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ORIGINAL ARTICLE

Comparative analyses of the hemostatic efficacy and surgical device performance of powdered oxidized regenerated cellulose and starch-based powder formulations

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Abstract

Background: Hemostatic powders offer unique therapeutic advantages over other formulations, including ease of application and rapid distribution over large bleeding surfaces. The efficacy of powder-based hemostats is dependent on device performance, which is rarely investigated independently from efficacy.

Objectives: The current study aimed to compare the hemostatic efficacy of an oxidized regenerated cellulose agent (Surgicel, Ethicon, Inc) and 3 starch-based biopolymers (Arista, Becton Dickinson; PerClot, Baxter International; and 4DryField, PlantTec Medical GmbH) and the performance of their delivery device applicators.

Methods: Efficacy was evaluated in a porcine model of bleeding using 2 study designs where the powder was delivered with (experiment 1) and without (experiment 2) device applicators. Device performance (powder expression) was examined *in vitro* at 3 device positions/angles: 90° (vertical, downward), 45° (slanted, downward), and 180° (horizontal).

Results: Surgicel efficacy rate was noninferior ($P \leq .0002$) and superior ($P \leq .004$) to that of any of the 3 starch-based agents regardless of whether the powder was delivered with their devices (experiment 1) or directly applied onto the bleeding sites (experiment 2). Surgicel required fewer applications ($P \leq .0002$) and less powder ($P < .0001$) to achieve hemostasis. The Surgicel device was the only one that consistently delivered precise amounts of powder over a critical range of applications in the 3 positions tested.

Conclusion: The oxidized regenerated cellulose powder was the most efficacious hemostat, and the Surgicel applicator exhibited the highest performance compared with any of the 3 starch-based devices investigated. The current study highlights the relevance of combining high-efficacy powder hemostats with innovative, high-performance applicators to effectively manage bleeding control in surgical settings.

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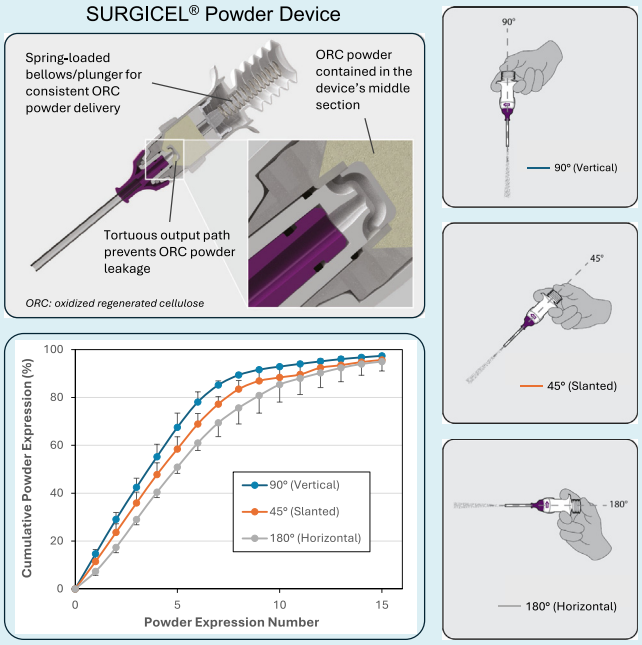

KEYWORDS
animal model, hemostasis, oxidized regenerated cellulose, polysaccharides, starch

Essentials

- Plant-based powdered hemostatic agents are commonly used in surgeries to control bleeding.
- This study evaluated the hemostatic efficacy of Surgicel (Ethicon), Arista (Becton Dickinson), PerClot (Baxter International) and 4DryField (PlantTec Medical GmbH).
- The study also assessed device performance: how effectively each device delivers powder.
- Surgicel and its device outperformed the others in both efficacy and effectiveness.

1 | INTRODUCTION

Adjunctive topical hemostatic agents are increasingly used to control mild to moderate bleeding of tissues that result from surgical procedures and reduce the risk of operative and postoperative complications [1,2] across a variety of surgical specialties, including cardiothoracic surgery [3], spinal and cranial surgery [4], and trauma surgery [5]. Agents from different inorganic and organic sources used to assist in controlling intraoperative bleeding when conventional methods are insufficient or impractical are broadly classified into 3 groups: hemostats that provide a physical surface to promote and accelerate clot formation (eg, cellulose, collagen, and starch), sealants that prevent blood leakage (eg, fibrin and silk fibroin), and synthetic adhesives that bond tissues (eg, cyanoacrylate) [2,6–8].

Hemostatic agents are manufactured into various physical forms, including dressings, pads, fibrillar tufts, woven strips, sponges, patches, gels, and powders, appropriate for a variety of clinical bleeding scenarios [9]. For example, hemostatic dressings and soft and microporous sponges are suitable for tamponade and compression over bleeding sites [10,11], whereas flowable hemostats (eg, gelatin matrices) can be delivered to small spaces or narrow wounds because they follow the topography of irregularly shaped injured tissue and have a low risk of swelling and compressing key tissue structures [12]. The small granular size of powdered hemostats and their shape plasticity make them suitable hemostatic agents for large and deep surface wounds [13].

Powdered-based hemostats containing polysaccharides (eg, cellulose and starch) or combinations of collagen, chondroitin sulfate, and thrombin have recently emerged as an option to treat mild to moderate bleeding as they effectively stick to rough wound surfaces [9,10]. However, while ordinarily safe, organic products derived from animals (eg, porcine/bovine gelatins and collagen) and humans (eg, thrombin) come with particular safety concerns, such as the risk of immunogenic reactions and viral or prion disease transmission [11]. Consequently, surgeons prefer the use of agents that do not contain animal products and agree that polysaccharide powder hemostats are unlikely to cause harm to patients [6].

Plant-derived polysaccharides including oxidized regenerated cellulose (ORC) and starch-based hydrophilic particles are the primary constituents of several powdered hemostats [2,5]. Polysaccharides are widely distributed in nature as linear biopolymer components of plant cell walls (eg, cellulose) and branched storage biopolymers (eg, starch) in plant tissues [14]. They are complex carbohydrates composed of monosaccharides joined by glycosidic bonds. Cellulose and starch are homopolysaccharides formed by beta-1,4 linked glucopyranose units and alpha-1,4 linked glucose monomers, respectively. While starch is the storage form of glucose in plant tissues, cellulose is an architectural component that provides mechanical strength and structural integrity to plants. Both are abundant natural biocompatible materials exhibiting low to no cytotoxicity [8]. ORC is manufactured by oxidizing natural cellulose with nitrous oxide and further converting it into cellulose fibers to generate a variety of hemostatic products for several indications [15].

ORC provides a substrate for clot formation by facilitating platelet adhesion and aggregation, and it exhibits bactericidal properties *in vitro* due to the low pH that results from cellulose chemical oxidation [16]. Polysaccharide particles derived from plant starch are hydrophilic hemostatic agents that accelerate blood clotting by absorbing water and retaining platelets and clotting proteins essential to the coagulation process [2,17].

Topical hemostats can be further classified into 3 distinct delivery methods: direct placement, syringe-based delivery systems, and device-based delivery systems. Direct placement entails the application of hemostatic dressings, sponges, fibrillar materials, and woven strips directly onto the bleeding site [11,18]. Syringe-based delivery systems are commonly utilized for collagen-based hemostats and gelatin matrices, with or without the addition of human thrombin. Flowable materials contained in prefilled syringes are applied directly over the bleeding wound via specialized applicator tips [9,13,19]. Typically prefilled with powdered hemostats, device-based delivery systems utilize bellows pumps to dispense the hemostatic powder. In this delivery method, the bellows are manually compressed to effectively distribute the powder onto the targeted bleeding area [20–23]. Overall, these delivery methods provide flexibility and precision, allowing for tailored applications that cater to specific hemostatic products and clinical scenarios.

The hemostatic capacity of polysaccharide-based agents derived from cellulose or starch has been previously evaluated [5,8,24]. However, no studies have simultaneously investigated both the efficacy of these hemostatic agents and the performance of their delivery devices—specifically, bellows pumps—that are employed to apply powder-based hemostats to bleeding areas.

In a surgical environment, the effectiveness of a device is primarily measured by the amount of hemostatic powder dispensed during successive applications. Several factors influence this performance, including the agent's physical properties (eg, particle size distribution), the speed of compression of the bellows-based device, the force exerted by the operator, and the orientation in which the device is held. Pilot studies conducted in our laboratory indicate that, although powder particle properties and compression speed and force are important, their impact on device performance is outweighed by the device's orientation (eg, vertical vs horizontal) during powder dispensing. This finding underscores the importance of the angle at which the surgeon holds the device to achieve consistent delivery of hemostatic powder following each compression of the device's bellows.

