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HEMOSTASIS

TRANSFUSION

Effects of pathogen reduction technology and storage duration on the ability of cryoprecipitate to rescue induced coagulopathies in vitro

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Abstract

Background: Fibrinogen concentrates and cryoprecipitate are currently used for fibrinogen supplementation in bleeding patients with dysfibrinogenemia. Both products provide an abundant source of fibrinogen but take greater than 10 min to prepare for administration. Fibrinogen concentrates lack coagulation factors (i.e., factor VIII [FVIII], factor XIII [FXIII], von Willebrand factor [VWF]) important for robust hemostatic function. Cryoprecipitate products contain these factors but have short shelf lives (<6 h). Pathogen reduction (PR) of cryoprecipitate would provide a shelf-stable immediately available adjunct containing factors important for rescuing hemostatic dysfunction.

Study Design and Methods: Hemostatic adjunct study products were psoralen-treated PR-cryoprecipitated fibrinogen complex (PR-Cryo FC), cryoprecipitate (Cryo), and fibrinogen concentrates (FibCon). PR-Cryo FC and Cryo were stored for 10 days at 20–24°C. Adjuncts were added to coagulopathies (dilutional, 3:7 whole blood [WB]:normal saline; or lytic, WB + 75 ng/ml tissue plasminogen activator), and hemostatic function was assessed by rotational thromboelastometry and thrombin generation.

Results: PR of cryoprecipitate did not reduce levels of FVIII, FXIII, or VWF. PR-Cryo FC rescued dilutional coagulopathy similarly to Cryo, while generating significantly more thrombin than FibCon, which also rescued dilutional coagulopathy. Storage out to 10 days at 20–24°C did not diminish the hemostatic function of PR-Cryo FC.

Discussion: PR-Cryo FC provides similar and/or improved hemostatic rescue compared to FibCon in dilutional coagulopathies, and this rescue ability is stable over 10 days of storage. In hemorrhaging patients, where every minute delay is associated with a 5% increase in mortality, the immediate availability of PR-Cryo FC has the potential to improve outcomes.

† Denotes equal contribution.

KEYWORDS
cryoprecipitate, dilutional coagulopathy, fibrinogen concentrate, hemostatic capacity, pathogen reduction

1 | INTRODUCTION

Mortality at 28 days is high in patients with severe traumatic hemorrhage, ranging between 20% and 24% in adults[1,2] and 36% and 50% in children.[3,4] Impaired fibrinogen function is highly associated with mortality in these patients with traumatic bleeding,[5,6] and transfusion of fibrinogen-containing products may improve outcomes.[7–10] Currently, there are two sources of exogenous fibrinogen used during resuscitation: fibrinogen concentrates and cryoprecipitate. To date, there are no published clinical studies comparing the impact of fibrinogen concentrates versus cryoprecipitate use on clinical outcomes in trauma patients with severe bleeding, resulting in a lack of consensus regarding which of these two products are optimal for severe traumatic bleeding.[11] In vitro studies suggest that cryoprecipitate attenuates hyperfibrinolysis more effectively than fibrinogen concentrates.[12] This is relevant to trauma patients as hyperfibrinolysis is a source of coagulopathy and a strong predictor of increased mortality.[13] Lastly, other studies have revealed that factor VIII (FVIII), factor XIII (FXIII), and von Willebrand factor (VWF) deficiencies are associated with worse outcomes in traumatic bleeding patients suggesting that cryoprecipitate may be an optimal product compared to fibrinogen concentrates in this population[14–18] and in other severely bleeding populations, such as cardiac and obstetric bleeding.[19]

Fibrinogen concentrates and cryoprecipitate each have advantages and disadvantages. Fibrinogen concentrates provide consistent amounts of fibrinogen between batches, are lyophilized allowing for long-term storage, and remove the risk of transfusion transmitted infections (TTIs).[20] However, fibrinogen concentrates take approximately 15 min to reconstitute[21] and are very expensive (current average price: $734/g of fibrinogen in United States).[22] In contrast, cryoprecipitate contains additional coagulation factors (fibrinogen [FGN], FVIII, FXIII, and VWF) important for hemostasis[23] and is 3–4 times cheaper than fibrinogen concentrates when compared with per gram of fibrinogen.[22,24] Disadvantages include increased batch-to-batch variation in fibrinogen content, a very small increased risk of TTIs due to pooling, and logistical issues: cryoprecipitate is frozen and requires 20 min to thaw and expires 6 h post-thaw, increasing waste. An immediately available (i.e., no reconstitution or thaw time) fibrinogen supplement with an extended shelf life could potentially improve outcomes in traumatically bleeding patients.[25]

Recent advances in the field of blood product safety have resulted in the implementation of pathogen reduction (PR) technology for multiple blood products as PR prevents replication of bacteria, viruses, and parasites associated with TTIs.[26] Multiple PR technologies exist globally for use on plasma products and can be adapted for use with cryoprecipitate products to generate a pathogen-reduced cryoprecipitate product. This would have the potential to extend cryoprecipitate product shelf life without the risk of pathogen growth over time, thereby improving product safety profiles. Very recently, the Food and Drug Administration (FDA) approved a pathogen-reduced cryoprecipitated fibrinogen complex product for use out to 5 days post-thaw,[27] which has been shown to rescue in vitro derived dilutional coagulopathies.[28,29] However, questions remain regarding the hemostatic efficacy of pathogen-reduced cryoprecipitate products, such as the maximum length of storage at which the product maintains functionality, the ability of the product to improve hyperfibrinolysis, and how long-term-stored pathogen-reduced cryoprecipitate products perform compared to current clinically used fibrinogen concentrates and cryoprecipitate.

Herein, we induced two distinct coagulopathies in vitro and resuscitated with hemostatic adjuncts (pathogen-reduced cryoprecipitate product, cryoprecipitate, or fibrinogen concentrate) then measured hemostatic rescue to determine whether the pathogen-reduced cryoprecipitate product was an effective and comparable adjunct. We induced a dilutional coagulopathy to mimic the iatrogenic effects of resuscitation with crystalloids, and a lytic coagulopathy (addition of tissue plasminogen activator [tPA]) to test the hypothesis that increased FXIII in cryoprecipitate-based products would stabilize fibrin crosslinking and reduce fibrinolysis. We also tested whether storing pathogen-reduced cryoprecipitate product out to 10 days would affect its hemostatic efficacy compared to fibrinogen concentrate and cryoprecipitate.

