^2 (at an irradiance of ~15 mW/cm^2), while the other PC containing transfer bag was designated as a control (no 405 nm light treatment via obscuring the transfer bag in a light impenetrable cover). This light dose was selected since the dose manifested a broad inactivation capacity for several bacteria, HIV-1 and a blood-borne parasite, *Trypanosoma cruzi,* and was compatible with platelet and plasma integrity [8]. Following the 5 hrs treatment, the PC was immediately used for thromboelastography assays and remaining sample was centrifuged at 4000 *x g* for 30 min to obtain platelet-poor-plasma-1 (PPP-1) and stored at -80°C for further use.

For experiments involving 405 nm light treatment of platelet-poor-plasma, upon receiving from NIH, PCs were centrifuged at 4000 *x g* for 30 min to obtain platelet-poor-plasma (PPP-2). These PPP-2 samples were dispensed into two transfer bags. The 405 nm light treatment was performed as described above. After treatment, samples were aliquoted and frozen at -80^{0}C for further analyses. The

treatments with 405 nm light were performed at ~22°C in a closed incubator shaker (60 rpm) that is equipped with high intensity narrow spectrum (HINS) 405 nm LED arrays (FWHM ~20 nm; LED Engin, CA, USA) (US Patent Application number 62/236, 706, 2015).

Coagulation Factor Activity Assessment

Activities of coagulation factors in plasma component of PCs were measured in either prothrombin time (PT) based potency assays (for FV, FVII, and FX) or in activated partial thromboplastin time (aPTT) based potency assays (for FVIII, FIX, and FXI). For each assay, samples (i.e., untreated controls, 405 nm light-treated PC-derived PPP (PPP-1), or PPP directly treated with the light (PPP-2)) were serially diluted in human plasma deficient by a factor of interest (either FV, FVII, FVIII, FIX, FX, or FXI). Then, samples were analyzed on an ACL TOP 550 coagulation analyzer per the manufacturer's protocol with minor modifications (manufacturer's lyophilized factor deficient plasma was replaced with frozen deficient plasma manufactured by HRF Inc., Raleigh, NC, USA). Commercial reagent kits were used: HemosIL SynthAFax for aPTT, and HemosIL RecombiPlasTin for PT assay (Werfen, Bedford MA, USA). Serially diluted CHP (in respective deficient plasma) was used to generate calibration curve(s) for potency assignment. Clotting times were recorded by the analyzer. Potency calculation was performed in OriginLab software (ver. 2023b 10.0.0.154), using in-house LabTalk script as described previously [12-14]. Potency was determined as an average of two best fit dilutions of each sample against a calibration curve of four points.

Thromboelastography (TEG)

Untreated or light-treated donor PC, PPP-1, or PPP-2 was assessed using the Global Hemostasis Cartridge (Haemonetics, Boston, MA, USA) on the TEG6s system. Approximately 700 µL of either untreated or light treated sample was pipetted into the TEG Global Hemostasis Cartridge, which was subsequently inserted to the TEG analyzer. TEG curve traces were made for the four tests associated

with the cartridge (CK; Citrated Kaolin TEG, CRT; Citrated Rapid TEG, CKH; Citrated Kaolin TEG with Heparinase, and CFF; Citrated Functional Fibrinogen). The following parameters were shown for the CK test: R (time to clot formation), K (time for clot to reach 20 mm), α angle (the rate of the TEG curve), and MA (maximum amplitude) of the clot.

Statistical Analysis

Statistical analysis was performed using an unpaired t-test to compare the means of 'Control' vs '405 Light' groups. Data is represented as mean ± standard deviation, and differences were considered as significant for p values < 0.05.

Results

Effect of 405 nm violet-blue light treatment on plasma coagulation factor activity

We evaluated the effect of PCs treatment with 405 nm light (270 J/cm^2 dose) on the functional activity of coagulation factors in the plasma component of PCs (PPP-1). Activities of the indicated coagulation factors were measured by aPTT- or PT- based potency assays for serially diluted samples (see Materials and Methods).

Fig. 3A-C, G-I shows dose response curves for the indicated coagulation factors, averaged for four donors for PC-treated PPP and control samples. Compared to donor-matched, untreated controls, 405 nm light treatment yielded dose response curves that were slightly shifted towards prolonged clotting times. This shift translated into a slight decrease in potencies of coagulation factors in light treated plasma (PPP-1) samples as compared to their donor-matched untreated control samples (Fig. 3 D-F, J-L), although a statistically significant reduction of activity was observed only for FVIII (Fig. 3F). CPP samples were used as an untreated control pooled for each donor to make an overall comparison against individual donor samples (to demonstrate donor-donor variance). Supplemental Table S1

summarizes the average potency for both control and 405 nm light treated samples, and associated Δ value for each coagulation factor.

We also studied the effect of light exposure on potency of coagulation factors for PPP samples directly treated with light (PPP-2; see Fig. 2). Observing a similar trend as before, 405 nm light-treated samples produced dose response curves shifted towards prolonged clotting times (Fig. 4A-C, G-I) and consequently lower potencies (Fig. 4D-F, J-L) for individual coagulation factors as compared to untreated, donor-matched controls. For each coagulation factor, the average potencies for both control and light treated samples, and associated Δ was calculated (see Supplemental Table S2).

Effect of 405 nm violet-blue light treatment on hemostasis

To elucidate the effect of 405 nm light treatment on overall hemostasis potential, untreated control and treated PCs, PPP-1, PPP-2 samples were separately assessed via thromboelastography (TEG). Overall, as shown in Tables 1-3, 405 nm light exposure induced slight modulation in the studied TEG parameters. The range of coefficients of variation (CVs) was quite broad amongst the different TEG parameters studied.

