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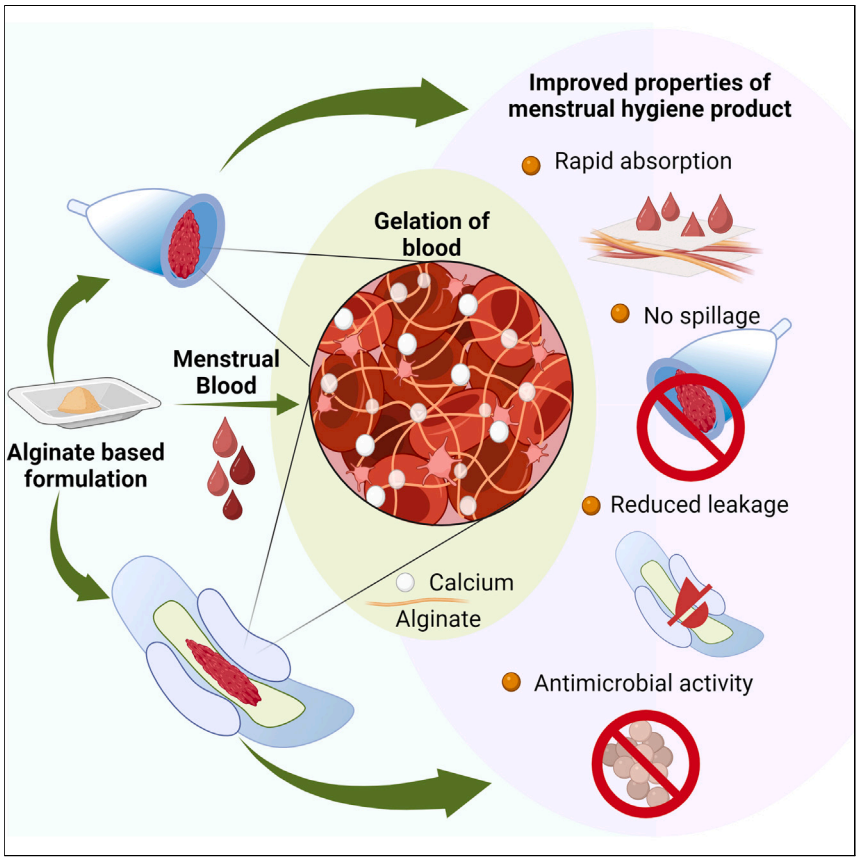
A naturally derived biomaterial formulation for improved menstrual care



Our work examines an alternative way to manage menstrual fluid. Instead of absorbing or retaining it, we aimed to "solidify" it so that it can be more easily managed with reduced leakage or spillage. We show that this environmentally friendly material can improve how menstrual pads and menstrual cups perform. We hope that our work will improve the quality of life for women and lead to further interest in advancing women's health.

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Highlights

Alginate can substantially increase the viscosity of defibrinated blood

Alginate-glycerol materials can readily absorb and retain blood

Alginate-glycerol materials improve the performance of menstrual care products

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4 **Demonstrate**
Proof-of-concept of performance with intended application/response

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Article

A naturally derived biomaterial formulation for improved menstrual care

Rogério Aparecido Bataglioli,[1] Harsimran Kaur,[1] John Muller,[2] Elizabeth Geddes,[1] Carrie Champine,[3] and Bryan B. Hsu[1,4,*]

SUMMARY

Adequately managing menstruation is an important factor in the overall quality of life for women. With a growing discussion of the global need for its improvement, it is clear that better management of menstruation can positively influence social, educational, and professional outcomes. Herein, we describe a biopolymer-based formulation that gels blood in a mechanism alternative to coagulation. We first tested several biopolymer mixtures with blood and quantified increases in viscosity, finding that high-molecular-weight alginate in combination with glycerol could rapidly absorb and gel blood. We then demonstrated that this powder could be deployed both as a traditional menstrual pad filler and as an additive to menstrual cups to reduce leakage and spillage, respectively. Finally, we include an antimicrobial polymer to impair the growth of *Staphylococcus aureus*, a bacterium associated with toxic shock syndrome. Collectively, our work describes a biodegradable formulation derived from renewable resources that can improve menstrual care.

