# Single-donor spray-dried plasma

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**BACKGROUND:** Dried plasma is logistically superior for hemostasis management because it can be transported and stored under nonfrozen conditions and quickly reconstituted at the point of care, enabling prehospital administration. Velico Medical has developed a spray-drying system to be integrated into routine blood center work streams for spray drying single donor plasma units. This study compared the quality of the spray-dried plasma (on-demand plasma [ODP]) with fresh frozen plasma (FFP).

**STUDY DESIGN AND METHODS:** ODP units (n = 60) were manufactured from never frozen fresh plasma, which was pretreated with glycine–hydrochloric acid and stored at 1to 6°C. Paired aliquots were frozen and stored at −18°C or less. After 31 to 33 days, ODP samples were reconstituted with water for injection and comprehensively characterized in parallel with paired FFP. The quantities of plasma dried and rehydration fluid were predetermined, ensuring comparable total protein concentration in ODP and paired FFP.

**RESULTS:** ODP is comparable to FFP in global coagulation function as assessed by activated partial thromboplastin time and prothrombin time and in clot formation evaluated by thrombelastography. Compared to FFP, ODP had greater than 80% levels of functional coagulation factors and related proteins and chemistry analytes except for Factor XIII (74%). Pretreatment mitigated cleavage of high-molecular-weight von Willebrand factor multimers by spray drying and resulted in 60% vWF:ristocetin cofactor activity in ODP compared to FFP.

**CONCLUSIONS:** ODP demonstrates coagulation function comparable to that of FFP. The spray drying system can be implemented in blood centers and is capable of producing units of ODP.

Hemostasis involves the interplay of a cellular constituent, platelet aggregation, and platelet plug formation referred to as primary hemostasis, and a protein constituent, the deposition of insoluble fibrin by the coagulation cascade, that is, secondary hemostasis.[1] Plasma contains all proteins involved in secondary hemostasis and the essential von Willebrand factor (vWF) for initiating primary hemostasis and stabilizing Factor (F) VIII in secondary hemostasis. Rapid replenishment of plasma loss is critical in the acute phase of trauma/hemorrhage under damage control resuscitation practice.[2–5] Currently, fresh frozen plasma (FFP) and plasma frozen within 24 hours are the most commonly used plasma products. However, it is impractical to use frozen plasma in certain environments including prehospital, mass casualty, and battlefield applications, as it requires complex frozen product logistics, prethawing, and delivery to the point of care.[6]

Plasma in a dry state can be stored under nonfrozen conditions, carried by first responders, and rapidly reconstituted at the point of care.[7] Freeze-dried plasma products have been used on a limited basis in Europe and South Africa for decades but have not been commercialized in the United States.[8–11] As an alternative to freeze-drying technology, spray drying is an established industrial

**ABBREVIATIONS:** aPTT = activated partial thromboplastin time; ELISA = enzyme-linked immunosorbent assay; F = factor; FFP = fresh frozen plasma; HCl = hydrochloric acid; HMW = high-molecular-weight; NFP = never-frozen plasma; ODP = on-demand plasma; PT = prothrombin time; RCo = ristocetin cofactor; TEG = thrombelastography; TT = thrombin time; vWF = von Willebrand factor

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processing technology commonly used in the food and pharmaceutical industries. However, it often requires extensive process optimization to avoid heat damage and loss of functionality of the product. This technology poses multifactorial challenges when applied to human plasma; a complex system requiring the retention of multiple functional proteins, especially the shear-sensitive vWF multimers, minimal zymogen activation, and protein aggregation; and an acceptable level of moisture content for extended storage. A pooled, solvent/detergent-treated, spray-dried plasma is under development.[7] The limited data on this product do not provide a complete picture of the impact of the drying process on the quality of the plasma. Nevertheless, spray-dried plasma appears to maintain thrombin generation potential[12] and is effective in modulating endotheliopathy and correcting coagulopathy in animal models.[7,12–14]

Feasibility studies on single-donor spray-dried plasma were previously reported.[15] We have advanced the state of the art of plasma spray-drying technology through formulation, which is critical for vWF recovery, and process optimization, and those results are described in this report. Partial characterizations of the development lots have been described.[7] The spray-drying system (FrontlineODP, Velico) consists of a spray-drying instrument and single-use disposables to facilitate the drying process. The system is designed to be integrated into routine blood center component laboratory workstreams for spray drying human plasma as an alternative to freezing. The purpose of this study was to comprehensively evaluate the quality of spray-dried plasma (on-demand plasma [ODP]) compared to FFP produced at a regional blood center.