For example, in PC samples (see Table 1), the CV was 15.39% in untreated control samples, and 17.86% in the light-treated group, indicating that both groups had relatively high donor-to-donor variation. That said, the average value for R parameter was 8.3 minutes (± 1.3) in the control group and 9.5 (± 1.7) minutes in the treated group, suggesting that light treatment delays clot reaction time by ~ 1 minute. The other time parameter (K; the time the clot reaches 20 mm amplitude) also had high CVs (25.34% for the control group and 39.74% for the treatment group), and their average K values being similar. The MA parameter (maximum clot amplitude) as a measure of clot strength was similar between the two groups (control = 72 mm ± 2.2 and treated = 69.6 mm ± 1.9), with much smaller CVs (control = 3.11% and treated = 2.73%). Fibrin polymerization kinetics did not differ much between control (α angle

= 78.9° ± 1.5; CV = 1.86%) and treated (α angle = 75.7° ± 3.9; CV = 5.09%) samples. No significant differences were observed between control and light-treated PC samples in any of the parameters studied.

We also assayed PPP-1 samples (derived from the light treated-PC samples) via TEG analysis to investigate potential differences in hemostasis capacity in the absence of platelets (Table 2). Similar to the treated PC samples, the associated PPP-1 samples had slightly higher R values in our treated group (R = 10.6 min ± 1.7; CV = 16.86%) as compared to controls (R = 9.2 ± 1.7; CV = 18.91%). Not surprisingly, many of the MA parameters were displayed as < 40 mm, indicating that the majority of these PPP-1 samples were lower than the reference range of the instrument most likely due to the lack of platelet contribution to clot formation. In contrast to the PC counterpart samples, fibrin accumulation was more varied between controls (α angle = 72.6° ± 4.9; CV = 6.8%) and treated (α angle = 65.4° ± 10.9; CV = 16.64%) samples.

Lastly, we investigated the hemostatic capacity of PPP-2 samples treated after platelets have been removed (Table 3). Interestingly, R values differed more so in this subset of samples; clot reaction time was several minutes faster in control samples (R = 9.6 minutes ± 1.3; CV = 13.55%) as compared to treated samples (R = 13.3 minutes ± 2.0; CV = 15.02%) suggesting that plasma devoid of platelets is more labile to the light exposure than platelet containing plasma (p = 0.055). Again, the majority of MA values for both control and treatment groups was < 40 mm, demonstrating the lack of clot strength in the absence of platelets. Interestingly, fibrin accumulation in these PPP-2 samples (directly exposed to 405 nm light) was similar between control (α angle = 68.4° ± 10.9; CV = 15.89%) and treated (α angle = 70.9° ± 4.3; CV = 5.99) samples.

Discussion

Coagulation can be triggered by either the extrinsic (through tissue factor; TF) or intrinsic (contact) pathway (Fig. 1). In the extrinsic pathway, TF becomes exposed to blood upon blood vessel damage and/or inflammation. Binding of FVIIa and TF (FVIIa-TF) initiates the cascade of reactions which results in clot formation. In the intrinsic pathway coagulation is triggered by blood/plasma contact with negatively charged surfaces or substances, promoting the activation of FXII, and following activation of FXI, FIX, FVIII. Ultimately, both pathways converge upon the common pathway, in which FVa interacts with FXa, forming the prothrombin complex and cleaving of thrombin to its active form [15]. Thrombin activation greatly enhances coagulation reactions through feedback mechanisms, cleavage of fibrinogen to fibrin and activation of platelets.

Loss of coagulation factor activity can lead to potentially life-threatening conditions, including excessive bleeding episodes or thrombotic events. Therefore, it is pivotal to maintain proper coagulation function during pathogen reduction treatment. Herein, for the first time through our comprehensive analyses, we demonstrated that exposure to microbicidal 405 nm light dose of 270 J/cm^2 does not drastically effect activity of coagulation factors in PCs. All potencies measured in this study were donor-matched and found to be < 0.5-fold difference between controls and treated samples. These potencies were assigned based on aPTT- or PT-based potency assays. The data demonstrates that for each coagulation factor studied, some differences are observed between untreated and light-treated groups. This was observed in both PPP-1 samples, and PPP-2 samples treated with 405 nm light after platelets were removed. For example, FVIII measured in light-treated PCs was significantly less potent than untreated controls. However, we observed more statistically significant differences in PPP-2 samples treated with 405 nm light, including FIX, FX, and FXI, suggesting that the absence of platelets prior to light treatment may contribute to more drastic losses of CF activity.

The effect of violet-blue light on the activity of the few coagulation factors that we studied here is comparable to previous studies by others involving chemical/UV-light based PRTs. For example, Smith

and Rock demonstrated that Mirasol PRT treatment preserves coagulation activity within the acceptable range compared to controls [16]. Similarly, treatment with amotosalen and UV light (the INTERCEPT PRT system) also maintains acceptable range of coagulation factor activity [17]. It has been previously reported that the potency of each coagulation factor per mL may vary 50% to 150% of a pooled standard control, suggesting that this is an is acceptable range [18]. The coagulation factor potency results we reported here certainly are within this reported normal range.