2 | MATERIALS AND METHODS

2.1 | Whole blood sources and coagulopathic model induction

Healthy donors of any ABO blood type were consented and enrolled according to Washington University in

St. Louis Institutional Review Board approval #201901135, using inclusion/exclusion criteria provided in Supplemental Methods. Whole blood (WB, 45 ml) was collected via venipuncture into sodium citrate vacutainers, and basic demographic data were collected (Table S1). Coagulopathic models were induced, and hemostatic function was measured using rotational thromboelastometry (ROTEM) and thrombin generation (Figure S1(A), Table S2). To create the dilutional coagulopathic model (dWB), WB was diluted in normal saline in a 3:7 ratio, resulting in a doubling of clot formation time (CFT) and a 50% reduction in the maximum clot firmness (MCF) after extrinsic activation (ExTEM). To induce the lytic coagulopathy model (lyWB), WB was dosed with tPA (75 ng/ml[30]), resulting in 10%–15% lysis after extrinsic activation. Both models recapitulate coagulopathies that have been demonstrated to be clinically relevant or associated with worse outcomes in patients with trauma-induced coagulopathy.[31]

2.2 | Hemostatic adjuncts

Hemostatic adjunct study products (n = 6 each group) were (i) psoralen-treated PR-Cryoprecipitated Fibrinogen Complex ("PR-Cryo FC"), (ii) Cryoprecipitate Antihemophilic Factor ("Cryo"), and (iii) fibrinogen concentrates ("FibCon"). PR-Cryo FC is produced through cryoprecipitation of proteins from plasma that has been treated with the INTERCEPT® Blood System for plasma. After treatment, the plasma is transferred to the storage container of the INTERCEPT set. The plasma storage containers containing INTERCEPT processed plasma are then sterile connected to the FIBRICEPT processing container (FPC), the proprietary container designed for the manufacturing of PR-Cryo FC. The plasma is transferred to the FPC, and the bundle is frozen at −18°C or colder and held for up to 30 days. The plasma is then thawed gradually at 1–6°C and then centrifuged using a hard spin at 1–6°C, resulting in the separation into a cryoprecipitated pellet (PR-Cryo FC) and plasma cryoprecipitate-reduced (PRPCR) supernatant. The PRPCR is transferred into two of the three final storage containers, with 60–100 ml of PRPCR supernatant remaining in the FPC for resuspension of PR-Cryo FC. The resuspended PR-Cryo FC is transferred to the remaining storage container and was pooled prior to freezing. The PR-Cryo FC was stored frozen at −18°C or colder. PR-Cryo FC and Cryo products were manufactured and provided by Cerus Corporation, stored at −20°C until thaw, and post-thaw, they were stored out to 10 days at 20–24°C. One gram of FibCon (RiaSTAP®, CSL-Behring) was reconstituted on the day of assay in 50 ml of sterile water for injection according to the manufacturer's protocol to obtain a 0.02 g/ml solution. The concentration was adjusted with normal saline to achieve a final fibrinogen concentration equal to the average fibrinogen concentration in all six Cryo units tested upon thaw (791.5 mg/dl, described in Supplemental Materials).

2.3 | Rescue of coagulopathy

Study products were combined with dWB or lyWB in a 1:5 fashion (PR-Cryo FC:dWB; Cryo:dWB; FibCon:dWB; PR-Cryo FC:lyWB; Cryo:lyWB; FibCon:lyWB), resulting in a study product fibrinogen dose of 158.3 mg/dl (roughly the equivalent of a 4g FC dose in an 80kg adult). Study product:coagulopathy mixtures were assessed by traditional hemostatic assays (Figure S1(A)). Mixtures were made, and assays performed on days 0, 2, 5, 7, and 10 of storage after thawing for cryoprecipitate-based products and within 6 h after reconstitution for FibCon.

2.4 | Traditional hemostatic assays

Study product:coagulopathy mixtures were assayed using ROTEM (ROTEM *delta* Analyzer; TEM International GmbH, Munich, Germany). ExTEM reagent (Instrumentation Laboratories World Wide [ILWW], Durham, NC, United States) was used for activation, and StarTEM reagent (ILWW) was used for recalcification. Assays were run for 60 min according to the manufacturer's suggested protocol. Clotting time (CT, seconds), CFT (seconds), alpha angle (α, °), MCF (millimeters [mm]), and lysis at 60 min (LY60, %) were recorded.

Platelet-rich plasma (PRP) was generated from study product coagulopathy mixtures (160 *g*, 10 min, 22°C) and used in thrombin generation assays. In brief, 80 µl of PRP was added to 20 µl of reconstituted PRP reagent (Tissue Factor (TF), 5 pM) and mixed with FluCa buffer and substrate, and thrombin generation was measured (absorbance at 460 nm, every 20 s for 60 min) using a calibrated automated thrombinoscope (Diagnostica Stago Inc., Maastricht, The Netherlands) according to the manufacturer's protocol. Endogenous thrombin potential (ETP, nM*min), lag time (time to initial thrombin generation, min), maximal thrombin generation (Peak, nM), and time to peak (Time to Peak, min) were reported.

Platelet-poor plasma (PPP) was generated from study product:coagulopathy mixtures (1600 *g*, 10 min, 22°C; then 2500 *g*, 10 min, 22°C) and frozen (−80°C) until batched analysis of coagulation factors (FGN, FVIII, FXIII, and VWF) using the Stago Compact Max coagulation analyzer (Diagnostica Stago, Parsippany, NJ, United States).

Variable	FibCon	Cryo	PR-Cryo FC	*p* values
FGN	730.5 (564.5–796.8)	758.5 (636.0–960.0)	760.5 (687.5–797.5)	ns,ns,ns
FVIII	10.00 (8.00–10.00)	229.5 (210.0–324.3)	175.5 (134.5–192.8)	***,ns,ns
FXIII	92.00 (51.75–109.8)	284.5 (163.8–303.3)	366.5 (42.50–377.3)	ns,ns,ns
VWF	154.5 (139.0–178.8)	404.5 (331.8–616.3)	491.5 (412.0–609.0)	*,**,ns

TABLE 1 Pathogen reduction of cryoprecipitate products does not decrease factor levels

Note: Fibrinogen (FGN), factor VIII (FVIII), factor XIII (FXIII), and von Willebrand factor (VWF) levels in thawed (PR-Cryo FC and Cryo) or reconstituted (FibCon) products. Data are reported as median (interquartile range [IQR; Quartile 1–Quartile 3]) and compared by Kruskal–Wallis analysis with Dunn's post hoc test to determine *p* values (n = 6 per treatment group). *p* values (ns $p > .05$; $^{*}p < .05$; $^{**}p < .01$; and $^{***}p < .001$) are reported in the order of these comparisons: FibCon to Cryo, FibCon to PR-Cryo FC, and Cryo to PR-Cryo FC.