## MATERIALS AND METHODS

### Study design and logistics

The comparator for ODP in the study was paired FFP. Prior to the study, we established the suitability of using a small aliquot of FFP in lieu of a typically sized FFP unit by conducting a two-arm study comparing the FFP stored as 4.5-mL aliquots in cryovials with paired standard-volume FFP units (data not shown). We used FFP aliquots in place of full FFP units as FFP controls, greatly simplifying the logistics and reducing the study cost and plasma waste. A prototype spray dryer was installed at MEDIC Regional Blood Center (Knoxville, TN) by Velico staff. Never-frozen plasma (NFP) was prepared by blood center staff according to their standard protocol for preparation of FFP. ODP units and paired FFP aliquots were prepared by Velico staff and shipped to Velico for storage. All assays/tests using commercial reagents/kits/platforms were performed on paired ODPs and FFP aliquots at Velico following the corresponding manufacturer's instructions. Additional ODP units were manufactured for the determination of moisture content and supplemental tests as indicated.

### Plasma collection, manufacture, and storage

Citrate-phosphate-dextrose whole blood was collected and processed to NFP. One FFP aliquot (4.5 mL) was prepared from each NFP, and 295 g of the remaining NFP was pretreated with glycine–hydrochloric acid (HCl) stock solution (1.4 M glycine +0.4 M HCl, 20×), and spray dried to a unit of ODP. ODPs (n = 60) and paired FFPs were stored, at 1 to 6°C and −18°C or less, respectively, prior to shipment to Velico for further storage. The amounts of plasma dried and water for injection for rehydration (190 mL) were predetermined to account for manufacturing losses and to yield a comparable total protein concentration in rehydrated ODP and its paired FFP, thereby ensuring comparability between ODP and FFP for various characterizations.

### Moisture determination and sample preparation for testing

Moisture content measurement was performed using the Karl Fischer titration method on a titrator (Mettler Toledo V30, Mettler Toledo AG, Analytical) per manufacturer's instruction. An ODP unit was rehydrated with 190 mL of water for injection, and a paired FFP control was thawed for 5 minutes in a 37°C water bath.

### Chemistry measurements

pH was measured using a pH meter (Accumet Basic Benchtop Meter, Fisher Scientific), and osmolality by freezing point depression using an osmometer (Model 3900 Osmometer, Advanced Instruments). Total protein and chemistry analytes were measured using a chemistry analyzer (Selectra ProM, ELITechGroup). Citrate concentration was determined using an enzymatic method (EnzyChrom Citrate Assay Kit, BioAssay Systems).

### Global coagulation tests, coagulation, and related factors

The following assays were performed on a fully automated coagulation analyzer (ACL TOP 700, Instrumentation Laboratory) using the reagents supplied by the manufacturer: activated partial thromboplastin time (aPTT; HemosIL SynthASil), prothrombin time (PT; RecombiPlasTin 2G reagent), thrombin time (TT; HemosIL Thrombin Time), factor assays based on modified aPTT (VIII, IX, XI, and XII) or modified PT (prothrombin, V, VII, and X) using corresponding factor-deficient plasma (HemosIL Factor Deficient Plasma), fibrinogen (HemosIL Q.F.A. Thrombin [Bovine]), and protein S (HemosIL Protein S Activity) by a photo-optical clot detection method; plasminogen (HemosIL Plasminogen), antithrombin III (HemosIL Liquid Antithrombin Kit), protein C (HemosIL Protein C), and plasmin inhibitor (HemosIL Plasmin Inhibitor) by a photometric method; and free protein S (HemosIL Free Protein S Kit) by latex-based immunoassay. FXIII activity (Berichrom FXIII, Siemens Healthcare) was performed on a coagulation analyzer (BCS XP, Siemens) and C1-esterase