It is known that some coagulation factors are more stable (fibrinogen, FVII, FIX, FX, FXI) whereas FV and FVIII are more labile factors [19]. It is of interest to note that when we measured clotting activity of coagulation factors in the PPP-2 samples, we observed very low potency of FV (see Supplemental Figure S2) in one of the donor samples (W-2541) in both untreated and treated conditions, while observing relatively normal activity of rest of the coagulation factors studied from donor W-2541. Although we did not pursue this phenomenon further at this time, the reduced FV activity in this donor could potentially be due to several reasons specific to the donor such as the presence of inhibitors to FV, which can be derived from a wide variety of sources, including treatment with bovine thrombin and other medications, or certain infections, autoimmune disorders, and pregnancy. Another possibility could be due to the regulation of normal FV gene by specific microRNAs in this donor as we have previously discovered for FVIII functional impediment in patients with Hemophilia A phenotype in which normal F8 was present [20, 21]. Furthermore, the labile nature of FVIII is reaffirmed again in our studies, where in the PPP samples derived from light-exposed PCs, the difference in activity between control and treated groups for FVIII was statistically significant.

In conclusion, PCs treated with 405 nm violet-blue light retain capacity for hemostasis. The activity of CFs of PCs stored in plasma are protected relative to the plasma prepared from PCs first and subjected to the light treatment later. While this study evaluated the effect of 405 nm light on PCs and

plasma *in vitro*, we believe future *in vivo* functional studies on global hemostasis function of 405 nm light treated PCs stored in plasma in a suitable animal model are highly warranted.

Author Contributions

Conceptualization, P.R.K., J.W.J., and C.A.D.; methodology, C.F.S., J.G.A., S.J.M., M.M., and C.A.D.; software, L.A.P.; validation, P.R.K. and J.W.J.; formal analysis, P.R.K. and J.W.J.; investigation, P.R.K. and J.W.J.; resources, L.A.P. and C.A.D.; data curation, P.R.K. and J.W.J.; writing—original draft preparation, P.R.K. and J.W.J.; writing—review and editing, P.R.K., J.W.J., L.A.P., M.M., and C.A.D.; visualization P.R.K. and J.W.J.; supervision, C.A.D.; project administration, C.A.D.; funding acquisition, C.A.D. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest Disclosure

All authors declare no conflicts of interest.

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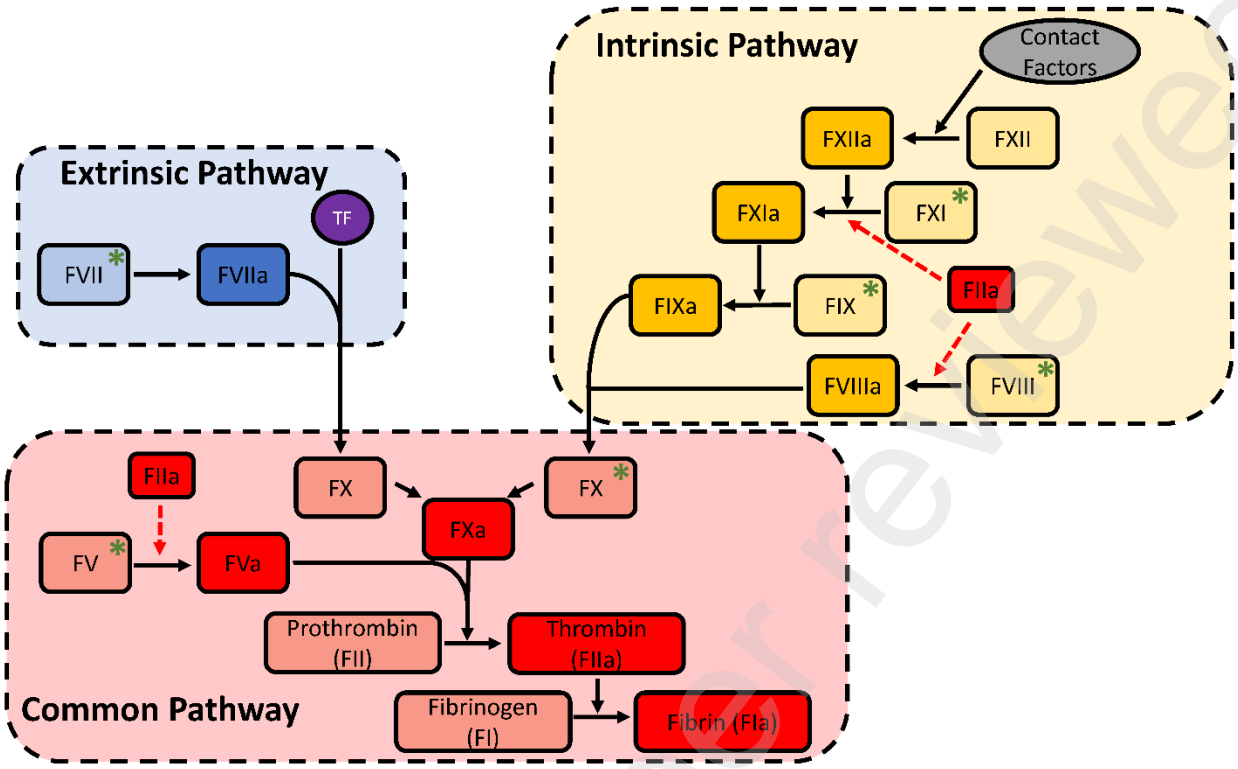


Figure 1. Schematic of coagulation cascade. Coagulation can be initiated by both the extrinsic pathway (shaded blue area) and the intrinsic pathway (shaded yellow area). Extrinsic activation is via exposure to tissue factor (TF) and interaction with activated FVII (FVIIa). Intrinsic activation begins with exposure to negatively charged surfaces or substances and results in subsequent activation of FXII, FXI, FIX, and FVIII. Both pathways converge to the common pathway (shaded red area), which consists of activation of FV and FX to form the prothrombin complex and eventual generation of thrombin. Green asterisks indicate factors investigated in this study.