The purpose of this study was to compare the hemostatic efficacy of ORC (SURGICEL Powder Absorbable Hemostatic Powder [Surgicel], Ethicon, Inc]) with 3 starch-based biopolymers—Arista Absorbable Hemostat AH (Arista; Becton Dickinson), PerClot Absorbable Hemostatic Powder (PerClot; Baxter International), and 4DryField PH Powder (4DryField; PlantTec Medical GmbH)—and to assess the performance of their respective delivery devices. While other powdered hemostats used in clinical practice (eg, Hemoblast [Biom'Up SA], Starsil [Hemostat Medical GmbH], and 4SEAL [Grena Biomed]) [25] could have been included in the study, we determined that the efficacy and device performance data from the 4 widely used products selected would provide surgeons in various specialties with reliable evidence. This would enable them to make informed decisions when selecting more effective formulations contained within high-performing delivery devices. Surgicel, Arista, PerClot, and 4DryField were specifically chosen due to their comparable powder formulations, indications as adjunctive powdered hemostats, and consistent device/applicator design (bellows pump), size, and capacity (3 g). Furthermore, all 4 products are plant-based, derived from cellulose and starch, and free from any animal or human-derived components.

The primary endpoint, hemostatic efficacy rate, was evaluated using a porcine punch-biopsy model of bleeding. The powder hemostats were tested with and without their device/applicator to ascertain the direct contributions of each agent to bleeding control in an *in vivo* setting. Device powder expression and whole blood clotting activity assays were conducted *ex vivo* to evaluate device performance and gain insight into the procoagulant capacity of the 4 powdered hemostats investigated.

2 | METHODS

2.1 | Test materials

The adjunctive hemostats (test articles) evaluated included 1 ORC-based product, Surgicel, and 3 starch-based agents: Arista, PerClot, and 4DryField. Surgicel is an aggregate of ORC fiber fragments [22], Arista is composed of microporous particles synthesized by cross-linking purified plant starch [20], PerClot is composed of purified starch polysaccharide granules [21], and 4DryField is made of microparticles purified from potato starch [23]. The 4 absorbable hemostatic powders come in prefilled applicators/devices that use manual bellows compression for powder expression (Figure 1) [20–23,26]. All the devices have bellows of similar sizes, with 5 convolutions per device, and can hold the same amount of powder.

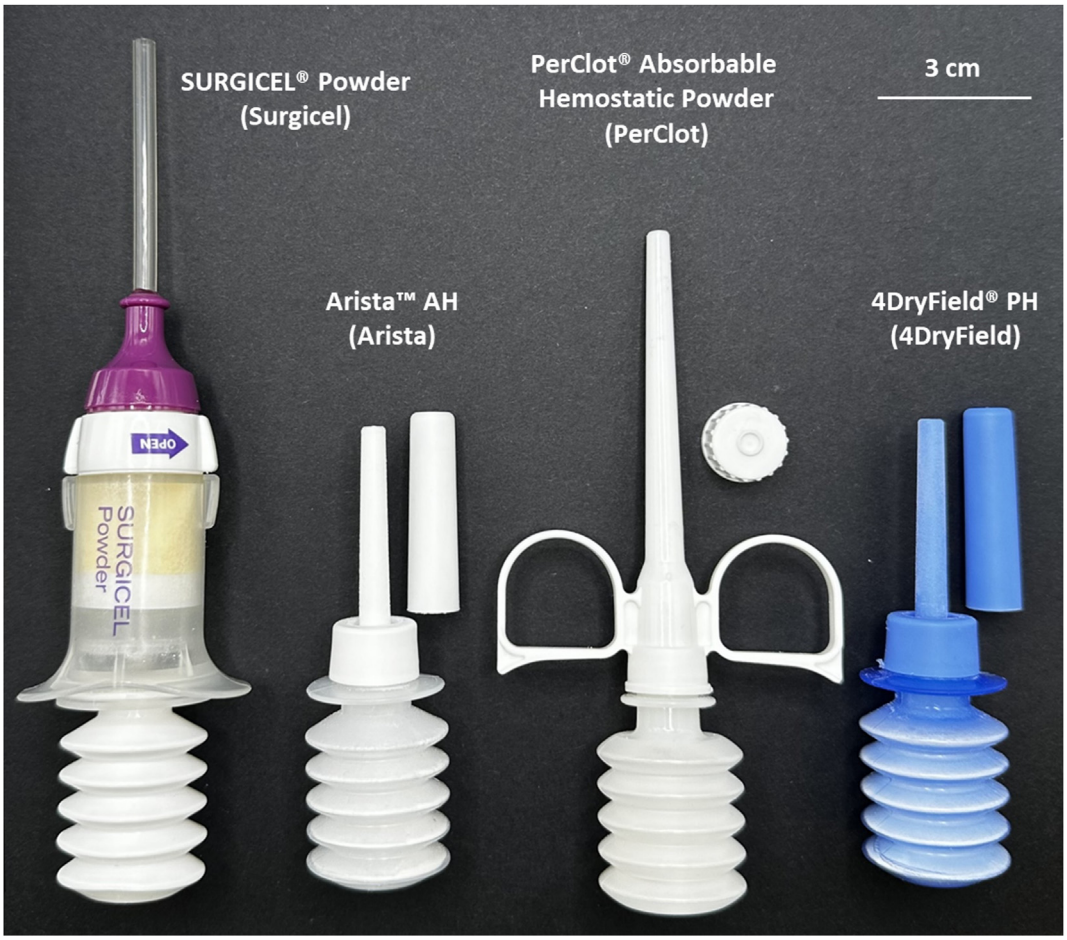

FIGURE 1 Hemostatic powder applicators/delivery devices. The 4 products are absorbable hemostatic powders supplied in prefilled applicators/delivery devices. Devices containing 3 g were used in the study. Device caps are shown next to the applicator. The Surgicel delivery device does not use a cap; the clockwise twisting of a rotary on/off valve in the body of the applicator opens the device [22]. The applicator caps of Arista and 4DryField must be removed before use [20,23]. The PerClot applicator's cap must be removed before attaching the applicator's tip [21], which is shown already attached to its device. The 4 devices operate under the same principle of manual air compression of the bellows, which have similar size and capacity. The Surgicel device is manufactured with a spring-loaded mechanism that allows for consistent powder delivery regardless of device position [26].

2.2 | *In vivo* experiments

2.2.1 | Experimental design/endpoints

The hemostatic efficacy of the 4 products was evaluated in porcine liver and spleen using a biopsy punch bleeding model [19,27]. The study was divided into 2 experimental designs, experiment 1 and experiment 2. Experiment 1 tested the hemostatic efficacy of the products when used with their proprietary delivery device/applicator as per the instructions for use (IFU). Experiment 2 tested the efficacy of the products when directly applied as 125 mg powder aliquots over the bleeding site without using the delivery device/applicator. This dual experimental approach allowed us to assess the efficacy of each of the 4 agents delivered following the product's IFU as performed in a real-world surgical setting (experiment 1) while also estimating their efficacy on a product weight basis independently of device performance (experiment 2).

The primary endpoint for both experimental designs was hemostatic efficacy, which is defined as the ability of the test articles to achieve hemostasis within 5 minutes. The rate of hemostatic efficacy was calculated as the number of sites that achieved complete hemostasis within 5 minutes divided by the total number of sites. Secondary endpoints for both study designs included a) the number of test article applications required to achieve hemostasis, b) the total cumulative amount of test article required to achieve hemostasis, and c) time to hemostasis (TTH; Table 1). Six animals were used, 3 for each study design. For experiment 1, 18 defect sites were created on each organ (16 treatment sites + 2 negative controls) for a total of 108 sites (36 per animal times 3 animals). For experiment 2, 17 defect sites were created on each organ of a single animal for a total of 102 sites (Table 1). Due to the substantial amounts of starch-based 4DryField powder required to achieve hemostasis in experiment 1, not enough material was left to be tested in experiment 2. Therefore, defect sites

TABLE 1 Study design and primary and secondary endpoints for the *in vivo* study.

Experiment	Group	Organ (spleen or liver) bleeding sites	Total bleeding sites per animal	Bleeding sites per group	Primary endpoint	Secondary endpoints
Experiment 1 (*n* = 3 swine)	Control	2[a]	4	12	Hemostatic efficacy[b]	- No. of test article applications required to achieve hemostasis. - Total cumulative amount of test articles required to achieve hemostasis (mg). - TTH (min).
	Surgicel	4	8	24		
	Arista	4	8	24		
	PerClot	4	8	24		
	4DryField	4	8	24		
	Total	18	36	108		
Experiment 2 (*n* = 3 swine)[c]	Control	2[a]	4	12	Hemostatic efficacy[b]	- No. of preweighed 125 mg test article aliquots required to achieve hemostasis. - Total cumulative amount of test articles required to achieve hemostasis (no. of applications ×125 mg). - TTH (min).
	Surgicel	5	10	30		
	Arista	5	10	30		
	PerClot	5	10	30		
	Total	17	34	102		

The efficacy of 4 powder hemostats was tested in bleeding lesions created in swine liver and spleen in 2 experiments where the powder hemostats were tested with (experiment 1) and without (experiment 2) their proprietary delivery devices.

TTH, time to hemostasis.