inhibitor assay (TECHNOCHROM C1-INH kit, Technoclone) on the Instrumentation Laboratory coagulation analyzer, both by photometric method. Alpha-1 proteinase inhibitor by immunoturbidimetric assay (Alpha-1 AT kit, Kamiya Biomedical Company) was implemented on a chemistry analyzer (Selectra ProM, EliTech). FV, FVII, and FVIII antigens were measured by enzyme-linked immunosorbent assay (ELISA) (ZYMUTEST Factor V ELISA kit, HYPHEN BioMed; VisuLize FVII and FVIII Antigen Kits, Affinity Biologicals).

### Coagulation and complement activation markers

Prothrombin fragment 1 + 2 (Fragment 1 + 2) and thrombin-antithrombin complex (Enzygnost F1.2 and TAT, Siemens), and complement fragments C3a and C5a (Microvue C3a des-Arg and C5a des-Arg Plus, Quidel) were determined by ELISA, and D-dimer (Innovance D-Dimer, Siemens) by a latex-based immunoassay on the Siemens coagulation analyzer.

### vWF and ADAMTS13

VWF:ristocetin cofactor activity (vWF:RCo; BC von Willebrand Reagent, Siemens) by measuring agglutination of fixed platelets was performed on the Siemens coagulation analyzer, and vWF:antigen (HemosIL von Willebrand Factor Antigen, IL) by the latex immunoassay on the Instrumentation Laboratory coagulation analyzer. Quantitative vWF multimer analysis by LiDS-agarose (0.65%) electrophoresis and ADAMTS13 activity by the fluorescence resonance energy transfer[16] were conducted by Blood Center of Wisconsin (Milwaukee, WI).

### Thromboelastography

Kaolin-activated citrated plasma thromboelastography (TEG) analysis was performed using a hemostasis analyzer (TEG 5000 Thrombelastograph, Haemonetics).

### Statistical analysis

The differences in the measured values of ODP and FFP were assessed by performing a two-tailed paired t test using computer software (GraphPad Prism 4, GraphPad Software) calculating standard 90% confidence intervals of the ratio of ODP/FFP using log-transformed data. Comparability was concluded for a 90% confidence interval between 0.8 and 1.25 (i.e., 80%-125% of the control).[17] For pH, osmolality, coagulation and complement activation markers, statistical analysis was performed using the t test in Excel (Microsoft).

## RESULTS

### Manufacture, storage, moisture content, and reconstitution

ODP units (n = 60) and paired FFPs were manufactured and stored for 31 to 33 days. One FFP control was excluded because of a handling error, and its paired ODP was analyzed but excluded in the data analyses except for the reconstitution time. ODP was reconstituted in 3.65 $\pm$ 0.44 minutes (n = 60) in water for injection to a transparent solution, visually indistinguishable from thawed FFP. Additional units of ODP were manufactured for measuring residual moisture content and supplemental studies. The moisture content was 2.2% $\pm$ 0.2% (n = 2), within the range recommended for dry biological products (<1%-3%).[18]

### Total protein, chemistry analytes, pH, and osmolality

Total protein and chemistry analytes were analyzed and the results are given in Table 1. ODP was similar to FFP in total protein, allowing a direct comparison of ODP to FFP in other measurements. ODP was comparable to FFP in the levels of citrate, albumin, calcium, immunoglobulins, lipoproteins, and lipids (94%-105%). ODP was significantly different from FFP in pH (7.03 $\pm$ 0.11 vs. 7.62 $\pm$ 0.06, $p < 0.0001$) and osmolality (394 $\pm$ 19 vs. 309 $\pm$ 4 mOsm/kg, $p < 0.0001$) as a result of the pretreatment. The European Pharmacopeia specifies pH between 6.5 and 7.6 and osmolality >240 mOsm/kg for a plasma product.[19] ODP was similar to LyoPlas in pH (7–7.2)[10] and Octaplas in osmolality (360 mOsm/kg; specification: 320–420).[20]