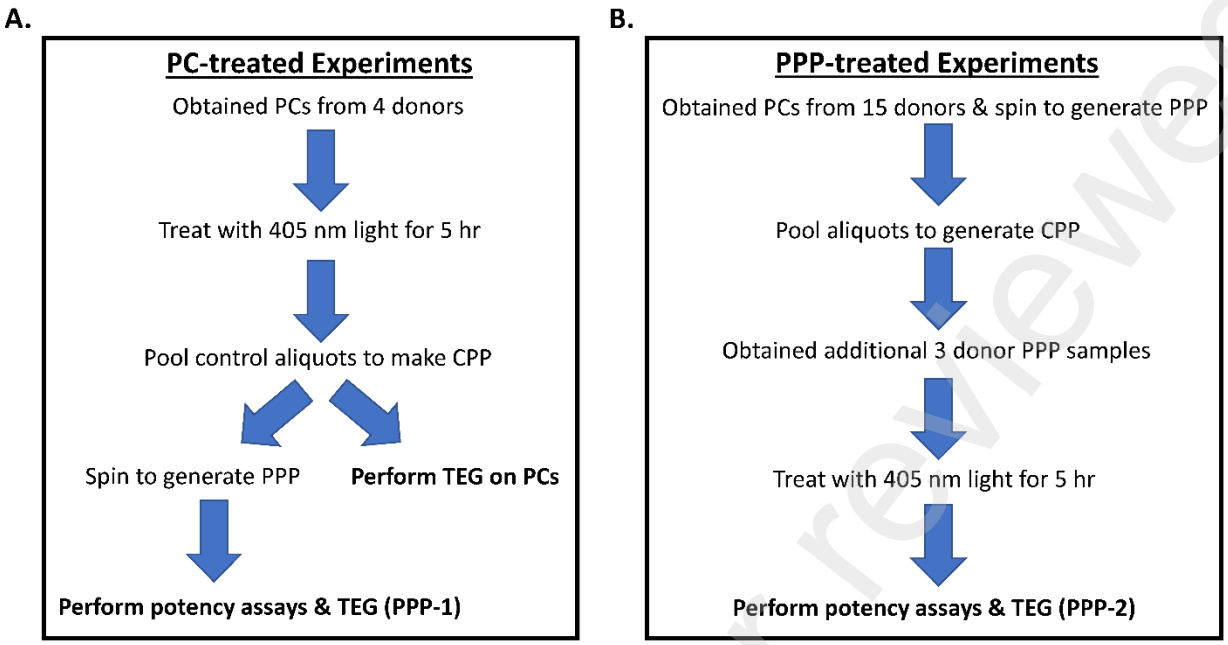


Figure 2. Schematic of experimental setup. We performed experiments assessing the effect of 405 nm light treatment on both PCs, PC-resident plasma, and PPP alone. In panel **A** "PC-treated Experiments" we obtained apheresis PCs from 4 individual donors, and subsequently centrifuged and pooled aliquots from each donor to generate controlled pooled plasma (CPP). Afterwards, each donor PC sample was treated, or not, with 270 J/cm^2 of 405 nm light for 5 hours and a small aliquot was immediately analyzed via thromboelastography. Simultaneously, the remaining PC samples were centrifuged to generate PC-resident plasma and analyzed for both potency assays and thromboelastography (PPP-1). In panel **B** "PPP -treated Experiments" we obtained PCs from 15 different donors, and subsequently centrifuged and pooled aliquots from each donor to generate CPP; three individual donor PPP samples were treated, or not, with 270 J/cm^2 of 405 nm light for 5 hours. These (PPP-2) samples were later analyzed via potency and thromboelastography assays. Here in this study, a total of 22 donor samples were used for different purposes, and different donors were used in both "PC-treated Experiments and "PPP-treated Experiments.