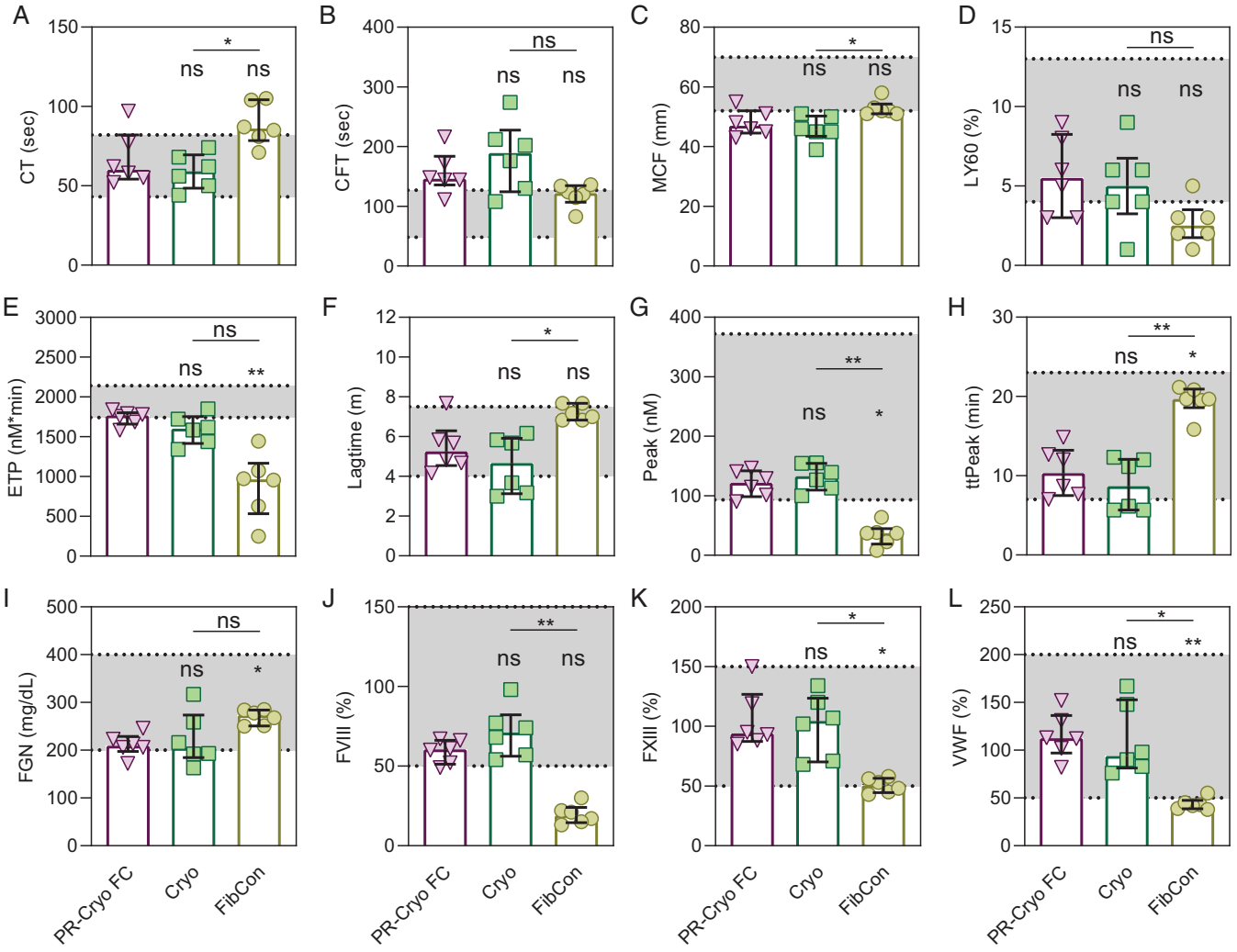

FIGURE 1 PR-Cryo FC and Cryo have improved thrombin generation compared to FibCon during hemostatic rescue of induced dilutional coagulopathy. Hemostatic adjuncts (fibrinogen concentrates, FibCon; cryoprecipitate, Cryo; and psoralen-treated PR-cryoprecipitated fibrinogen complex, PR-Cryo FC) were added to the induced dilutional coagulopathy (dWB), and the following measurements were made: (A) clotting time (CT, seconds), (B) clot formation time (CFT, seconds), (C) maximal clot firmness (MCF, mm), (D) percent lysis at 60 min (LY60, %), (E) endogenous thrombin potential (ETP, nM*min), (F) lag time (min), (G) peak (nM), (H) time to peak (ttPeak, min), (I) fibrinogen (FGN, mg/dl), (J) factor VIII (FVIII, %), (K) factor XIII (FXIII, %), and (L) von Willebrand factor (VWF). Data are represented as individual points and with median ± interquartile range (IQR), and Kruskal–Wallis analysis between groups determine *p* values (n = 6 per treatment group); ns, $p > .05$; *, $p < .05$; and **, $p < .01$. Significance values directly above each column are comparing the given column to PR-cryo FC, whereas bars above given columns indicate the comparison being made. Dotted lines bound the whole blood reference ranges

At the time of assay, PPP was thawed at 37°C for 10 min, then assayed for FGN (STA FIBRINOGEN 5), FVIII (STA Deficient VIII), FXIII (K-assay FXIII), and VWF (STA LIATEST VWF:AG). All additional Stago reagents were used as indicated by package inserts in the associated tests.

2.5 | Statistical analyses

Data were analyzed using GraphPad Prism software (version 9.0.0 for Windows; GraphPad Software, La Jolla, CA, United States, www.graphpad.com) and R 3.4.3 (2017-11-30, Vienna, Austria). Data are reported as median (interquartile range [IQR]) and represented as individual data points, with a bar at the median value and error bars denoting IQR. Comparison between adjuncts used to rescue coagulopathies was performed by one-way analysis of variance (ANOVA, Kruskal–Wallis test), and if the p value was significant ($p < .05$), then Dunn's multiple comparison test was performed to compare adjuncts to one another. Comparison of a given variable over the course of storage in a single study product group was performed by one-way ANOVA (Kruskal–Wallis test), and if the p value was significant ($p < .05$), then Dunn's multiple comparison test was performed to compare values on different storage days to one another. Mann–Whitney tests were used to compare predefined storage days of one study product to predefined storage days of another adjunct (e.g., PR-Cryo FC Day 10 to FibCon Day 0).