### Coagulation and fibrinolytic factors/inhibitors, and protein inhibitors

Compared to FFP, ODP had greater than 80% levels of functional coagulation factors (fibrinogen, prothrombin, V, VII, VIII, IX, X, XI, and XII) and inhibitors (antithrombin III, protein C, and protein S), plasminogen, plasmin inhibitor, C1 esterase inhibitor, and alpha 1-proteinase inhibitor (Table 2). ODP was similar to FFP in the levels of protein S, FV, FVII, and FVIII evaluated by antigen assays. ODP had

**TABLE 1. Total protein, citrate, and chemistry analytes (n = 59)**

| Analyte/Test | ODP* | FFP* | Ratio† |
|---|---|---|---|
| Protein (g/dL) | 6.30 $\pm$ 0.35 | 6.13 $\pm$ 0.35 | 103% |
| Citrate (mM) | 27.3 $\pm$ 2.0 | 26.2 $\pm$ 1.8 | 102% |
| Albumin (g/dL) | 3.79 $\pm$ 0.25 | 3.76 $\pm$ 0.22 | 99% |
| Calcium (mg/dL) | 6.58 $\pm$ 0.35 | 6.45 $\pm$ 0.28 | 104% |
| IgA (mg/dL) | 187 $\pm$ 88 | 184 $\pm$ 87 | 102% |
| IgG (mg/dL) | 802 $\pm$ 148 | 783 $\pm$ 145 | 102% |
| IgM (mg/dL) | 83 $\pm$ 57 | 81 $\pm$ 56 | 103% |
| LDL (mg/dL) | 71 $\pm$ 20 | 82 $\pm$ 25 | 94% |
| HDL (mg/dL) | 37 $\pm$ 11 | 40 $\pm$ 14 | 100% |
| Total cholesterol (mg/dL) | 146 $\pm$ 32 | 145 $\pm$ 31 | 101% |
| Triglycerides (mg/dL) | 122 $\pm$ 66 | 120 $\pm$ 62 | 102% |

* Data are presented as mean $\pm$ standard deviation.
† The geometric mean ratios of ODP and FFP with 90% confidence interval.
FFP = fresh frozen plasma; HDL = high-density lipoprotein; IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; LDL = low-density lipoprotein; ODP = on-demand plasma.

**TABLE 2. Coagulation and fibrinolytic factors/ inhibitors, vWF, and other proteins (n = 59)**

| Analyte | ODP* | FFP* | Ratio† |
|---|---|---|---|
| Fibrinogen (mg/dL) | 214 ± 39 | 263 ± 52 | 82% |
| Prothrombin (IU/dL) | 85.5 ± 11.0 | 96.3 ± 11.7 | 89% |
| FV (IU/dL) | 104.4 ± 20.2 | 98.8 ± 20.4 | 106% |
| FVII (IU/dL) | 92.3 ± 20.2 | 100.3 ± 20.7 | 91% |
| FVIII (IU/dL) | 106.5 ± 27.2 | 127.4 ± 33.3 | 83% |
| FIX (IU/dL) | 109.4 ± 17.0 | 119.6 ± 17.6 | 91% |
| FX (IU/dL) | 88.9 ± 14.6 | 101.0 ± 17.4 | 88% |
| FXI (IU/dL) | 105.4 ± 19.8 | 110.3 ± 20.4 | 95% |
| FXII (IU/dL) | 88.8 ± 21.4 | 103.5 ± 23.4 | 85% |
| FXIII (IU/dL) | 98.2 ± 18.3 | 131.3 ± 21.4 | 74%‡§ |
| Plasminogen (IU/dL) | 103 ± 13 | 100 ± 13 | 103% |
| Protein C (IU/dL) | 98 ± 20 | 104 ± 20 | 93% |
| Protein S (IU/dL) | 98.0 ± 19.2 | 106.0 ± 20.0 | 92% |
| Antithrombin (IU/dL | 91 ± 13 | 92 ± 12 | 99% |
| Plasmin inhibitor (IU/dL) | 99 ± 8 | 100 ± 7 | 99% |
| C1 esterase inhibitor IU/dL) | 98 ± 12 | 97 ± 13 | 101% |
| Alpha 1-proteinase inhibitor (mg/dL) | 140.5 ± 25.5 | 139.6 ± 20.8 | 100% |
| Free protein S antigen (IU/dL) | 96.4 ± 20.5 | 103.6 ± 22.9 | 93% |
| FV antigen (IU/dL) | 67 ± 14 | 70 ± 13 | 96% |
| FVII antigen (IU/dL) | 99.8 ± 18.1 | 97.3 ± 18.8 | 102% |
| FVIII antigen (IU/dL) | 142.5 ± 34.5 | 140.6 ± 36.7 | 102% |
| vWF:RCo (IU/dL) | 62.3 ± 23.5 | 105.0 ± 40.5 | 60%‡ |
| vWF antigen (IU/dL) | 141.9 ± 42.0 | 135.6 ± 42.7 | 106% |
| ADMATS13 (IU/dL) (n = 6)§ | 135.0 ± 5.3 | 128.5 ± 9.3 | 105% |