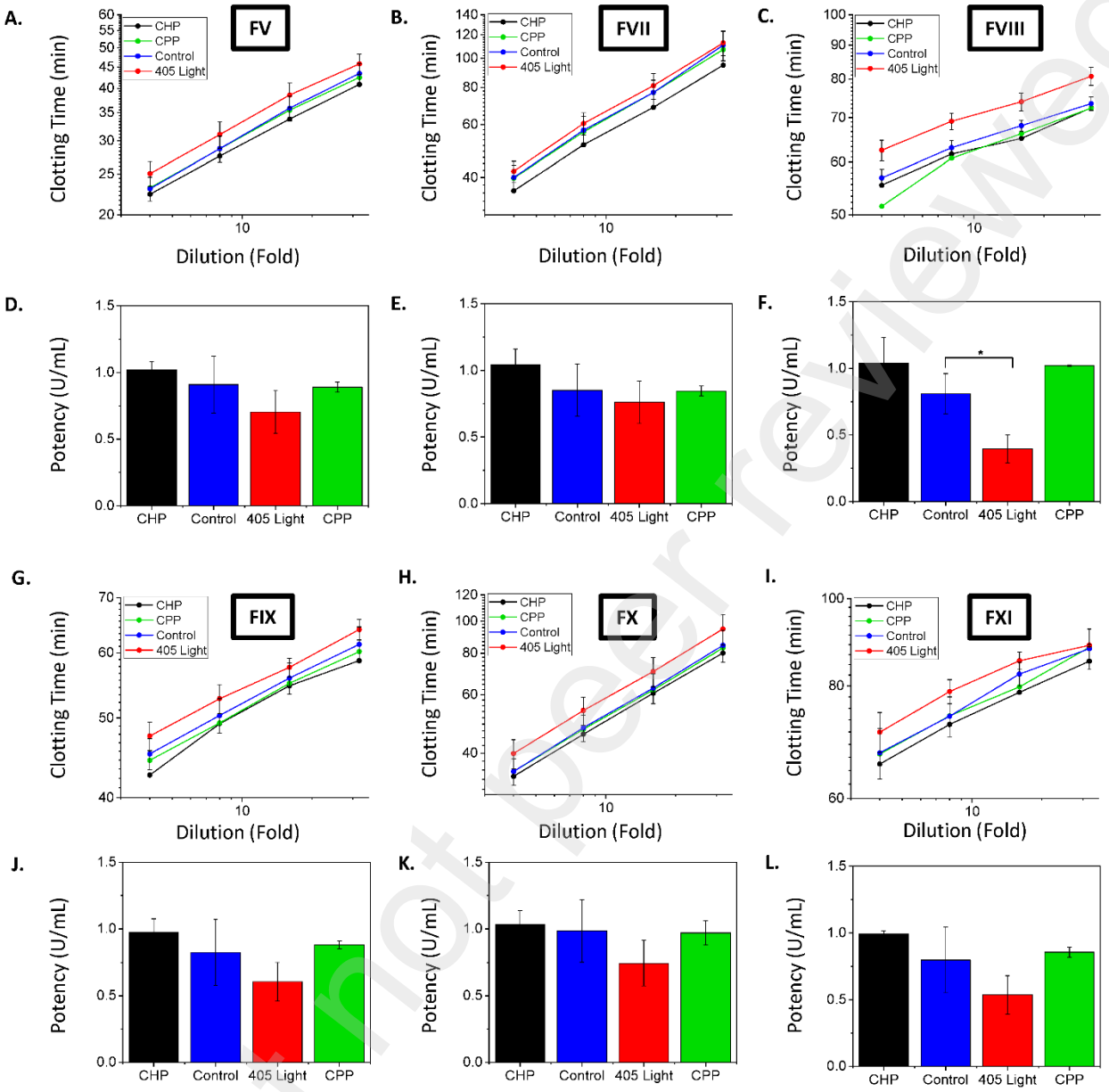


Figure 3. Effect of 405 nm light on potency of coagulation factors on PC samples. Dose response curves and potency of coagulation factors were assessed in untreated (control) or 405 nm light-treated PC-resident plasma (PPP-1) from four individual donors (average representative data is shown for 'Control' and '405 light' groups). The indicated coagulation factors: **(A, D)** FV, **(B, E)** FVII, **(C, F)** FVIII, **(G, J)** FIX, **(H, K)** FX, and **(I, L)** FXI were assessed. Statistical significance comparison of means between 'Control' and '405 nm Light' groups were performed via an unpaired t-test, where * denotes $p<0.05$. CHP= characterized human plasma (used as a calibration reference), CPP = controlled pooled plasma.