[a]First and last bleeding sites for each organ.

[b]Efficacy was defined as the ability to achieve hemostasis within 5 minutes of test article application (pass/fail scoring).

[c]Due to the large amounts of 4DryField powder needed to achieve hemostasis in experiment 1, not enough powder remained to be tested in experiment 2; therefore, 4DryField was tested only in experiment 1. Defect sites in experiment 2, originally planned for 4DryField testing, were redistributed among the remaining 3 test articles.

originally planned for starch-based 4DryField in experiment 2 were distributed among the 3 remaining groups.

2.2.2 | Animals

Female crossbreed swine from Oak Hill Genetics weighing 75 to 100 kg were used for the hemostasis testing. Female swine were chosen because their anatomy facilitates performing a longer ventral midline laparotomy incision, which allows for easier access to the liver and spleen. Animals were ear-tagged with a unique identification number and acclimated at the testing facility for 4 to 6 days before any procedure was conducted. Animals were assigned to the 2 experimental groups (experiment 1 and experiment 2) in consecutive order as they arrived at the facility. No substitutions were made after the assignment. Healthy pigs were housed individually, fed once a day with standard swine chow, and had access to water *ad libitum*. The housing and care of the animals followed the standards set by the Guide for the Care and Use of Laboratory Animals [28]. The study was conducted by Ethicon Endo-Surgery, Inc, in their Association for Assessment and Accreditation of Laboratory Animal Care International-accredited facility. The animal protocols were reviewed and approved by the facility's Institutional Animal Care and Use Committee and followed the recommendations of the Animal Research: Reporting of *In Vivo* Experiments guidelines 2.0 (The Animal Research: Reporting of *In Vivo* Experiments Essential 10) [29]. The Supplementary Methods describes in detail the general anesthetic procedures, drugs used, and maintenance and monitoring of blood pressure during the surgical procedure.

2.2.3 | Porcine biopsy punch model of hemostasis

The porcine biopsy punch-induced bleeding model utilized to compare the hemostatic efficacy of the test articles in the liver and spleen has been previously described [19,27]. Similarly sized wound defects 6 mm in diameter, and 3 mm in depth were created sequentially on the liver and spleen surface using 6 mm diameter biopsy punches (Integra Life Sciences). Identification bands (Integra Life Sciences) were fitted to the biopsy punches to ensure that a 3 mm depth was achieved. After defect creation, bleeding sites were scored using a validated bleeding severity scale [30]. Only mild (grade 1) and moderate (grade 2) bleeding sites were included in the study.

2.2.4 | Test article application protocol

All test articles are indicated for adjunctive management of diffuse or localized surgical bleeding areas and should be delivered after blotting excess blood from the target sites. They were all provided in sterile packages. Test article powder was applied using the device applicator (experiment 1) or a preweighed 125 mg aliquot (experiment 2) to a freshly created defect site, as detailed below. Additional information on the biopsy punch model and the test article application protocol are provided in the Supplementary Methods and Supplementary Figure S1.

2.2.5 | Experiment 1

Test articles were used according to the manufacturer's IFU. A single new device per bleeding site was used. Since the test article powder in experiment 1 was applied using its own, unconcealed proprietary device, the surgeon was not blinded to the test article application. However, test articles were applied to bleeding sites in a randomly generated order that was not disclosed to the surgeon until after the creation of the bleeding site. Test devices were weighed before and after each powder application to each bleeding site to determine the total mass of article required—in single or multiple applications—to achieve hemostasis, as previously described [19].

2.2.6 | Experiment 2

No proprietary devices were used for experiment 2. Instead, 125-mg aliquots of test article powder were dispensed out of the delivery devices into 3 cc glass vials and stored in an amber nitrogen-purged desiccator box until use. The 125-mg dose was selected based on published data indicating that such an amount adequately covers defect sites of 6 mm diameter by 3 mm depth [24]. For each hemostatic agent, single or multiple 125-mg powder aliquots were applied directly onto the bleeding site as needed to achieve hemostasis. For experiment 2, the surgeon was blinded to the test article applied, and the order of test article application onto the bleeding site was randomized before application.

2.3 | *In vitro* experiments

2.3.1 | Powder expression assay

Powder delivery device performance assessments were conducted for the 4 devices using an *in vitro* powder expression assay. The assay tested the effectiveness of the device in consistently delivering hemostatic powder while held in 3 different positions: 90° (right angle, vertical, downward position), 45° (acute angle, slanted, downward position), and 180° (straight angle, horizontal position) angles. Proprietary precapped bellows applicators for Surgicel, Arista, PerClot, and 4DryField were used following their IFU [20–23] (Figure 1). All devices contained 3 g of preloaded material each. Six new devices for each test article (24 devices in total) were tested. Each powder expression in any of the 3 positions consisted of a full stroke of the device bellows. Expressed powder amount was calculated based on the weight of the device before and after each application. Twenty expressions for each device position were performed for a total of 60 expressions per device. Device performance was defined as the ability to express a consistent amount of powder over a range of consecutive applications in a specific device position.

2.3.2 | Blood clotting assays

To gain insight into the mechanism of action of ORC aggregates and starch-based polysaccharide particles, 2 *in vitro* blood clotting assays, blood clotting mass (BCM) and blood clotting index (BCI), were performed.

BCM assay: The *in vitro* clotting activity of the test articles was determined by a BCM assay that measured the total mass of clotted blood retained in glass vials following the application of the topical hemostats as previously described [31]. The full methodological steps of the BCM assay are provided in the Supplementary Methods.

BCI assay: A BCI assay in whole blood was performed to assess the *in vitro* procoagulant ability of the test articles using a free hemoglobin absorbance-based method [32,33]. Sample absorbance at 540 nm in the BCI assay is directly proportional to the amount of free hemoglobin from red blood cells (RBCs) and inversely proportional to clotting capacity. Therefore, high absorbance values indicate a low clotting capacity as readily available free hemoglobin is released from burst RBCs upon incubation with water. The BCI was calculated using the following equation:

$$BCI\ (\%) = \frac{OD\ control - OD\ sample}{OD\ control} X\ 100$$

where the optical density (OD) sample and OD control represent supernatant absorbances in the tested samples and negative controls, respectively. Higher BCI values indicate better coagulation capacity. A detailed description of the BCI assay is provided in the Supplementary Methods.

2.4 | Statistical analysis

2.4.1 | *In vivo* experiments

Primary endpoint: For the primary endpoint (hemostatic efficacy within 5 minutes), data from all bleeding sites in both the liver and spleen were pooled for hypothesis testing of noninferiority and superiority. Based on previous studies using the same animal model [19,27], a sample size of 24 pooled liver and spleen defect sites for each test article provided at least 80% power to detect statistical significance using a 1-sided test at a type 1 error rate of 5%. Hypothesis testing was conducted in a prespecified sequence using a gatekeeping strategy to preserve the overall type I error rate at 5%. A total of 10 statistical hypotheses, 6 for experiment 1 and 4 for experiment 2, were tested separately, as described in detail in the Supplementary Methods. For the noninferiority hypotheses, the null hypothesis was rejected if the 1-sided 95% lower confidence limit of the difference between the hemostasis rate within 5 minutes between Surgicel and the corresponding test article was greater than −0.15. For the superiority hypotheses, the null hypothesis was rejected if the 95% lower confidence limit was greater than zero.

Secondary endpoints: For all secondary endpoints, Surgicel was compared with Arista, PerClot, and 4DryField in experiment 1 and Arista and PerClot in experiment 2. Both sets of comparisons were performed using *t*-tests with data pooled from the liver and spleen models. The survival distributions of hemostasis were compared between Surgicel and each of the 3 starch-based test articles using the long-rank test. The Kaplan–Meier method was used to generate the median survival time (TTH). Sites that did not achieve hemostasis at 5 minutes were censored at that time.

2.4.2 | *In vitro* experiments

A linear regression model was used to fit cumulative powder expression to the number of powder expressions for the first 6 expressions. Pearson's correlation coefficients (r) were obtained for each product at each of the 3 device positions (90° [vertical], 45° [slanted], and 180° [horizontal] angles). Analysis of variance tests were conducted for data obtained from the BCM and BCI assays. If the overall analysis of variance F value was statistically significant ($P < .05$), post hoc Tukey-adjusted *t*-tests (alpha = 0.05) comparing the means of the 4 groups were conducted.

3 | RESULTS

3.1 | *In vivo* experiments

A total of 123 and 121 bleeding defects were created in the spleen and liver of 3 animals for experiment 1 and experiment 2, respectively. Out of the 123 sites created in experiment 1, 88% (108/123) were scored as mild (grade 1) or moderate (grade 2) bleeding and used, whereas 12% (15/123) were discarded: 14 as severe (grade 3) bleeding and 1 due to incorrect lesion size. For experiment 2, 84% (102/121) of sites were scored as mild or moderate and 15% (18/121) as severe. One bleeding site (1%, 1/121) was replaced due to incorrect compression time. Assessments of untreated negative control sites confirmed that tamponade alone, applied repeatedly over the 5-minute period, did not stop blood loss from the tissue defect.