3 | RESULTS

3.1 | Pathogen reduction does not alter coagulation factor levels in cryoprecipitate

PR-Cryo FC had similar levels of FGN, FVIII, FXIII, and VWF compared to Cryo (Table 1), suggesting that PR treatment does not alter the baseline levels of these important coagulation factors. Notably, while FGN levels were similar across all three hemostatic adjuncts (Table 1), FibCon contained at least a third of the FVIII, FXIII, and VWF compared to PR-Cryo FC (Table 1).

3.2 | Pathogen-reduced cryoprecipitate provides similar hemostatic function to cryoprecipitate in both dilutional and lytic coagulopathies

At Day 0, PR-Cryo FC, Cryo, and FibCon all rescued dWB to the same degree, as the CTs (Figure 1(A)), CFTs (Figure 1(B)), MCF (Figure 1(C)), and lysis (LY60, Figure 1(D)) were not significant from each other. ETP was similar in both PR-Cryo FC:dWB and Cryo:dWB (Figure 1(E)). However, ETP was reduced by 49% when FibCon was used to resuscitate dWB compared to PR-Cryo FC (Figure 1(E)). Lag time was similar no matter the adjunct used (Figure 1(F)), whereas FibCon:dWB resulted in peak thrombin levels almost a third of that generated in PR-Cryo FC:dWB or Cryo:dWB (Figure 1 (G)), and the time to peak thrombin generation was significantly delayed (2-fold) in FibCon:dWB compared to PR-Cryo FC:dWB or Cryo:dWB (Figure 1(H)). FGN levels were slightly yet significantly elevated in FibCon: dWB compared to PR-Cryo FC:dWB (Figure 1(I)). While not statistically different, there was a 3-fold reduction in FVIII levels in FibCon:dWB compared to PR-Cryo FC: dWB (Figure 1(J)). Importantly, FibCon use failed to bring FVIII levels back to normal healthy WB levels, whereas PR-Cryo FC and Cryo both resuscitated dWB back to healthy WB levels. FibCon:dWB also resulted in decreased FXIII (Figure 1(K)) and VWF (Figure 1(L)) levels when compared to PR-Cryo FC:dWB or Cryo:dWB.

At Day 0, PR-Cryo FC, Cryo, and FibCon all rescued lytic coagulopathy in a comparable manner (Figure 2). CT, CFT, MCF, and LY60 (Figure 2(A)–(D)) of study product:lyWB mixtures were similar between study groups. ETP (Figure 2(E)) and lag time (Figure 2(F)) were approximately the same in PR-Cryo FC:lyWB, Cryo:lyWB, and FibCon:lyWB. While peak thrombin generation and time to peak thrombin generation in resuscitated lyWB were similar to the dWB profile, these trends of reduced peak and delayed time to peak in FibCon:lyWB were not significant from PR-Cryo FC: lyWB (Figure 2(G),(H)). There was again an increase in FGN levels in FibCon:lyWB compared to PR-Cryo FC: lyWB (Figure 2(I)), although not significant. Similar to the dWB model, there was a 2-fold reduction in FVIII in FibCon:lyWB compared with PR-Cryo FC:lyWB, although this was not significant (Figure 2(J)). FXIII levels were reduced in FibCon:lyWB compared with PR-Cryo FC:lyWB, but FXIII levels in both treatment groups were well above normal healthy WB levels (Figure 2(K)). Lastly, VWF levels were increased by 2-fold in PR-Cryo FC:lyWB compared to FibCon:lyWB (Figure 2(L)).

3.3 | Long-term storage of pathogen-reduced cryoprecipitate does not decrease hemostatic function

Storage of PR-Cryo FC at 20–24°C out to 10 days had no effect on the ability of PR-Cryo FC to serve as an