* Data are presented as mean ± standard deviation.
† The geometric mean ratios of ODP and FFP with 90% confidence interval.
‡ Data outside the 80–125% range; p < 0.0001 (t test: two-sample assuming equal variances).
§ Performed on ODPs prepared separately from the n = 60 study.
F = Factor; FFP = fresh frozen plasma; ODP = on-demand plasma; RCo = ristocetin cofactor; vWF = von Willebrand factor.

74% FXIII activity (range 73%–76%; 90% confidence interval) relative to FFP, slightly lower than other factors.

## vWF and ADAMTS13

VWF multimers exist in plasma in various sizes, referred to as high-molecular-weight (HMW), intermediate-molecular-weight, and low-molecular weight multimers, respectively. ODP had a reduced vWF:RCo at 60% relative to FFP (62.3 ± 23.3 vs. 104.3 ± 40.1 IU/dL; p < 0.0001), but comparable levels of vWF antigen (Table 2). These data were consistent with the reduction in HMW multimers and corresponding increase in smaller multimers in vWF multimer analysis (data not shown). Spray drying cleaves HMW vWF multimers and often reduces vWF:RCo to less than 30% compared with the untreated starting plasma (Meledeo et al., submitted for peer review). ODP had FVIII levels comparable to other coagulation factors, albeit at the low end, indicating that the ability of vWF to stabilize FVIII was largely unaffected by the change of size distribution. ODP had the same levels of ADAMTS13 activity as FFP.

We have noted high vWF activity (75%) for dry plasma rehydrated in 1.5% glycine compared with FFP in a proof-of-concept study using a ristocetin-dependent platelet agglutination method on a platelet aggregometer.[15] In the current study, we performed all vWF:RCo measurements using a fully automated coagulation analyzer to minimize assay variations.

## Coagulation activation and complement activation

ODP and FFP were analyzed for Fragment 1 + 2, thrombin-antithrombin complex, and D-dimer to assess coagulation activation (Table 3) and were similar in all three measurements. Spray drying does not cause coagulation activation. C3a and C5a were analyzed to assess complement activation (Table 3). ODP was similar to FFP in the C3a level and slightly elevated in C5a (9.6 ± 3.7 vs. 7.3 ± 3.6 ng/mL; p < 0.001). ODP remained low in the C5a level compared with solvent/detergent-treated plasma at 16.1 (14.7–17.8) ng/mL or apheresis plasma at 17.1 (14.3–26.2) ng/mL[21] and 26.6 (4.9–74) ng/mL.[22]

## aPTT, PT, and TT

Compared to FFP, ODP had similar aPTT, slightly extended PT (10%), and prolonged TT (49%) (Table 4). TT assesses the central step of coagulation, the conversion of fibrinogen to fibrin, bypassing all the preceding reactions through the use of a limited amount of exogenous thrombin. Thus, TT prolongation can occur in conditions affecting either fibrinogen or thrombin. ODP had normal levels of fibrinogen (239 ± 39 IU/dL) and did not have a significant amount of fibrinogen degradation products to inhibit thrombin as implied by the lack of coagulation activation. A kit was used for measuring thrombin time (HemosIL Thrombin Time, Instrumentation Laboratory), which uses bovine thrombin with a pH optimum of 8.3.[23] It was plausible that the reduced pH in ODP (7.03 ± 0.11) compared to FFP (7.62 ± 0.06) shifted the pH of the reaction farther from the pH optimum of thrombin and prolonged TT.