3.2 | Hemostatic efficacy rates

3.2.1 | Experiment 1

A total of 24 defect sites per test article were tested in 3 animals in experiment 1 (Table 1). Hemostasis was achieved within 5 minutes for 100%, 75%, 71%, and 21% of defect sites treated with Surgicel (24/24), Arista (18/24), PerClot (17/24), and 4DryField (5/24), respectively (Figure 2A, Table 2). Noninferiority tests (tests 1-3) were all significant ($P = .0002$ for test 1 [Surgicel vs Arista], $P < .0001$ for test 2 [Surgicel vs PerClot], and $P < .0001$ for test 3 [Surgicel vs 4DryField]). The lower bound of the 1-sided 95% CI for the difference in hemostasis

efficacy rates between Surgicel and each of the test articles (tests 4-6) was greater than zero in all cases ($P = .0044$ for Surgicel vs Arista [test 4], $P < .002$ for Surgicel vs PerClot [test 5], and $P < .0001$ for Surgicel vs 4DryField [test 6]). Thus, the hemostatic efficacy of Surgicel was both noninferior and superior to that of Arista, PerClot, and 4DryField when the hemostats were applied using their proprietary delivery device/applicator (Supplementary Table S1).

TTH mean values for Surgicel, Arista, PerClot, and 4DryField are shown in Figure 2B and Supplementary Table S2. Two-sample *t*-tests of the mean differences in TTH between Surgicel and each of the 3 starch-based agents tested showed that Surgicel required significantly less time to achieve hemostasis compared with Arista ($P = .0002$), PerClot ($P = .0003$), and 4DryField ($P < .0001$; Supplementary Table S2). Furthermore, the median survival time (TTH) of Surgicel was lower than that of Arista, PerClot, and 4DryField, and the log-rank test showed that the difference in time to achieve hemostasis between Surgicel and each of the test articles was also highly significant ($P < .0001$; Supplementary Table S3). Surgicel required fewer applications to achieve hemostasis compared with Arista ($P < .0001$), PerClot ($P = .0002$), and 4DryField ($P < .0001$; Figure 2C, Supplementary Table S2), as well as significantly lower amounts of powder than Arista ($P < .0001$), PerClot ($P < .0001$), and 4DryField ($P < .0001$) to stop bleeding from surgical defects in porcine liver and spleen (Figure 2D, Table 2, Supplementary Table S2). The Surgicel device could treat an average of 9.1 bleeding sites with the 3-g device compared with 1.9, 2.2, and 1.2 for the Arista, PerClot, and 4DryField devices, respectively, when considering all sites (Table 2).

3.2.2 | Experiment 2

A total of 30 defect sites per test article were assessed in 3 animals in experiment 2. Hemostasis was achieved within 5 minutes for 100% (30/30), 57% (17/30), and 53% (16/30) of defect sites treated with Surgicel, Arista, and PerClot, respectively (Figure 3A). Noninferiority tests (tests 1 and 2) were significant ($P < .0001$ for test 1 [Surgicel vs Arista], and $P < .0001$ for test 2 [Surgicel vs PerClot]). The lower bound of 1-sided 95% CI for the difference in efficacy rates between

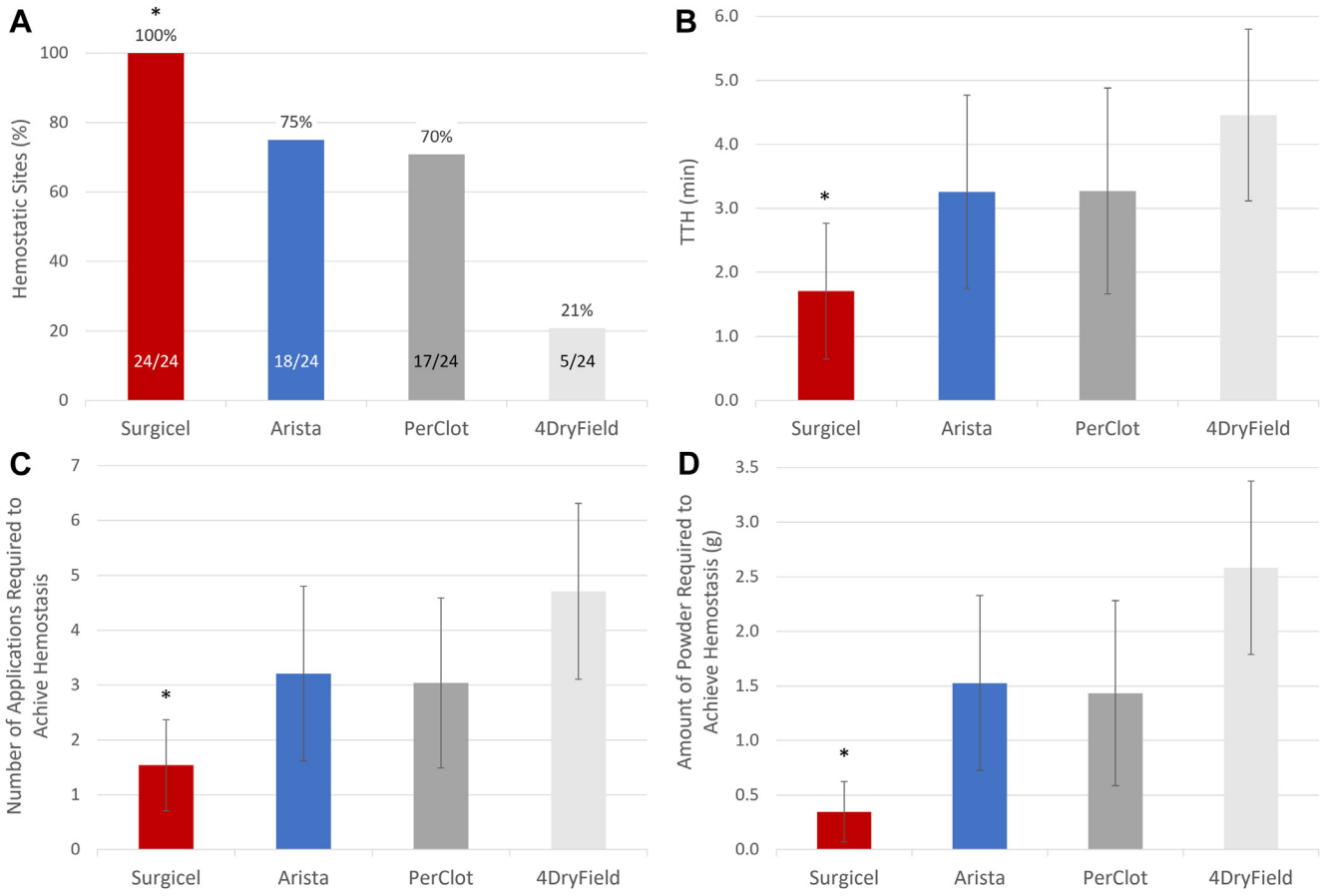

FIGURE 2 (A) Hemostatic efficacy, (B) time to hemostasis (TTH), (C) number of applications, and (D) amount of hemostat powder required to achieve hemostasis for experiment 1. (A) All defect sites (100%) achieved hemostasis after Surgicel treatment, whereas 75%, 70%, and 21% of defect sites were hemostatic after applying Arista, PerClot, and 4DryField, respectively. (B) Surgicel required significantly less time (*$P \leq .0002$), (C) a lower number of powder applications (*$P \leq .0002$), and (D) smaller amounts of powder (*$P \leq .0001$) to achieve hemostasis compared with Arista, PerClot, and DryField. Data are expressed as mean ± SD. Refer to Supplementary Table S2 for the full set of comparisons and their *P* values.

TABLE 2 Powder amount per bleeding site required to achieve hemostasis and number of bleeding sites that can be treated with preloaded test article devices.

	Total amount of powder contained in test article devices		Powder amount per bleeding site required to achieve hemostasis[a]						No. of bleeding sites that can be treated with a 3-g device	
			All sites (pass and fail)			Hemostatic sites (pass)			All sites (pass and fail)	Hemostatic sites (pass)
Test article	n[b]	mg (mean ± SD)	n[b]	mg (mean ± SD)	% (mean ± SD)	n[b]	mg (mean + SD)	% (mean ± SD)	A/B[c]	A/B[c]
Surgicel	24	3167.5 ± 61.9	24	346.7 ± 278.4	10.9 ± 8.8	24	346.7 ± 278.4	10.9 ± 8.8	9.1	9.1
Arista	24	2878.8 ± 284.4	24	1526.7 ± 801.0	54.2 ± 47.5	18	1318.9 ± 794.2	47.5 ± 30.0	1.9	2.2
PerClot	24	3083.8 ± 56.6	24	1432.5 ± 847.0	46.3 ± 27.2	17	1096.5 ± 724.8	35.7 ± 23.5	2.2	2.8
4DryField	24	3058.3 ± 30.9	24	2583.3 ± 793.1	84.4 ± 25.8	5	1292.0 ± 278.4	42.4 ± 31.2	1.2	2.4

All data shown are derived from experiment 1.