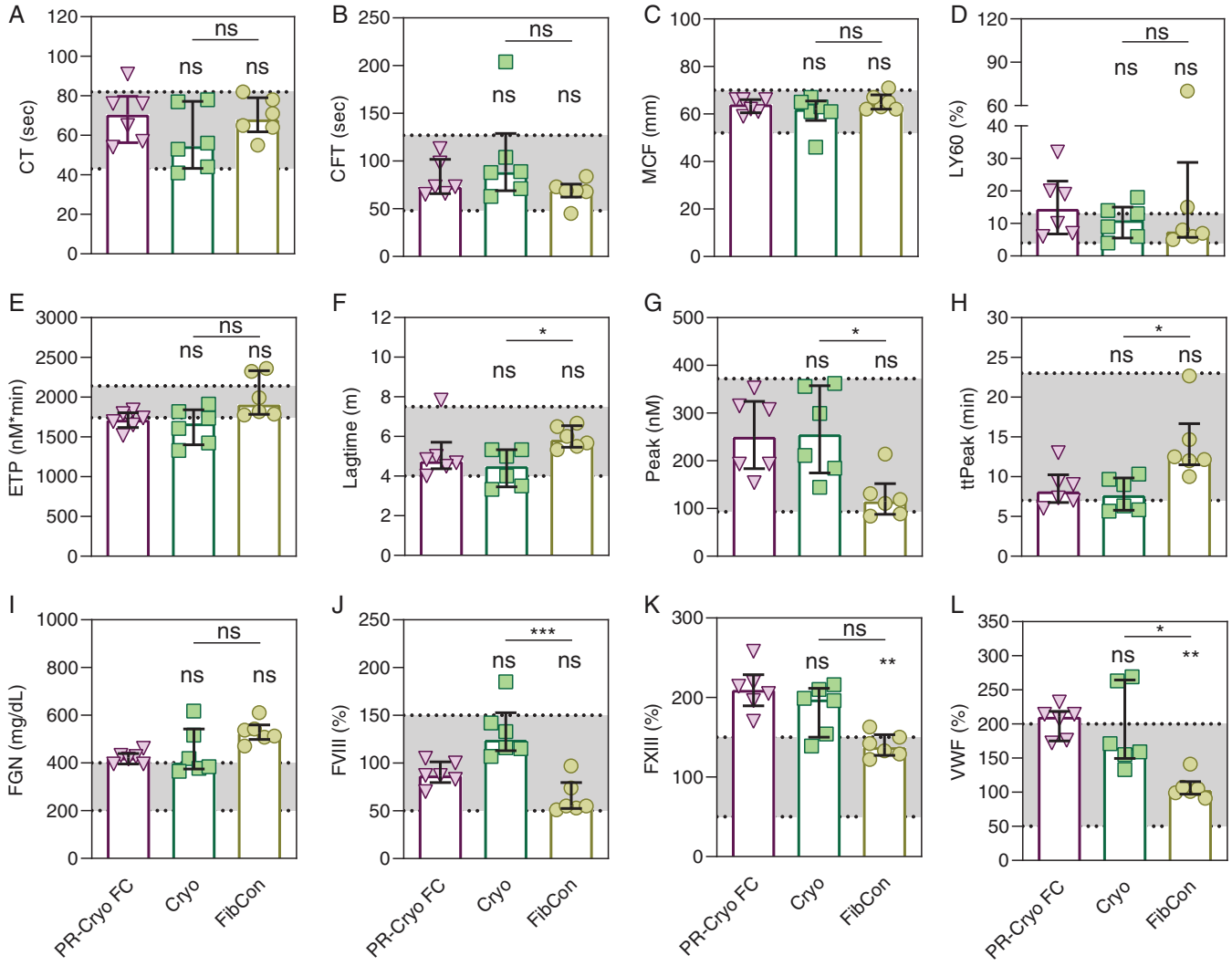

FIGURE 2 PR-Cryo FC and Cryo provide similar hemostatic rescue of induced lytic coagulopathy compared to FibCon. Hemostatic adjuncts (fibrinogen concentrates, FibCon; cryoprecipitate, Cryo; and psoralen-treated PR-cryoprecipitated fibrinogen complex, PR-cryo FC) were added to induced lytic coagulopathy (lyWB), and the following measurements were made: (A) clotting time (CT, seconds), (B) clot formation time (CFT, seconds), (C) maximal clot firmness (MCF, mm), (D) percent lysis at 60 min (LY60, %), (E) endogenous thrombin potential (ETP, nM*min), (F) lag time (min), (G) peak (nM), (H) time to peak (ttPeak, min), (I) fibrinogen (FGN, mg/dl), (J) factor VIII (FVIII, %), (K) factor XIII (FXIII, %), and (L) von Willebrand factor (VWF). Data are represented as individual points and with median ± interquartile range (IQR), and Kruskal–Wallis analysis between groups determine p values (n = 6 per treatment group); ns, $p > .05$; *, $p < .05$; **, $p < .01$; and ***, $p < .001$. Significance values directly above each column are comparing the given column to PR-Cryo FC, whereas bars above given columns indicate the comparison being made. Dotted lines bound the whole blood reference ranges

effective hemostatic adjunct for both dilutional and lytic coagulopathies (Figure 3). While there was a consistent 30% reduction in CFT when stored (D2-10) PR-Cryo FC rather than fresh (D0) PR-Cryo FC was used to rescue dWB, the reduced CFT was still greater than that of healthy WB (Figure 3(B)). In addition, rescue of lyWB with stored PR-Cryo FC led to increases in FGN, FXIII, and VWF that were beyond the upper limit of the reference ranges (Figure 3(F), (H), (I)).

3.4 | Stored pathogen-reduced cryoprecipitate improves clotting initiation and thrombin generation in dilutional coagulopathy compared to fibrinogen concentrates

Lastly, we compared hemostatic function between coagulopathies rescued by D5 or D10 PR-Cryo FC versus either D0 Cryo or D0 FibCon (Table 2). FVIII, FXIII, VWF, thrombin generation (ETP, peak, and time to