**TABLE 3. Coagulation and complement activation markers (n = 59)**

| Analyte | ODP* | FFP* |
|---|---|---|
| Fragment 1+ 2 (pmol/L) | 184.4 ± 84.7 | 180.1 ± 81.8 |
| Thrombin-antithrombin complex (μg/L) | 3.6 ± 6.7 | 3.9 ± 6.9 |
| D-dimer (mg/L) | 0.46 ± 0.23 | 0.51 ± 0.25 |
| C3a (ng/mL) | 186.2 ± 108.8 | 169 ± 98.8 |
| | 74.3-680.1 | 62-556.3 |
| C5a (ng/mL)† | 9.6 ± 3.7 | 7.3 ± 3.6 |
| | 3.5-19.4 | 1.5-16.2 |

* Data are presented as mean ± standard deviation and range (C3a and C5a).
† p < 0.001 (t test: two-sample assuming equal variances).

**TABLE 4. Global coagulation and hemostatic characteristics of ODP and FFP evaluated by aPTT, PT, TT, and TEG (n = 59)**

| Test | ODP* | FFP* | Ratio† |
|---|---|---|---|
| aPTT (sec) | 29.9 ± 3.4 | 29.3 ± 3.0 | 102% |
| PT (sec) | 12.5 ± 0.9 | 11.3 ± 0.9 | 110% |
| TT (sec) | 19.5 ± 2.0 | 13.0 ± 0.9 | 149%‡ |
| Clot time (min) | 6.0 ± 1.0 | 4.6 ± 0.8 | 132%‡ |
| Angle (deg) | 74.9 ± 3.4 | 77.5 ± 2.8 | 97% |
| Maximum amplitude (mm) | 25.4 ± 5.1 | 27.4 ± 5.1 | 93% |

* Data are presented as mean ± standard deviation.
† The geometric mean ratios of ODP and FFP with 90% confidence interval.
‡ Data outside the 80%–125% range; $p < 0.0001$ (t test: two-sample assuming equal variances).
aPTT = activated partial thromboplastin time; FFP = fresh frozen plasma; ODP = on-demand plasma; PT = prothrombin time; TT = thrombin time.

### Global hemostasis evaluation by TEG

TEG analysis of plasma assesses the interaction between fibrinogen and clotting factors with a single test and thereby produces a composite dynamic picture of the entire coagulation process. ODP was similar to FFP in clot rate as measured by angle (α) and clot strength as measured by maximum amplitude (Table 4), reflecting the comparability of ODP to FFP in clotting kinetics and clot strength. ODP had a 32% increase in clot time compared to FFP, which may be attributed to the pH difference. ODP was comparable to FFP in all three parameters when ODP was pH matched to FFP, as described in the companion paper (Meledeo et al., submitted for peer review). A 25% increase in clot time and reduction in maximum amplitude at pH 7.0 versus 7.4 has been reported in the viscoelastic study using rotational thromboelastometry.[24]