[a]Individual values were calculated by dividing the amount of powder used by the total amount contained in each device.

[b]Sample size (n) = the total number of liver and spleen bleeding sites per group. Each bleeding site was treated with a single device.

[c]This parameter was calculated as the ratio of (A) the total amount of powder (mg) contained in each device over (B) the average amount (mg) required to achieve hemostasis. All test article devices were preloaded with approximately 3 g of powder.

Surgicel and each of the test articles (tests 4 and 5) was greater than zero in both cases ($P < .0001$ for Surgicel vs Arista [test 4], and $P < .0001$ for Surgicel vs PerClot [test 5]). Thus, the hemostatic efficacy of Surgicel in experiment 2 was noninferior and superior to that of Arista and PerClot (Supplementary Table S1). TTH mean values for Surgicel, Arista, and PerClot are shown in Figure 3B and Supplementary Table S2. Two-sample t-tests of the mean differences in TTH between Surgicel and Arista and Surgicel and PerClot showed that Surgicel required significantly less time to achieve hemostasis compared with Arista ($P < .0001$) and PerClot ($P < .0001$; Supplementary Table S2). Furthermore, the median survival time (TTH) of Surgicel was lower than that of Arista and PerClot, with the log-rank test showing that the difference in time to achieve hemostasis between Surgicel and Arista and Surgicel and PerClot was also highly significant ($P < .0001$; Supplementary Table S3). Additionally, Surgicel required fewer applications than Arista ($P < .0001$) and PerClot ($P < .0001$; Figure 3C, Supplementary Table S2), as well as lower amounts of hemostat powder than Arista ($P < .0001$) and PerClot ($P < .0001$), to achieve hemostasis (Figure 3D, Supplementary Table S2).

3.2.3 | Cumulative hemostatic efficacy

Following the first product application in experiment 1, 63% of Surgicel-treated sites achieved hemostasis, whereas only 21%, 25%, and 13% of sites treated with Arista, PerClot, and 4DryField, respectively, had achieved a successful outcome (Figure 4A). After 4 consecutive applications, 100% of Surgicel-treated sites achieved hemostasis compared with 67%, 71%, and 21% of sites treated with Arista, PerClot, and 4DryField, respectively. No additional sites in experiment 1 reached hemostasis after either 5 powder applications of PerClot and 4DryField or 6 applications of Arista (Figure 4A). Similar cumulative efficacy profiles were observed in experiment 2, with 100% of Surgicel-treated sites achieving hemostasis after 3 applications. Arista and PerClot achieved maximum hemostatic rates of 57% and 53% after 5 and 4 applications, respectively (Figure 4B).

3.3 | *In vitro* experiments

3.3.1 | Powder expression efficacy

Powder expression data for the 4 topical hemostats in the 3 device positions assessed is shown in Figure 5, where cumulative expression is displayed against the number of applications. To investigate whether the full preloaded amount of powder (~3 g) could be expressed by each device, 20 powder expressions per device position were performed. In the vertical position, Surgicel expressed 78% of the total powder content after 6 applications, with increasingly smaller amounts expressed from applications 7 to 20, reaching a maximum cumulative expression of 99% (Figure 5A). Arista, PerClot, and 4DryField exhibited nonlinear expression profiles that increased rapidly in the first 2 to 3 applications and then plateaued out to a maximum cumulative expression of 92% (Arista and 4DryField) and 97% (PerClot) after 20 applications. The Surgicel device expressed a mean of 15% of the total powder content after the first expression (Figure 5A), whereas Arista, PerClot, and 4DryField expressed 74%, 48%, and 73%, respectively (Figure 5B–D).

The performance of the 3 starch-based devices decreased significantly when the devices were held at 45° and 180° angles (Figure 5B–D). The expression profile at a 45° angle was similar for the 3 starch-based devices, which also expressed similar maximum cumulative powder amounts, 82%, 75%, and 73% for Arista, PerClot, and 4DryField, respectively, after 20 expressions. In the slanted 45° angle position, the Surgicel device delivered a consistent amount of powder in the first 6 applications and a maximum cumulative amount of 98% after 20 applications (Figure 5A). In the horizontal position

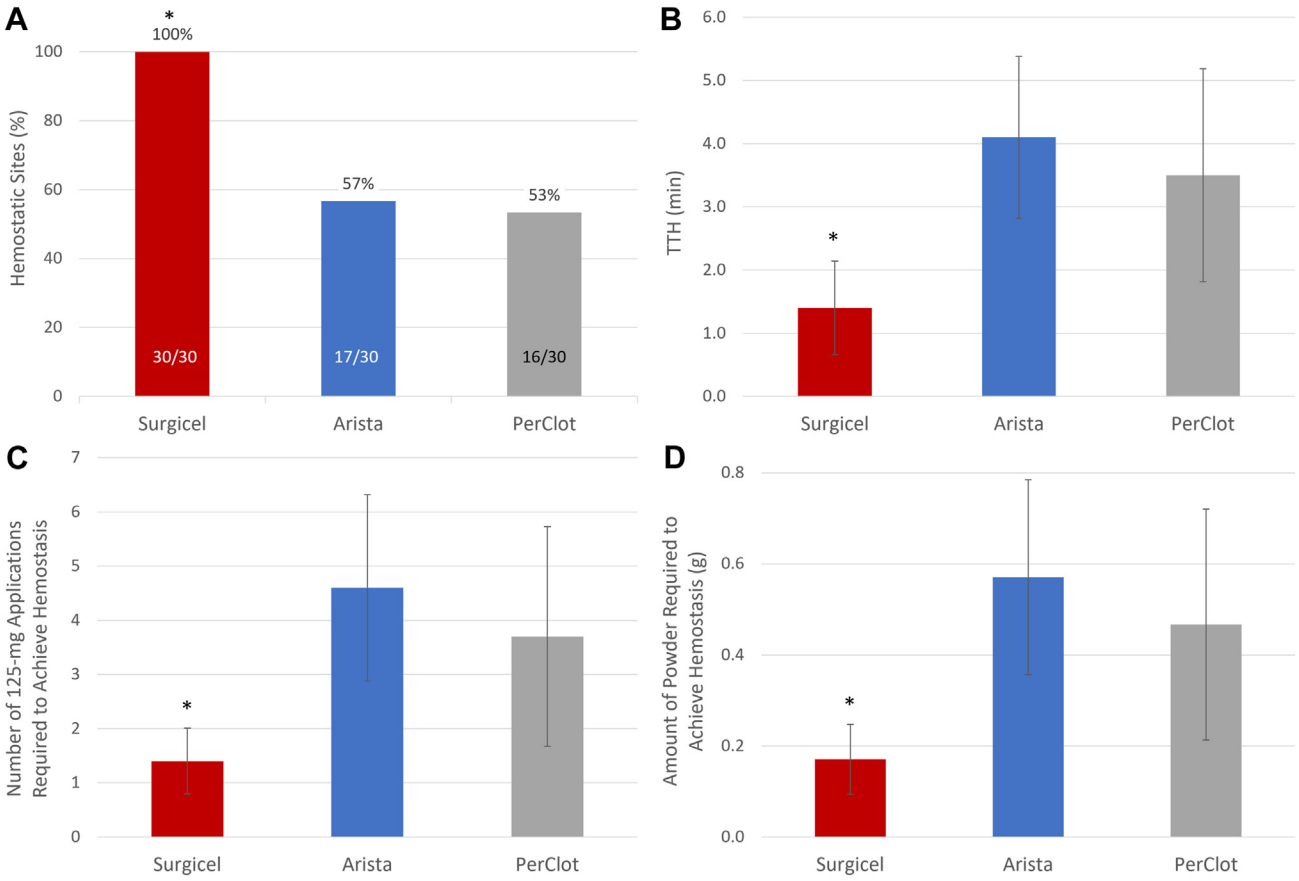

FIGURE 3 (A) Hemostatic efficacy, (B) time to hemostasis (TTH), (C) number of applications, and (D) amount of hemostat powder required to achieve hemostasis for experiment 2. (A) All defect sites (100%) achieved hemostasis after Surgicel treatment, whereas 57% and 53% of defect sites were hemostatic after the application of Arista and PerClot, respectively. (A) Differences in hemostatic efficacy rates between Surgicel and Arista or PerClot were statistically significant for noninferiority (*$P < .0001$) and superiority (*$P < .0001$; see also Supplementary Table S1). (B) Surgicel required less time (*$P < .0001$), (C) a lower number of powder applications (*$P < .0001$), and (D) smaller amounts of powder (*$P < .0001$) to achieve hemostasis compared with Arista and PerClot (see also Supplementary Table S2 for specific comparisons and their P values). Data are expressed as mean ± SD.