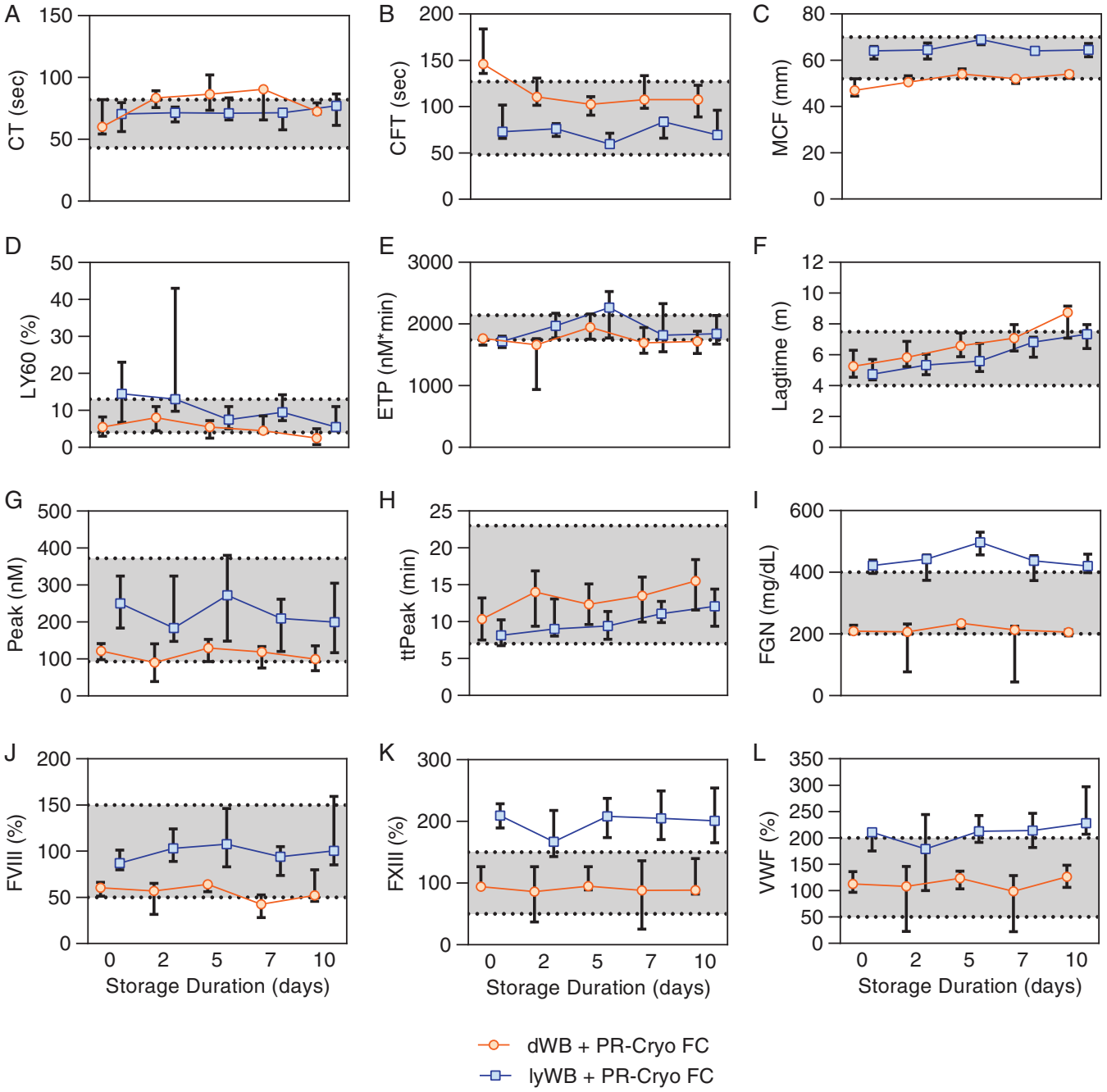

FIGURE 3 PR-Cryo FC stored out to 10 days does not significantly affect hemostatic rescue of dilutional or lytic coagulopathies. PR-Cryo FC stored out to 10 days was added to dilutional (dWB) or lytic (lyWB) coagulopathies, and the following measurements were made: (A) clotting time (CT, seconds), (B) clot formation time (CFT, seconds), (C) maximal clot firmness (MCF, mm), (D) percent lysis at 60 min (LY60, %), (E) endogenous thrombin potential (ETP, nM*min), (F) fibrinogen (FGN, mg/dl), (G) factor VIII (FVIII, %), (H) factor XIII (FXIII, %), and (I) von Willebrand factor (VWF). Data are represented as summary data points and with median ± interquartile range (IQR); n = 6 per group

peak), and CT were all significantly improved in D10 PR-Cryo FC:dWB when compared to FibCon:dWB. CT and thrombin generation parameters (lag time and time to peak) were elongated, and CFT and MCF reduced in D10 PR-Cryo FC:dWB compared to D0 Cryo:dWB. There were no significant differences in functional hemostatic parameters between D10 PR-Cryo FC:lyWB and D0 FibCon:lyWB, while factor levels in FibCon:lyWB remained significantly lower than the levels in both D5 and D10 PR-Cryo FC:lyWB. There was little difference

TABLE 2 Comparison of D5 or D10 PR-Cryo FC to D0 Cryo or D0 FibCon in all assays

Variable	FibCon D0	Cryo D0	PR-Cryo FC D5	PR-Cryo FC D10	*p* values
dWB					
ExTEM CT (sec)	86.0 (78.5–104.3)	59.0 (48.5–69.5)	86.5 (73.5–102.0)	72.5 (70.8–79.5)	‡‡,#,$
ExTEM CFT (sec)	122.0 (107.0–134.5)	189 (124.5–227.5)	102.5 (90.8–110.8)	107.5 (89.0–123.0)	‡‡,$
ExTEM MCF (mm)	52.0 (51.0–54.3)	45.5 (43.5–50.3)	54.0 (52.3–56.3)	54.0 (52.3–55.0)	‡‡,$$
ExTEM LY60 (%)	2.5 (1.8–3.5)	5.0 (3.3–6.8)	5.5 (2.5–7.3)	2.5 (0.8–5.0)	
TG ETP (nM*min)	964.4 (532.6–1166)	1601 (1414–1753)	1947 (1754–2162)	1720 (1521–1883)	**,‡,##
TG Lagtime (min)	7.09 (6.83–7.67)	4.67 (3.13–5.92)	6.59 (5.88–7.42)	8.75 (7.08–9.17)	‡,$$
TG Peak (nM)	37.2 (19.0–44.6)	132.9 (109.6–154.6)	129.5 (93.2–153.2)	99.4 (68.6–135.9)	**,##
TG ttPeak (min)	19.7 (18.6–20.9)	8.7 (5.7–12.1)	12.3 (9.6–15.1)	15.5 (11.6–18.4)	**,$
FGN (mg/dl)	273.0 (250.8–284.3)	204.5 (184.8–273.5)	235.0 (217.8–240.3)	206.0 (193.8–213.0)	**,##,¶
FVIII (%)	19.0 (14.5–24.0)	71.00 (56.3–82.3)	64.0 (56.0–68.5)	52.0 (45.8–79.8)	**,##
FXIII (%)	50.5 (44.5–56.5)	104.5 (70.3–123.5)	95.0 (89.0–126.5)	88.5 (82.3–139.8)	**,##
VWF (%)	42.5 (38.8–47.5)	94.0 (81.3–152.8)	123.5 (103.3–137.0)	126.0 (106.3–148.0)	**,##
lyWB					
ExTEM CT (sec)	68.0 (61.8–79.0)	54.5 (43.3–77.3)	71.0 (65.8–83.5)	77.0 (61.3–86.8)	
ExTEM CFT (sec)	69.0 (62.3–75.8)	88.5 (69.0–129.0)	59.5 (55.5–71.3)	69.5 (65.8–96.0)	‡
ExTEM MCF (mm)	64.0 (62.0–68.0)	62.0 (57.3–65.5)	69.0 (66.8–70.0)	64.5 (61.5–67.3)	‡‡,¶
ExTEM LY60 (%)	7.5 (5.8–28.8)	11.0 (5.5–15.0)	7.5 (5.0–11.0)	5.5 (4.5–11.0)	
TG ETP (nM*min)	1906 (1783–2333)	1671 (1401–1842)	2267 (1775–2528)	1841 (1674–2137)	‡
TG Lagtime (min)	5.84 (5.46–6.54)	4.50 (3.46–5.33)	5.59 (4.92–6.75)	7.34 (6.42–7.96)	$$
TG Peak (nM)	114.8 (87.6–152.1)	255.3 (174.4–357.6)	272.0 (148.1–380.7)	199.8 (116.7–304.8)	**
TG ttPeak (min)	12.3 (11.5–16.7)	7.7 (5.8–9.8)	9.4 (7.6–11.4)	12.1 (9.4–14.4)	$
FGN (mg/dl)	525.5 (498.8–559.3)	401.5 (374.8–542.3)	497.0 (456.0–529.8)	420.0 (399.0–458.5)	#
FVIII (%)	55.0 (52.5–79.8)	124.5 (113.0–152.8)	107.5 (83.0–146.3)	100.5 (85.3–159.3)	*,#
FXIII (%)	137.5 (127.3–153.3)	197.5 (150.3–211.5)	208.0 (173.8–237.5)	201.0 (165.3–254.3)	**,##
VWF (%)	103.0 (97.0–115.5)	165.0 (149.5–264.5)	212.5 (191.5–242.5)	228.0 (207.0–297.3)	**,##

Note: Values reported as median (interquartile range [IQR; Quartile 1–Quartile 3]). Mann–Whitney tests comparing rank sums for comparing FibCon to D5 PR-Cryo FC ($^{*}p < .05$; $^{**}p < .01$), FibCon to D10 PR-Cryo FC ($^{\#}p < .05$; $^{\#\#}p < .01$), D0 Cryo to D5 PR-Cryo FC ($^{\ddagger}p < .05$; $^{\ddagger\ddagger}p < .01$), D0 Cryo to D10 PR-Cryo FC ($^{\$}p < .05$; $^{\$\$}p < .01$), or D5 PR-Cryo FC to D10 PR-Cryo FC ($^{\P}p < .05$).

between D5 and D10 PR-Cryo FC in rescue of both coagulopathies. FGN levels were slightly elevated in D5 PR-Cryo FC:dWB compared to D10 PR-Cryo FC:dWB, and while the difference in MCF between D5 PR-Cryo FC:lyWB and D10 PR-Cryo FC:lyWB was significant, the absolute difference was only 2.5 mm and both MCF values remained above 60 mm.