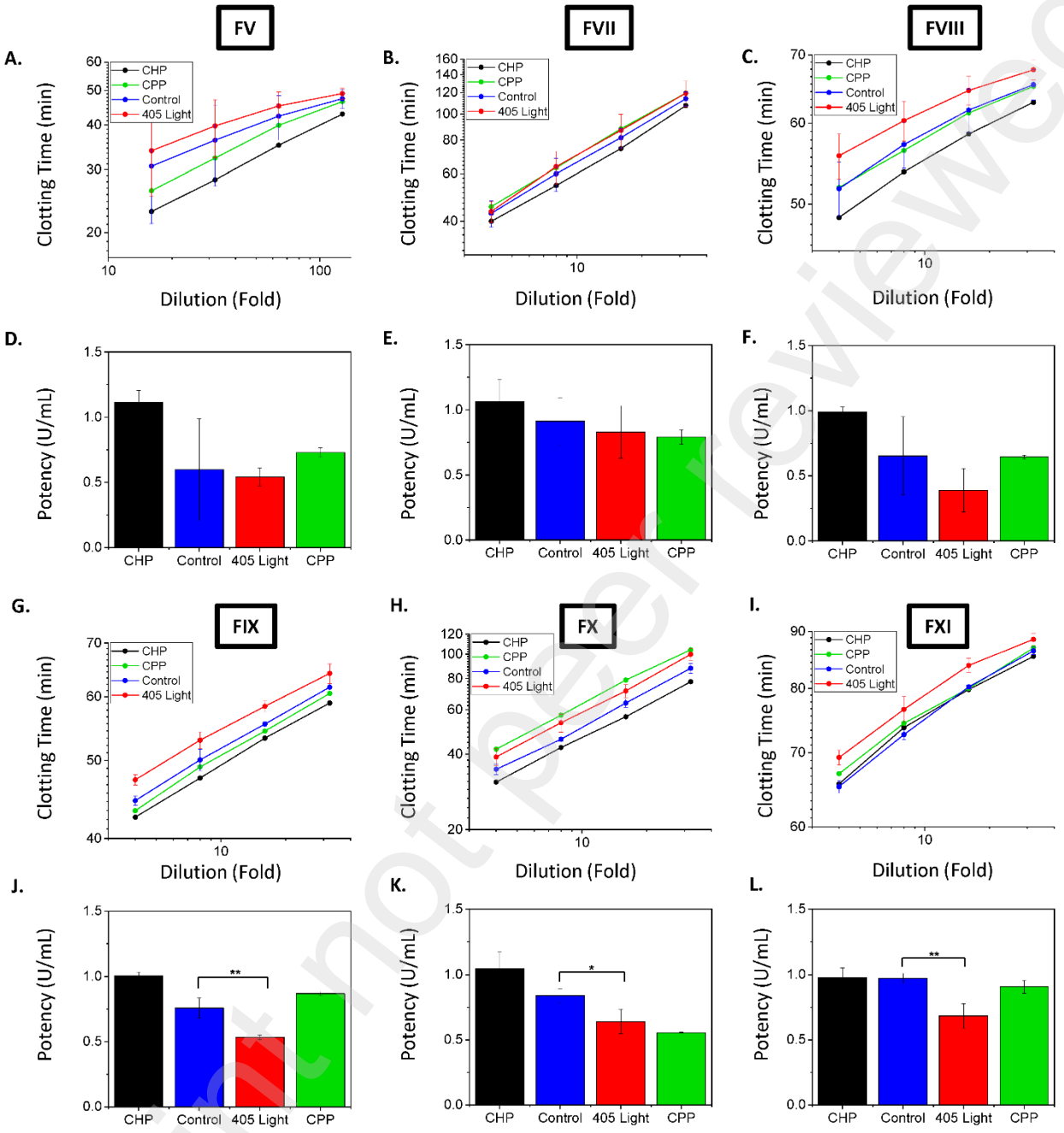


Figure 4. Effect of 405 nm light on potency of coagulation factors in PPP samples. Dose response curves and coagulation factor potencies were assessed in untreated (control) or 405 nm light-treated PPP (PPP-2) of three individual donors (average representative data is shown for 'Control' and '405 light' groups). The indicated coagulation factors: **(A, D)** FV, **(B, E)** FVII, **(C, F)** FVIII, **(G, J)** FIX, **(H, K)** FX, and **(I, L)** FXI were assessed. Statistical significance comparison of means between 'Control' and '405 nm Light' groups were performed via an unpaired t-test, where * denotes $p<0.05$ and ** denotes $p<0.01$. CHP= characterized human plasma (used as a calibration reference), CPP = controlled pooled plasma.

	R (min)		K (min)		α Angle (°)		MA (mm)	
Donor	**Control**	**405 nm Light**	**Control**	**405 nm Light**	**Control**	**405 nm Light**	**Control**	**405 nm Light**
W-2578	9.6	10.7	0.8	1.7	80.2	70.6	70	68
W-2689	8.1	7.5	0.7	0.8	*	78.1	*	>75
W-2800	6.6	8.7	0.8	1	79.1	75	71.5	69.1
W-3005	8.8	11.1	1.2	0.8	77.3	79.2	74.4	71.7
Average	8.3	9.5	0.9	1.1	78.9	75.7	72.0	69.6
p Value	0.292		0.438		0.245		0.235	
SD	1.3	1.7	0.2	0.4	1.5	3.9	2.2	1.9
CV, %	15.39	17.86	25.34	39.74	1.86	5.09	3.11	2.73

Table 1. TEG parameters in 405 nm light-treated PC samples. The measured TEG parameters: R (clot reaction time), K (time until clot reaches 20 mm), α angle (measure of clot kinetics), and MA (maximum clot amplitude) of PRP samples from the indicated donors. SD: Standard Deviation, CV: coefficient of variation. An '*' symbol indicates the value is beyond the detection range of the TEG6S instrument. Statistical significance comparison between 'Control' and '405 nm Light' groups for each parameter were performed via an unpaired t-test, where associated p values are listed.

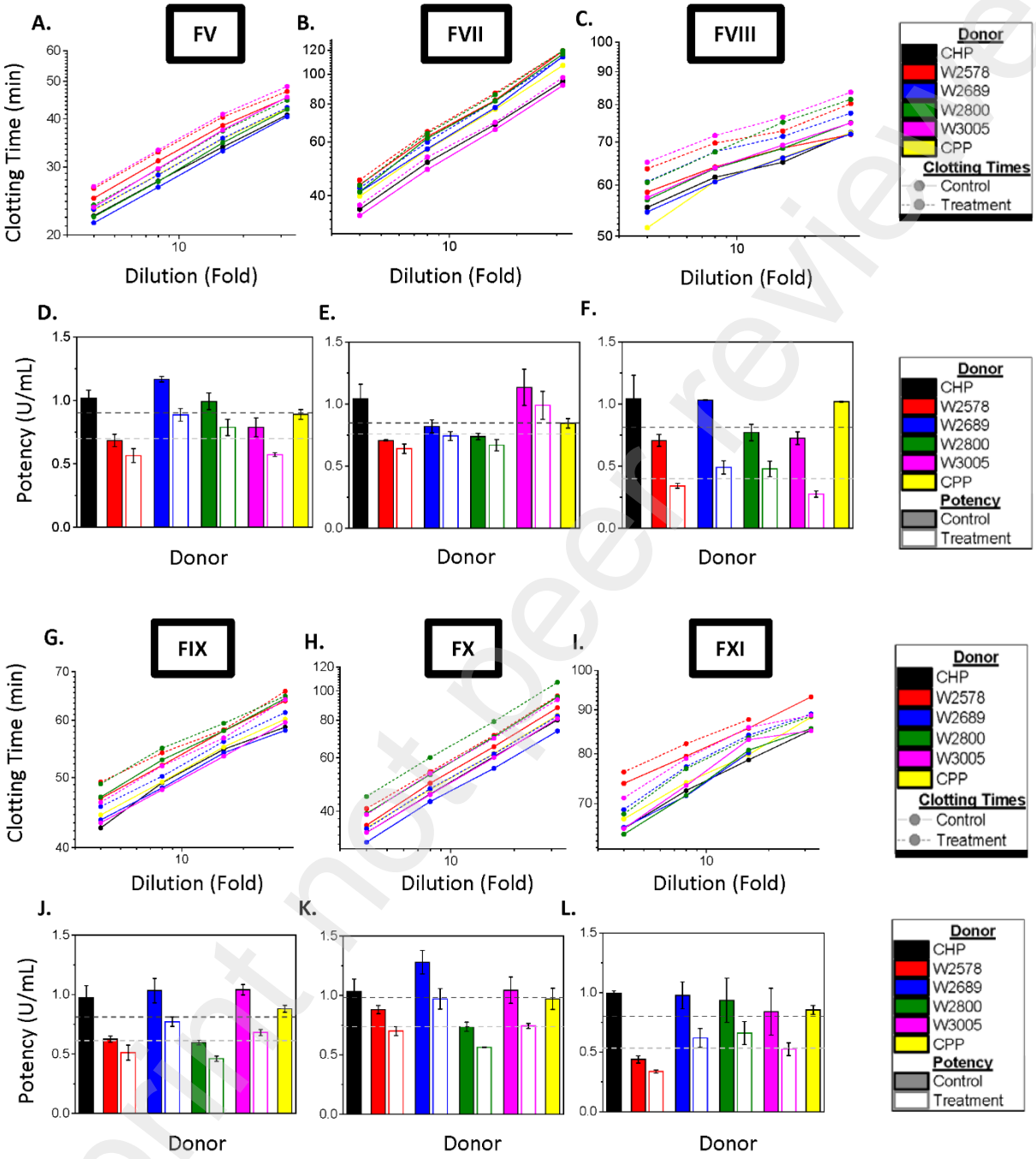
	R (min)		K (min)		α Angle (°)		MA (mm)	
Donor	**Control**	**405 nm Light**	**Control**	**405 nm Light**	**Control**	**405 nm Light**	**Control**	**405 nm Light**
W-2578	10.8	11.1	*	2.5	65.2	66	<40	<40
W-2689	7.3	8.2	1.3	1.1	75.3	74.7	<40	47.9
W-2800	8.2	10.7	1.3	2.1	75.2	71	<40	<40
W-3005	10.6	12.5	1.4	3.6	74.7	50	<40	<40
Avg	9.2	10.6	1.3	2.3	72.6	65.4	*	47.9
p Value	0.306		0.166		0.275		n/a	
SD	1.7	1.8	0.1	1.0	4.9	10.9	*	*
CV, %	18.91	16.86	4.33	44.47	6.80	16.64	*	*