(180° angle), the Surgicel device maintained a linear expression profile in the first 6 applications and a maximum cumulative expression of 98% (Figure 5A). In the same horizontal position, the PerClot device expressed <1% of powder over the entire expression range of 20 applications, whereas the Arista and 4DryField devices expressed a maximum of 42% and 13%, respectively.

The cumulative expression of Surgicel for the first 6 data points at each device position (90°, 45°, and 180° angles) was fitted to the number of expressions using a linear regression model. Pearson's correlation coefficients (r) and their 95% CIs are shown in Figure 6. Surgicel showed the strongest correlation at each device position among the 4 powder hemostats evaluated. Significant differences between Surgicel and the 3 starch-based devices were found as Surgicel's 95% CIs did not overlap with those of Arista, PerClot, and 4DryField in any of the 3 device positions assessed (Figure 6). The 95% CIs of the correlation coefficients for Arista, PerClot, and 4DryField overlapped at each device position; hence, differences between the 3 groups were not significant. The linear regression data showed that the Surgicel device was more effective at delivering consistent amounts of powder per expression (11%-15%) over a critical application range (first 6 applications) in the 3 device positions.

3.3.2 | Blood clotting efficacy

BCM assay: The *in vitro* clotting efficacy of the 4 test articles was evaluated by measuring the mass of clotted blood that remained in the sample vial after a 2-minute incubation period with hemostat powder. BCM efficacy was significantly higher for Surgicel compared with Arista, PerClot, and 4DryField ($P < .05$; Figure 7A). Over 90% (92.6% ± 2.5%) of Surgicel-treated blood was effectively clotted after the incubation period. Blood incubated with Arista, PerClot, and 4DryField resulted in 15.3% ± 3.2%, 35.1% ± 3.7%, and 33.4% ± 1.9% of clotting efficacy, respectively (Figure 7A).

BCI assay: A free hemoglobin absorbance assay that provides a quantitative measure of the degree of blood clotting induced by different substrates was used to test the clotting efficacy of the 4 test

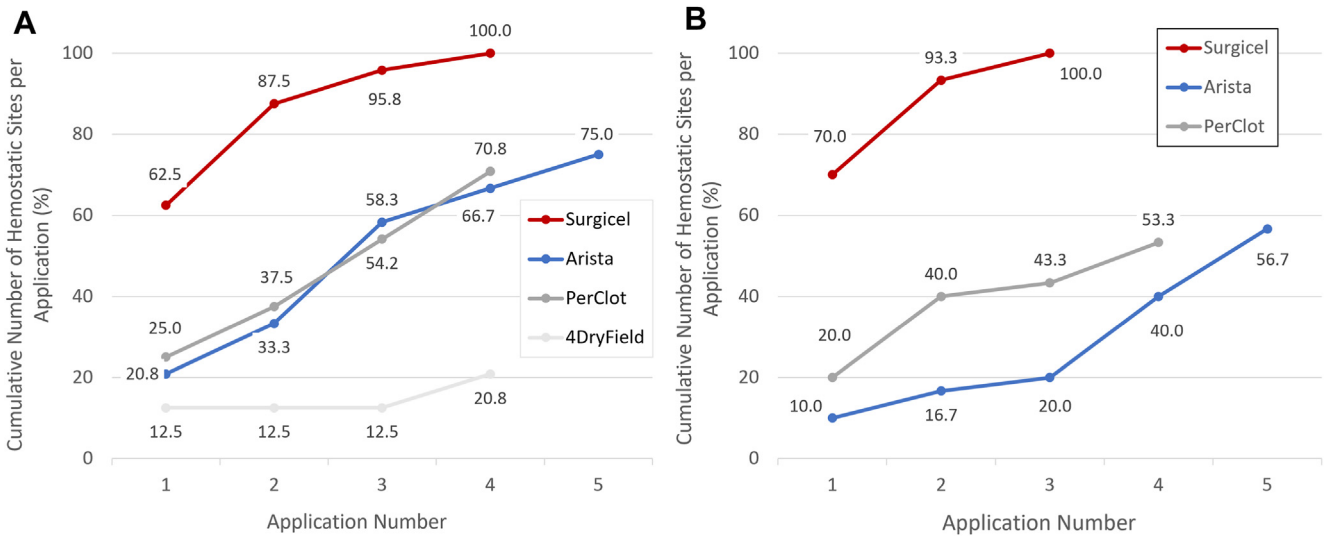

FIGURE 4 Cumulative percentage of sites that achieved hemostasis after 1 to 5 applications of the test articles for (A) experiment 1 and (B) experiment 2. In experiment 1, 15 Surgicel-treated sites (62.5%, 15/24) achieved hemostasis after a single powder application, whereas 5 (20.8%, 5/24), 6 (25.0%, 6/24), and 3 (12.5%, 3/24) defect sites achieved hemostasis following the first powder application of Arista, PerClot, and 4DryField, respectively. After 4 consecutive applications, 100.0% of Surgicel-treated sites were hemostatic compared with 66.7%, 70.8%, and 20.8% of sites treated with Arista, PerClot, and 4DryField, respectively. Experiment 2 showed a pattern similar to that observed in experiment 1, with Surgicel achieving 100% (30/30) hemostasis in all treated sites after 3 applications.

powder articles. Free hemoglobin concentration was measured at 2 minutes, and the BCI was calculated. Blood clotting was stimulated to different degrees by the 4 hemostatic agents (Figure 7B). The efficacy of Surgicel was significantly greater than the efficacy observed for Arista, PerClot, and 4DryField ($P < .05$). Surgicel achieved 86.2% ± 1.1% of clotting efficacy at 2 minutes. Efficacy values for Arista and PerClot were 4.2% ± 3.3% and 38.2% ± 5.8%, respectively, whereas those for 4DryField were 3.1% ± 3.4%, the lowest BCI values of the 4 test articles (Figure 7B).

4 | DISCUSSION

Hemostatic powders are a subclass of topical hemostats that can be applied over large regular or irregular surface bleeding areas, providing rapid and efficient hemostatic powder distribution and adherence. The 2 most common powder-based agents providing minimally invasive hemostasis are composed of ORC and starch polysaccharides [6]. Surgicel is safe and effective for mild to moderate bleeding control in a wide range of surgical procedures [34]. Arista, PerClot, and 4DryField have also been shown to be effective hemostats in various surgery types, including cardiothoracic [35], head and neck [36], and gynecological [37] surgeries, respectively. However, no comparative clinical or nonclinical studies have independently investigated the hemostatic efficacy and the device performance of ORC vs starch-based products.

Data from the current dual experimental approach demonstrated that ORC was the most efficacious powder agent tested, regardless of whether the product was delivered with (experiment 1) or without (experiment 2) its proprietary device. Surgicel displayed significantly higher hemostatic efficacy rates than Arista, PerClot, and 4DryField, and it was also a faster hemostat (lower TTH), requiring fewer applications and smaller amounts of powder to achieve hemostasis. 4DryField, an antiadhesive agent that also exhibits hemostatic actions [37,38], displayed the lowest *in vivo* efficacy of the 4 formulations tested and required the largest amount of powder per bleeding site to achieve hemostasis. Cumulative efficacy rates evidenced large efficacy gaps between Surgicel and each of the starch-based agents following 1 to 5 powder applications, with 4DryField exhibiting the largest gaps from applications 1 to 4. Estimates using experiment 1 data indicate that the Surgicel device can treat more bleeding sites than the starch-based devices. Specifically, the Surgicel device can treat 4.8 (9.1/1.9), 4.2 (9.1/2.2), and 7.7 (9.1/1.2) additional bleeding sites than the Arista, PerClot, and 4DryField devices, respectively.

The hemostatic efficacy of powder-based agents is intrinsically linked to the performance of its delivery device/applicator. A device loaded with a highly efficacious powder hemostat but a low-performance applicator may limit the device's effectiveness at critical times during surgery. Thus, the optimal device must perform reliably over a wide range of positions used by surgeons in real-world scenarios, from the nearly horizontal to the fully vertical. In addition, the optimal device must deliver consistent amounts of powder over multiple expressions to achieve fast and durable bleeding control. Pilot studies from our laboratory indicated that other variables, including compression speed and force applied by the operator, were less impactful on the amount of powder dispensed/expressed in consecutive applications than device position (eg, vertical or horizontal).