To identify if hemostatic adjunct type correlated with any particular hemostatic profile of rescued coagulopathies, we performed Pearson's correlations for all reported parameters and adjuncts on D0 in both dilutional (Figure 4(A)) and lytic coagulopathies (Figure 4(B)). We found the variable "adjunct" was strongly and significantly correlated to factor levels and thrombin generation in dilutional coagulopathy rescue (Figure 4(A), (C)) but not as strongly in lytic coagulopathy rescue (Figure 4(B),(D)). Specifically, CT, lag time, and time to peak thrombin generation all positively and significantly correlated with study product in the dilutional coagulopathy (Figure 4(A),(C)), but less so in the lytic coagulopathy (Figure 4(B),(D)). Factor levels were more significantly associated with thrombin generation in the dilutional coagulopathy (dark pink lower right quadrant, Figure 4(C)) compared to the lytic coagulopathy (Figure 4(D)).

4 | DISCUSSION

This study is unique in a few ways: (i) the hemostatic efficacy of PR-Cryo FC was compared to both Cryo and FibCon within the same set of experiments;

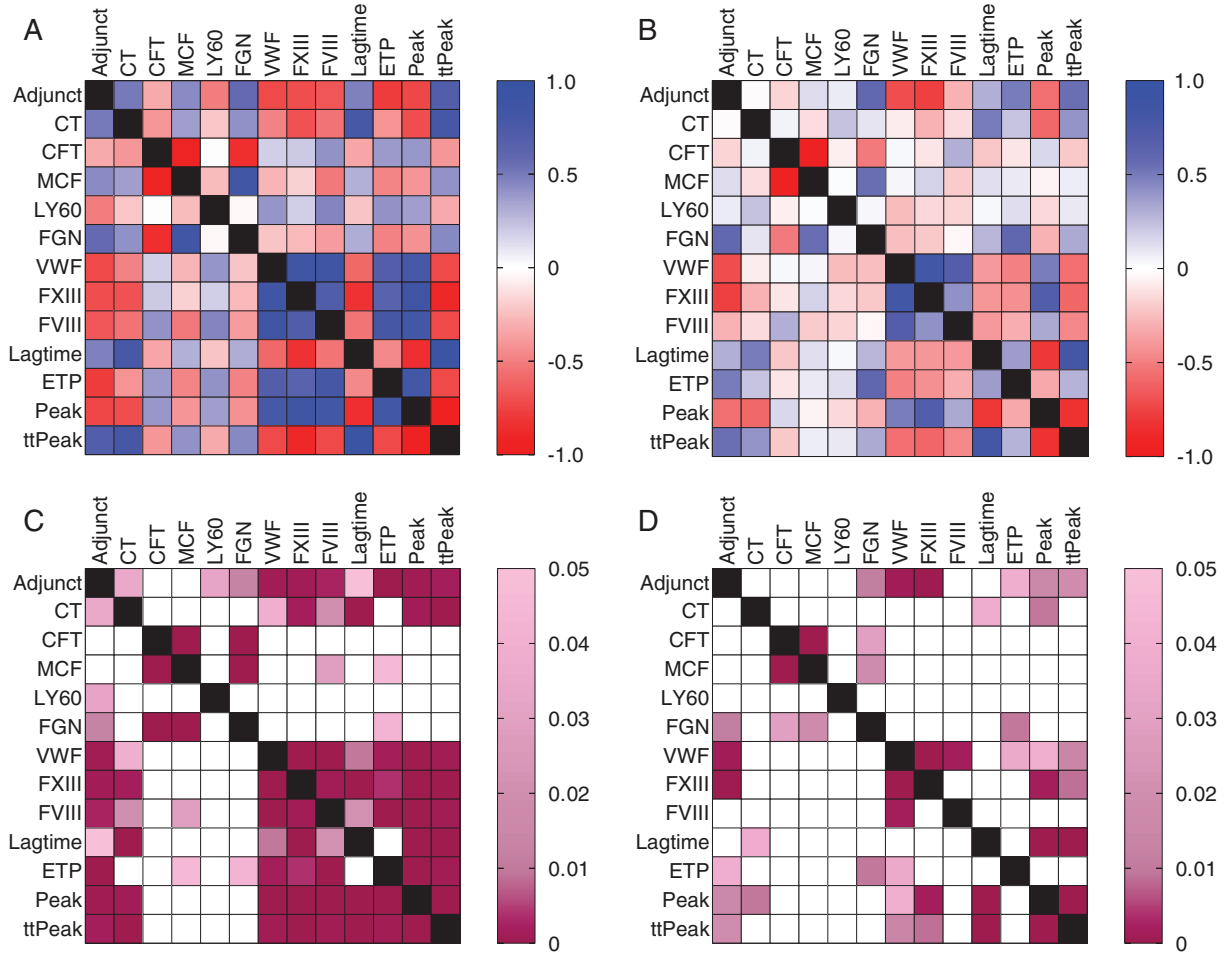

FIGURE 4 Correlation of hemostatic parameters in adjunct rescued coagulopathies. Parameters: adjunct (PR-Cryo FC D0, Cryo D0, and FibCon D0), clotting time (CT, seconds), clot formation time (CFT, seconds), maximal clot firmness (MCF, mm), percent lysis at 60 min (LY60, %), endogenous thrombin potential (ETP, nM*min), fibrinogen (FGN, mg/dl), factor VIII (FVIII, %), factor XIII (FXIII, %), and von Willebrand factor (VWF). (A) Matrix of Pearson's correlation coefficients (−1.0 to 1.0) between given parameters in dilutional coagulopathies rescued by D0 adjuncts. (B) Matrix of Pearson's correlation coefficients (−1.0 to 1.0) between given parameters in lytic coagulopathies rescued by D0 adjuncts. (C) Matrix of p-values (0.0–0.05) associated with Pearson's correlation coefficients between given parameters in dilutional coagulopathies rescued by D0 adjuncts. White squares represent nonsignificant Pearson's correlations. (D) Matrix of p-values (0.0–0.05) associated with Pearson's correlation coefficients between given parameters in lytic coagulopathies rescued by D0 adjuncts. White squares represent nonsignificant Pearson's correlations