Table 2. TEG parameters in PC-resident plasma samples. The measured TEG parameters: R (clot reaction time), K (time until clot reaches 20 mm), α angle (measure of clot kinetics), and MA (maximum clot amplitude) of PRP-derived PPP samples from the indicated donors. SD: Standard Deviation, CV: coefficient of variation. An '*' symbol indicates the value is beyond the detection range of the TEG6S instrument. Statistical significance comparison between 'Control' and '405 nm Light' groups for each parameter were performed via an unpaired t-test, where associated p values are listed.

	R (min)		K (min)		α Angle (°)		MA (mm)	
Donor	**Control**	**405 nm Light**	**Control**	**405 nm Light**	**Control**	**405 nm Light**	**Control**	**405 nm Light**
W-2541	9.2	11.4	*	2.1	56.4	66.6	<40	<40
W-097	11.1	15.4	1.9	1.1	71.1	75.1	<40	40.5
W-1184	8.6	13.2	0.9	2.2	77.6	71.1	<40	<40
Avg	9.6	13.3	1.4	1.8	68.4	70.9	*	40.5
p Value	0.055		0.544		0.723		n/a	
SD	1.3	2.0	0.7	0.6	10.9	4.3	*	*
CV, %	13.55	15.02	50.51	33.79	15.89	5.99	*	*

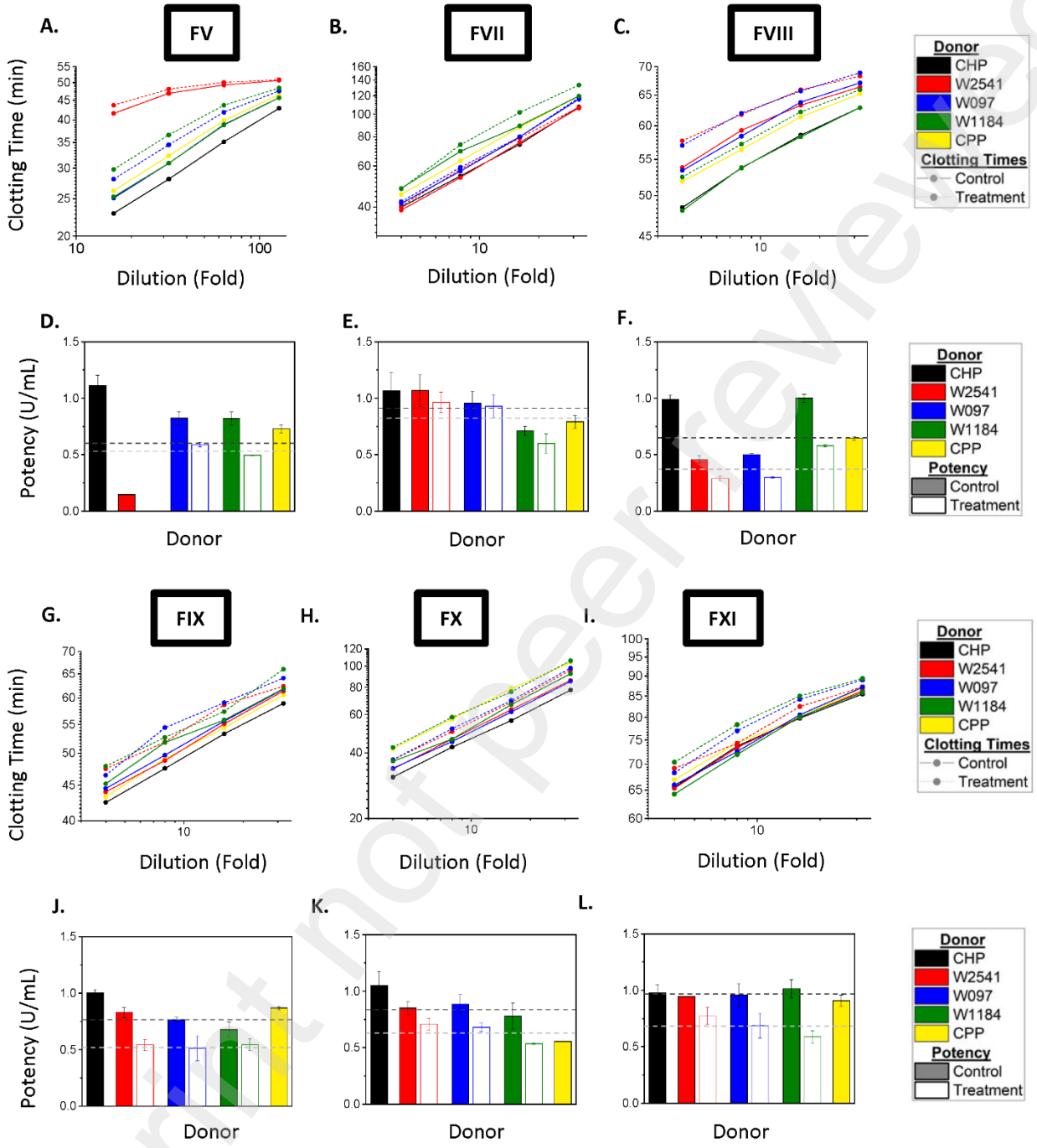
Table 3. TEG parameters in PPP alone treated samples. The measured TEG parameters: R (clot reaction time), K (time until clot reaches 20 mm), α angle (measure of clot kinetics), and MA (maximum clot amplitude) of PRP-derived PPP samples from the indicated donors. SD: Standard Deviation, CV: coefficient of variation. An '*' symbol indicates the value is beyond the detection range of the TEG6S instrument. Statistical significance comparison between 'Control' and '405 nm Light' groups for each parameter were performed via an unpaired t-test, where associated p values are listed.