## DISCUSSION

Commonly recognized deleterious factors inherent in the spray drying of protein molecules are adsorption, shear, and thermal and dehydration stresses, which can result in protein unfolding, aggregation, and denaturation.[25–27] However, for spray drying plasma, pH elevation attributable to the loss of carbon dioxide is probably one of the most important single stress factors. pH increase has been associated with the freeze drying of plasma.[28] The combined effects of extensive aeration and heating during spray drying result in a very alkaline plasma product, typically at pH 8.5 to 9.0 upon reconstitution in water (data not shown). Spray drying simultaneously exposes the proteins to high pH and heat, the most recognized influential factors for protein instability. The alkaline condition may adversely impact the stability of the product during processing and storage, which is particularly concerning for a product with higher moisture content. Numerous studies have related product instability to the moisture content.[29,30] Based on these considerations, we hypothesized that mitigation of the spray drying–induced alkalinization will improve the in-process and storage stability of the product, which can be accomplished by pretreating the plasma with a nonvolatile acid prior to spray drying. HCl in combination with glycine was preferably chosen for pretreating the plasma because of its nonvolatility, nontoxicity, and low cost. Importantly, glycine has been used at up to 80 mM as an osmolarity adjuster in a Food and Drug Administration–approved plasma product,[31] which may reduce the regulatory concern for the use of glycine (total 70 mM) in ODP. Spray drying cleaves HMW vWF multimers and often reduces vWF:RCo to less than 30% compared with the untreated starting plasma (Meledeo et al., submitted for peer review). The current study shows that pretreatment with glycine-HCl greatly improved preservation of vWF multimers and resulted in a sharp increase of vWF:RCo to 60%, while retaining greater than 80% levels of almost all other functional proteins without coagulation and complement activation. Additionally, the relatively low moisture content (<3%) may be beneficial for product storage stability. The high-level recoveries of albumin, immunoglobulins, lipoproteins, and coagulation protein antigens are suggestive of the absence of the formation of protein aggregates, which is reflected by the rapid reconstitution of the product. To complement the current data, we have collaborated with US Army Surgical Research Institute to perform extensive functional studies. The result is described in a separate manuscript (Meledeo et al., submitted for peer review). Highlights include demonstration of biological equivalence in thrombin generation, platelet adhesion and aggregation, and clot formation.

HMW vWF multimers are most effective in interacting with collagen and platelet receptors to facilitate wound healing under conditions of shear stress.[32] Deficiency of HMW vWF multimers positions ODP on the low end of normal levels of vWF:RCo (62.3 ± 23.5 vs. 50–200 IU/dL; $p < 0.0001$) with the vWF:RCo/vWF:antigen ratio, the surrogate marker for HMW multimers, reduced to 0.44 (62.3/141.9) as opposed to 0.77 for paired FFP (105.0/135.6) (Table 2). Diminished HMW multimer levels have been reported in commercial solvent/detergent-treated, pooled plasma products as reflected by low vWF:RCo/vWF:antigen ratios (0.55)[33] or reduced levels of vWF activity relative to FFP (43% vs. 97%).[34] Shortage of HMW multimers in a plasma product may be of clinical relevance in cases of very low vWF. Paradoxically, vWF/FVIII concentrates derived from plasma do not necessarily have a high percentage of HMW vWF multimers. The vWF:RCo/vWF:antigen ratios in some vWF/FVIII concentrates are below 0.5.[35] The possible mechanism underling the impact on vWF by spray drying and pH deserves some elaboration. In a separate study aimed to dissect steps leading to vWF fragmentation, we observed that vWF degeneration occurs mainly in the aerosolization step. Under physiologic conditions, vWF multimers have ADAMTS13 proteolytic sites buried in each

dimer and are resistant to ADAMTS13 cleavage.[36,37] Unfolding of the mechanosensitive vWF multimers in response to the shear forces exerted by the flowing blood exposes the cryptic proteolytic sites and renders them vulnerable to enzymatic cleavage. The conformation of vWF dimers is pH dependent and more compact at lower pH.[38,39] Taken together, it is tempting to analogize the vWF cleavage during spray drying to the in vivo process as a combination of physical and enzymatic actions, that is, aerosolization-induced unfolding and action of ADAMTS13. This process is accelerated at alkaline pH where individual vWF dimers are more relaxed, and rectification of the alkalinization via acid pretreatment reduces the enzymatic cleavage. This mechanism could explain the apparent high specificity of vWF fragmentation incurred during spray drying.

In conclusion, ODP demonstrates in vitro characteristics comparable to that of FFP and this spray-drying system can be implemented in component manufacturing laboratories to produce a product suitable for transfusion. Future studies will evaluate the long-term stability of ODP at refrigerated and room temperatures and clinical studies are being developed to evaluate the safety and efficacy of the product.

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### CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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