(ii) cryoprecipitate products were analyzed out to 10 days of storage at room temperature; (iii) we measured PR-Cryo FC efficacy in rescuing in vitro induced lytic and dilutional coagulopathies; and (iv) we incorporated the use of thrombin generation capacity as a functional measure of hemostasis for both dilutional and lytic coagulopathies. Our results indicate as expected that PR-Cryo FC and Cryo provided more VWF, FVIII, and FXIII upon mixture with dilutional or lytic coagulopathies compared to FibCon. This was maintained throughout storage as D10 PR-Cryo FC dilutional and lytic coagulopathic mixtures had significantly increased levels of VWF, FVIII, and FXIII compared to FibCon:dWB or FibCon:lyWB coagulopathic mixtures, but not Cryo:dWB or Cryo:lyWB coagulopathic mixtures. Notably, the dilutional coagulopathy provided an optimal platform to assess the hemostatic function of study products compared to the lytic coagulopathy due to the inherently lower concentrations of coagulation factors in saline-diluted WB. This was recapitulated in our correlation analyses, which revealed significant relationships between factor levels and thrombin generation in dilutional but not lytic coagulopathies. These data highlight that in settings where multiple factors may need to be restored, such as dilutional coagulopathy, that cryoprecipitate-based products may serve as a better hemostatic adjunct than fibrinogen concentrates.

Our data show that the additional FXIII available in cryoprecipitate products did not greatly improve hemostatic rescue in the lytic coagulopathy. The lytic model

was created to target 10%–15% lysis (via ROTEM) using exogenous tPA; the quantity of FXIII in the cryoprecipitate products may not have been enough to overcome the effects of 75 ng/ml tPA. Future studies evaluating cryoprecipitate product dosage in conjunction with various tPA concentrations may identify a key range of lysis where standard cryoprecipitate dosing would be beneficial.

This study is the first to measure thrombin generation induced by PR-Cryo FC rescue of WB-based coagulopathy models, as Kamyszek et al. demonstrated the intrinsic ability of PR-Cryo FC to promote thrombin generation (adjunct alone).[29] We found thrombin generation was greater when PR-Cryo FC or Cryo were mixed into dilutional coagulopathy, compared to FibCon, irrespective of storage duration. However, this difference was not apparent in study product:lytic coagulopathic mixtures. In both coagulopathies, there were no differences in thrombin generation between PR-Cryo FC and Cryo, suggesting both products support similar hemostatic capacity with respect to thrombin generation. These data support the theory that cryoprecipitate products provide a more efficacious hemostatic resuscitation than fibrinogen concentrates alone via the ability to promote thrombin generation.[19,23]

Both PR-Cryo FC:dWB and Cryo:dWB had reduced time to initial fibrin formation (CT) compared to FibCon:dWB, reaffirming that additional factors available in Cryo products are important for clot initiation.[28] There were no significant differences between additional ROTEM variables in PR-Cryo FC and Cryo dilutional coagulopathic mixtures, suggesting that PR has minimal effect on clot formation, as seen before.[28] These data demonstrate that supplementation with factors upstream of fibrinogen will increase the kinetics of clot formation, but if FGN concentration is similar between adjuncts, then terminal clot formation will be relatively equivalent.

Our study was designed in a reductionist manner to understand the relative contributions of each adjunct to both dilutional and lytic coagulopathies. Hemostatic adjuncts are rarely transfused alone when treating hemorrhaging patients; as such, future work assessing hemostatic adjunct efficacy in combination with other transfused products such as packed red blood cells and/or platelets will help to inform on the efficacy of hemostatic adjuncts. Specifically, our data demonstrating enhanced thrombin generation upon resuscitation with cryoprecipitate products suggest a potential for additional augmented thrombin generation. This may be beneficial for patients who have a dilutional or consumptive coagulopathy where the concentration of thrombin is potentially lower than required for hemostasis. In addition, we used PRP derived from adjunct-resuscitated coagulopathies to measure the maximal ETP upon resuscitation—to include adjunct interaction with platelets. To measure the thrombin potential associated with adjuncts alone in absence of platelets, one would need to measure thrombin generation in PPP derived from the adjunct-resuscitated coagulopathies. Our study did not assess hemostatic rescue by PR-Cryo FC, Cryo, and FibCon under physiologically relevant flow conditions. Flow regimes are a significant component of clot formation in vivo[32]; therefore, future studies of hemostatic adjunct efficacy should incorporate flow, such as the use of microfluidic models of bleeding. Logistical constraints of our study (product storage times, WB volumes required, and assay/workflow throughput) limited the number of each product tested (n = 6 for each group) and hindered us from using the same WB donors for the duration of each storage period, leading to large amounts of variability in data generated from resuscitated samples. Lastly and unfortunately, there is a lack of established relevance of these in vitro functions in context of patient outcomes. How differences we identify here can be extrapolated in vivo in actively bleeding patients with complex coagulopathies remains to be determined.

Providing fibrinogen to traumatically bleeding patients is clearly beneficial, yet it is important to recognize that fibrinogen supplementation alone may not be effective if upstream pathways regulating fibrinogen use are also dysregulated due to trauma-associated coagulopathies. Our results demonstrate that cryoprecipitate products provide an advantage with respect to reducing the time to clot formation compared to fibrinogen concentrates and may be more helpful in cases of iatrogenic dilutional coagulopathy. Moreover, PR-Cryo FC is hemostatically stable over 10 days of storage at 20–24°C, and D10-stored PR-Cryo FC provides similar hemostatic function compared to FibCon. The ability to increase the post-thaw storage duration to 10 days at room temperature without risk of bacterial growth would allow for PR-Cryo FC products to be immediately available for use and provide a product that may be superior for patients with a dilutional coagulopathy. This has great potential to improve outcomes in patients with severe traumatic bleeding as immediate access to a hemostatic agent is essential for resuscitation, and every single minute delay in providing any blood product to a severely bleeding trauma patient increases mortality by 5%.[33] As FibCon reconstitution time is approximately 15 min, the immediate availability and use of PR-Cryo FC or Cryo products could have a significant effect on outcomes in traumatically injured patients with severe bleeding.

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CONFLICT OF INTEREST

Dr. Spinella is on the Board of Directors of the THOR Network Foundation and is a consultant for Secure Transfusion Services.

AUTHOR CONTRIBUTIONS

KAT and SMS designed and performed the study, analyzed data, prepared figures, and drafted the first version of the manuscript. PCS designed the study, analyzed data, edited the manuscript, and provided funding. All authors critically contributed to finalization of the article.

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SUPPORTING INFORMATION

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