Data from experiment 1 showed that no bleeding site treated with any of the 4 hemostats required more than 6 powder applications to achieve hemostasis. Hence, this range of applications was deemed clinically relevant for further analysis. The Surgicel device was the

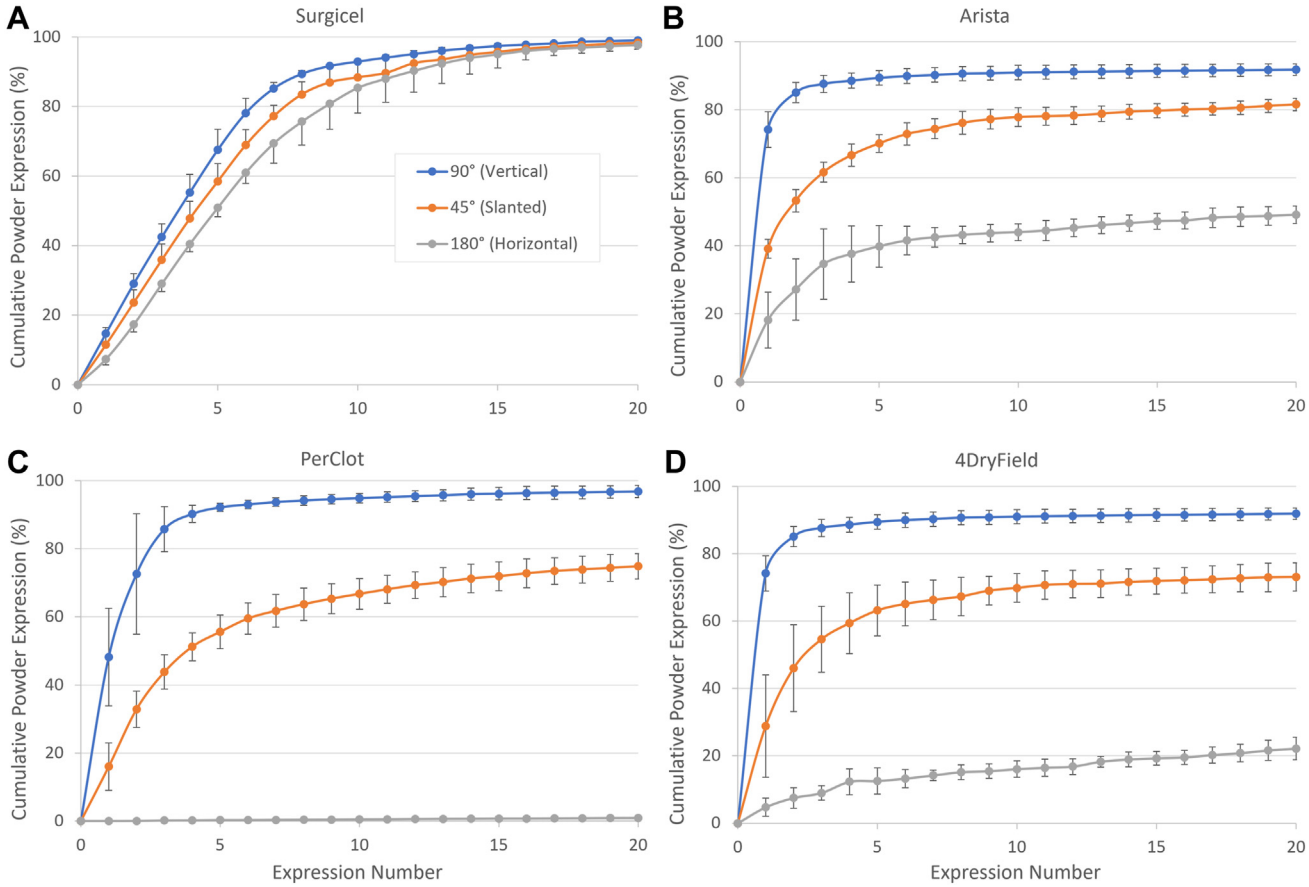

FIGURE 5 *In vitro* powder expression for the 4 test articles using 3 device positions (90°, 45°, and 180° angles) and 20 expressions per device. Surgicel exhibited a consistent linear powder expression profile over the first 6 consecutive applications for any device position. Significantly lower amounts of powder were released when the Arista, PerClot, 4DryField devices were held horizontally (gray markers/lines in panels B–D) or at 45° angles (orange markers/lines in panels B–D) compared with (A) Surgicel in the same positions. (C) Furthermore, negligible amounts of powder were released when the PerClot device was tested horizontally. While 14% of the total amount of Surgicel powder was released after the first expression in the vertical position, 74%, 48%, and 72% of the device's total powder content for Arista, PerClot, and 4DryField, respectively, were released after their first expression in the same vertical position. After 3 expressions in the vertical position, 88%, 86%, and 90% of the total amount of power was expressed for Arista, PerClot, and 4DryField, respectively, leaving only 14%, 12%, and 10% of hemostatic powder available for further use, respectively.

only applicator able to express consistent amounts of powder over this range of applications at each of the 3 device positions (90°, 45°, and 180° angles) examined. Performance of all 3 starch-based devices was limited by the excessive wasteful amounts of powder delivered in the first 2 to 3 applications when the device was held at either 90° or 45° angles, which represent the upper and lower bounds, respectively, of a typical device inclination range used during surgical procedures in humans. Of note, powder expression for PerClot at 180° was negligible over the entire range of expressions evaluated.

Several factors may be involved in the efficiency differences in powder expression observed between the devices tested, including powder flowability, particle size distribution, powder density, and particle sphericity [31]. However, in our experience prototyping delivery devices, the most influential factor leading to differences in powder expression is the design of the delivery device. Unlike the starch-based devices tested, which store the hemostatic powder within the bellows, the Surgicel device stores the ORC powder in its middle section, and it is equipped with a unique spring-loaded mechanism that allows for precise and consistent delivery of powder, regardless of device position [26]. The device also features a rotary on/off valve and a built-in tortuous output path in the applicator, which prevents wasteful powder leakage when the device is held vertically or at slanted angles [26]. The bellows pump used by the Arista, PerClot, and 4DryField devices serves not only as a pump but also as a powder reservoir. This dual function can result in powder accumulation within the device convolutions, leading to wastage due to incomplete emptying, regardless of the device's orientation or the compression force applied to the pump. The design engineering features of the Surgicel device translate into consistent device performance, ease of application, and precise control of powder delivery across a broad range of surgical approaches, including difficult-to-reach anatomical spaces requiring holding the delivery device at slanted or horizontal positions.

The BCM and BCI assays demonstrated that Surgicel was more than twice as efficacious as any of the 3 starch-based hemostats tested

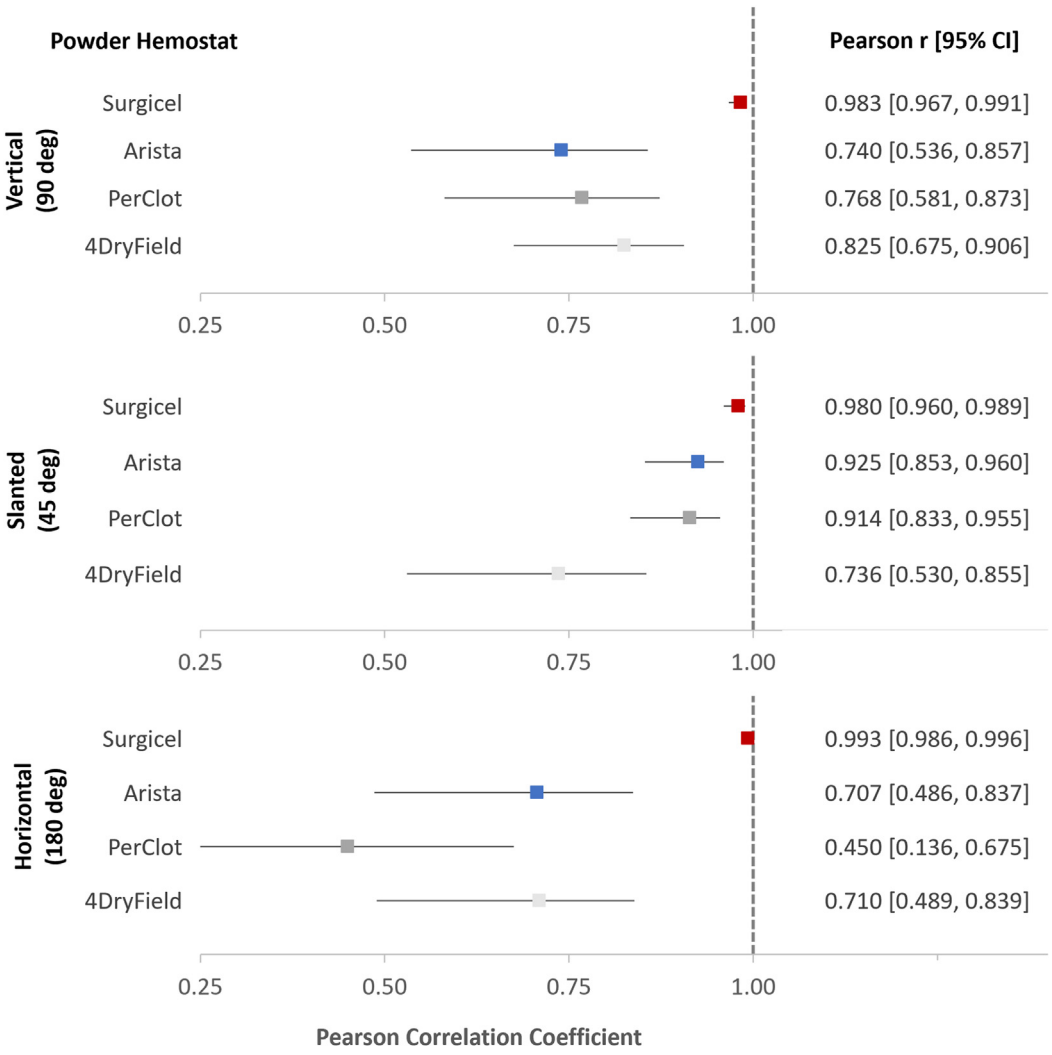

FIGURE 6 Linear regression analysis of cumulative powder expression and number of expressions. Independent analyses were conducted for each device held at 3 positions: vertical (90°), slanted (45°), and horizontal (180°). Six devices were tested at each position for a total of 18 devices per product. To assess powder expression consistency, the first 6 consecutive expressions for each powder expression curve shown in Figure 5 were used to fit the data into a linear equation to generate Pearson correlation coefficients (r). The first 6 data points were chosen because no bleeding site required more than 6 applications to achieve hemostasis for any of the products evaluated. deg, degree angle.