Supplemental Figures



Supplemental Figure S1. Effect of 405 nm light on potency of coagulation factors on PC samples. Dose response curves and potency of coagulation factors were assessed in untreated (control) or 405 nm light-treated PC-resident plasma (PPP-1) of four individual donors. The indicated coagulation factors: **(A, D)** FV, **(B, E)** FVII, **(C, F)** FVIII, **(G, J)** FIX, **(H, K)** FX, and **(I, L)** FXI were assessed. For clotting times untreated control samples are denoted with solid lines, whereas light-treated samples are denoted with dashed lines. For potencies, untreated control samples are denoted with solid bars, whereas light-treated samples are denoted with open bars. The dark gray dashed line denotes the average potency of

untreated control samples, whereas the light gray dashed line denotes the average potency of 405 nm light-treated samples. CHP= characterized human plasma (used as a calibration reference), CPP = controlled pooled plasma.



Supplemental Figure S2. Effect of 405 nm light on potency of coagulation factors in PPP samples. Dose response curves and coagulation factor potencies were assessed in untreated (control) or 405 nm light-treated PPP (PPP-2) of three individual donors. The indicated coagulation factors: **(A, D)** FV, **(B, E)** FVII, **(C, F)** FVIII, **(G, J)** FIX, **(H, K)** FX, and **(I, L)** FXI were assessed. For clotting times, untreated control samples are denoted with solid lines, whereas light-treated samples are denoted with dashed lines. For potencies, untreated control samples are denoted with solid bars, whereas light-treated samples are denoted with open bars. The dark gray dashed line denotes the average potency of untreated control samples, whereas the light gray dashed line denotes the average potency of 405 nm light-treated

samples. CHP= characterized human plasma (used as a calibration reference), CPP = controlled pooled plasma.

Coagulation Factor	Control	405 nm Light	Δ (Control vs Treatment)	p Value
FV (U/mL)	0.91	0.7	0.21	0.176
FVII (U/mL)	0.85	0.76	0.09	0.502
FVIII (U/mL)	0.81	0.4	0.41	0.004 (**)
FIX (U/mL)	0.82	0.61	0.22	0.177
FX (U/mL)	0.98	0.74	0.24	0.146
FXI (U/mL)	0.8	0.54	0.26	0.115

Table S1. Average potency values and delta between control and PC resident plasma samples. Normal reference potency values, as well as average potencies from control and 405 nm light treated groups are shown. The Δ between control and treatment groups is also calculated. Statistical significance comparison of means between 'Control' and '405 nm Light' groups were performed via an unpaired t-test, where * denotes $p<0.05$ and ** denotes $p<0.01$.

Coagulation Factor	Control	405 nm Light	Δ (Control vs Treatment)	p Value
FV (U/mL)	0.60	0.54	0.05	0.388
FVII (U/mL)	0.91	0.83	0.08	0.932
FVIII (U/mL)	0.65	0.39	0.26	0.189
FIX (U/mL)	0.76	0.53	0.22	0.008 (**)
FX (U/mL)	0.84	0.64	0.20	0.033 (*)
FXI (U/mL)	0.97	0.68	0.29	0.008 (**)

Table S2. Average potency values and delta between control and PPP alone treated samples. Normal reference potency values, as well as average potencies from control and 405 nm light treated groups are shown. The Δ between control and treatment groups is also calculated. Statistical significance comparison of means between 'Control' and '405 nm Light' groups were performed via an unpaired t-test, where * denotes $p<0.05$ and ** denotes $p<0.01$.