in their capacity to accelerate whole blood clotting. ORC decreases the local pH and causes RBC lysis, an event evidenced by the brown discoloration of blood samples incubated in its presence [18]. The cytosolic content of ORC-mediated RBC lysis, comprised mainly of hemoglobin, is released to the local environment, where it limits the availability of nitric oxide, prevents vasodilation, and promotes platelet adhesion [39–41]. These ORC-triggered actions contribute to local hemostasis at the bleeding sites. The higher clotting efficacy of ORC may be due to its lower surface area and wettability values and the greater capacity of high-sphericity Surgicel aggregates to penetrate the blood interface, resulting in a more efficient clot formation process [31]. ORC has been shown to accelerate clot formation by providing a matrix for platelet adhesion and aggregation [17]. Some of the aforementioned actions may be linked to the physical and mechanical properties of cellulose, the primary structural component of plant cell walls, including its high tensile strength and elasticity [10]. Interestingly, the functional groups present in the cellulose molecule have triggered novel experimental approaches to augment its hemostatic actions while preserving its mechanical and procoagulant properties [10].

The lower hemostatic efficacy of starch-based products in the *in vivo* setting reported here may be partially accounted for by the limited procoagulant activity of starch. Like cellulose, starch is a natural, low-cost, plant-derived polysaccharide. It is composed of 2 fractions, amylopectin (~70%) and amylose (~30%), and can be sourced from corn, rice, wheat, and potatoes [42]. Starch exhibits high swelling power (water-holding capacity) and hemostatic properties [43,44]. However, because of its fast dissolution in biological fluids, starch degrades rapidly and cannot form stable structures. To enhance the mechanical strength of starch-based agents, research efforts have been directed toward chemical modifications of starch via copolymerization and the development of polyvinyl alcohol/starch polymer blend hydrogels [45]. Compellingly, cellulose nanofibers have

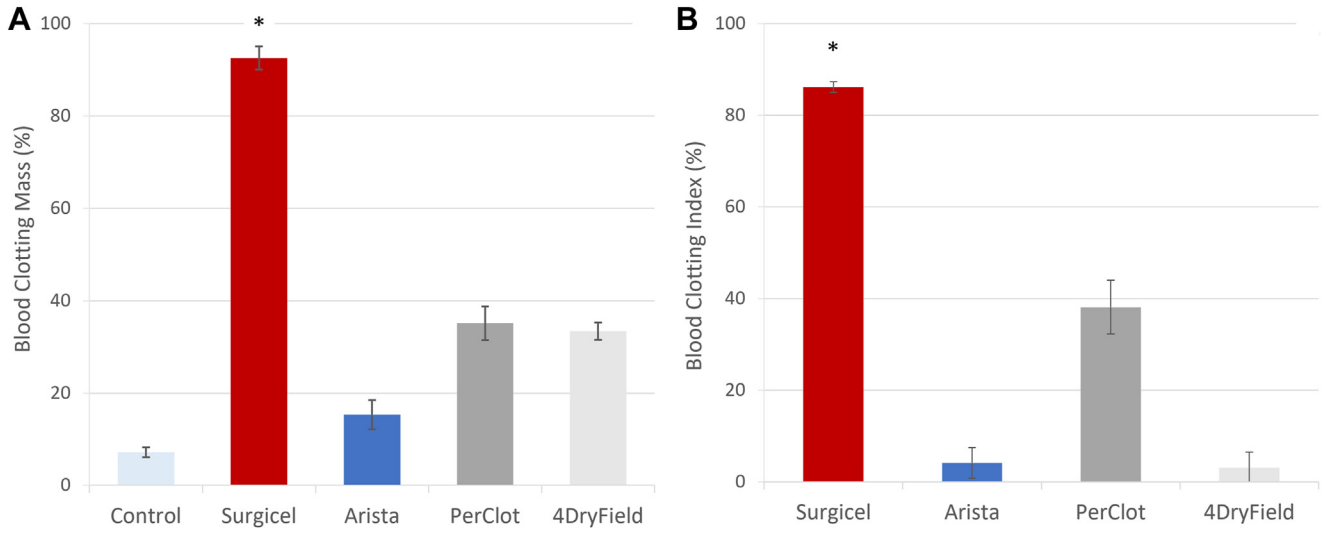

FIGURE 7 (A) Blood clotting mass and (B) blood clotting index assays. (A) Over 90% (92.6% ± 2.5%) of Surgicel-treated blood was effectively clotted, whereas blood incubated with Arista, PerClot, and 4DryField resulted in 15.3% ± 3.2%, 35.1% ± 3.7%, and 33.4% ± 1.9% of clotting efficacy, respectively. Untreated blood used as negative controls showed low levels of spontaneous clotting. (B) Blood clotting index efficacy of Surgicel was significantly greater compared with Arista, PerClot, and 4DryField ($P < .05$). Surgicel achieved 86.2% ± 1.1% of clotting efficacy at 2 minutes. Clotting efficacy values for Arista, PerClot, and 4DryField were 4.2% ± 3.3%, 38.2% ± 5.8%, and 3.1% ± 3.4%, respectively. *$P < .05$.

been recently used in the development of novel starch-based biopolymers with hemostatic properties, the rationale being to improve the mechanical properties of starch hydrogels and reduce the rapid degradation rates of the starch component [42].

4.1 | Study limitations

For experiment 1, the *in vivo* study was randomized to the order of articles tested, and the surgeon was blinded to treatment identity until the bleeding site was scored and recorded; however, the device's physical features precluded masking device identity. For experiment 2, the surgeon was also blinded to the powder being tested without the use of proprietary devices. Experiment 1 was conducted before experiment 2 so that the powder remaining in the devices could be aliquoted for use in experiment 2. Due to the substantially high amounts of starch-based 4DryField powder per bleeding site required to achieve hemostasis in experiment 1, not enough 4DryField powder was available to be evaluated in experiment 2, and thus experiment 2 was performed with 3 hemostats, Surgicel, Arista, and PerClot. Nonetheless, based on the low efficacy of 4DryField when the powder was applied with its proprietary device (experiment 1), we would not expect this test article to perform better when applied directly over the bleeding sites (experiment 2).

5 | CONCLUSION

The Surgicel hemostat has been demonstrated to be superior to 3 starch-based hemostats – Arista, PerClot, and 4DryField – in both efficacy and speed in stopping bleeding. This study is groundbreaking in that it proves that regardless of whether hemostatic powders were applied using proprietary devices or delivered directly over the bleeding site, Surgicel outperforms all other hemostats. Moreover, the device's performance, which is typically not tested independently of hemostatic efficacy, has shown that Surgicel is the only surgical device capable of reliably delivering sufficient hemostatic powder in 3 clinically relevant device positions (90°, 45°, and 180° angles). Additionally, the Surgicel device consistently delivers adequate powder quantities required to successfully treat multiple bleeding sites with a single device. The effective management of mild to moderate tissue bleeding encountered by surgeons across various surgical specialties relies heavily on the successful combination of hemostatic powder efficacy and device performance.

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AUTHOR CONTRIBUTIONS

G.C., M.S., H.D.L., B.C., R.K., and A.Y.W. conceived the study design and planned the *in vivo* and *in vitro* experiments. M.S. and B.C. performed the *in vivo* experiments (experiment 1 and experiment 2). M.S., B.C., G.C., and H.D.L. contributed to the interpretation of results from all *in vivo* experimentation. A.Y.W. and S.A. performed all *in vitro*

experiments (BCM, BCI, and powder expression assays) and provided data analysis and interpretation. H.G.W. conducted statistical analyses for all *in vivo* and *in vitro* experiments. H.D.L. wrote the manuscript with support from M.S. and A.Y.W. All authors discussed the results, provided critical feedback, and contributed to developing the final version of the manuscript.

RELATIONSHIP DISCLOSURE

All authors are Ethicon, Inc employees. H.D.L. is a professional medical writer also employed by Ethicon, Inc.

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SUPPLEMENTARY MATERIAL

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