INTRODUCTION

Menstruation is a natural biological process for which cultural stigmas and an absence of societal or infrastructural support can have profoundly negative effects for women and girls. Menstruation can last between 3 and 7 days and occurs at monthly intervals roughly from age 13.6 to 49.6, with a median of 451 cycles per lifetime.[1] Among several menstruation-associated factors that disproportionately impact women, poor performance of menstrual products is a major factor in absenteeism,[2] especially in regions where menstruation is considered taboo.[3] In low- to middle-income countries (LMICs), as well as low-income areas in the United States,[4] the inaccessibility or unaffordability of menstrual products can lead to alternatives (newspapers, rags, leaves) that increase the risk of vaginal infection.[5]

Another concern with current menstrual products is the environmental consequences of waste generated by single-use disposable pads and tampons. A woman can use up to 15,000 disposable menstrual products in her lifetime,[6] with most products containing synthetic materials as supporting components (topsheets and backsheets in pads or strings in tampons), as packaging, or as the absorbent component for some products (i.e., superabsorbent polymers like cross-linked polyacrylic acid [PAA]).[7] PAA, which is also used in other absorbent products like diapers and absorbent pads, is non-degradable, with <1% degrading under landfill conditions.[8] Because of environmental concerns, reusable menstrual cups have become more popular. Regardless of the type of menstrual product, the challenges associated

PROGRESS AND POTENTIAL

Menstruation is a natural biological process experienced by women. Inadequate menstrual care can significantly impact a woman's quality of life, leading to potential setbacks in education and career advancement. The principal strategies of current menstrual products have relied on the absorption or retention of menstrual fluid, which frequently results in leakage or spillage. In addition, single-use menstrual care products are an environmental concern. In this work, we describe a different strategy for managing menstrual fluid by turning it into a gel so that it can be more easily managed with reduced leakage or spillage. We show that by "solidifying" blood with a naturally derived and biodegradable biomaterial, we can improve the use of hygiene products in a pad or cup format. We hope that our work can improve menstrual care and lead to further advancements in improving the quality of life for women.

with menstrual care, such as leakage and spillage during use and management, can lead to shame and distress that leads to withdrawal from daily activities.[9]

To improve the quality of life in women undergoing menstruation, we aimed to develop a biomaterial-based menstrual product that would improve the management of menstrual fluid by reducing its leakage during use, by minimizing its spillage during changing, and by simplifying the process to change/replace menstrual products in the absence of appropriate facilities. In using a biodegradable material to absorb this fluid and increase its resultant viscosity, we hypothesized that we could improve menstrual hygiene and care with broadly familiar products in a more sustainable manner.

In this study, we first characterized an array of polysaccharides for their ability to increase the viscosity of blood. Among these materials, we found that high-molecular-weight (HMW) alginate in combination with a glycerol additive could rapidly absorb and gel blood to produce a highly viscous gel that maintained stiffness for long durations. We then incorporated this powder formulation into common menstrual care products. When used as a menstrual pad filler, we found that this formulation substantially reduced blood permeation with increased blood retention and reduced transfer compared to other common absorptive fillers. We then used this formulation as an additive for menstrual cups and showed that it eliminated blood spillage during *in vitro* menstrual cup removal. Finally, to minimize bacterial growth, we showed that the incorporation of an antimicrobial polymer into this powder formulation inhibits *Staphylococcus aureus* without impairing the formulation's ability to solidify blood. Collectively, our work describes a biodegradable, naturally derived biomaterial product capable of improving menstrual care.

RESULTS

Polysaccharide solutions mixed with blood can substantially increase viscosity

As a practical assessment of viscosity, we developed an assay in which we measured the time required for an 80 μL droplet to flow down an acid-cleaned glass test tube (Figure 1A). Using increasing concentrations of low-molecular-weight (LMW) alginate, we confirmed that droplet flow time was dependent on the tube tilt angle (Figure S1) and had a good correlation with kinematic viscosity as measured by a Cannon-Fenske viscometer (Figure 1B). Menstrual fluid is a complex mixture of blood, tissue, and mucus that is unable to coagulate[10] due to an abundance of fibrinolytic proteases.[11] To mimic human menstrual blood, we used defibrinated porcine blood, which has previously been used as the basis for simulated menstrual fluids.[12] To determine whether polysaccharides can increase the viscosity of blood, we prepared well-mixed equivolume mixtures using 1% w/v polysaccharide solutions combined with water or blood (0.5% w/v final polysaccharide concentration) and measured their flow time. Several polysaccharides had high flow times when mixed with water or blood (Figure 1C), but only kappa-carrageenan, HMW alginate, xanthan gum, and iota-carrageenan had significantly greater flow times when mixed with blood compared to water. Alginates in this test were in the sodium form, lacking divalent cations, and are hereafter referred to as alginate. When testing LMW alginate samples that would be more sensitive to degradation over time than HMW alginate, we found that flow times had an expected dependence on concentration, which remained consistent over time (Figure S2). Despite the increased viscosity of well-mixed polysaccharide-blood mixtures, their practical application would depend on their miscibility with menstrual fluid. To test this, we added blood to select polysaccharide solutions without mechanical agitation and found that only alginates could reach homogeneity (Figure 1D). Inversion of tubes after the 4 h incubation showed the formation of a cohesive hydrogel when blood was mixed with HMW alginate, but not the other

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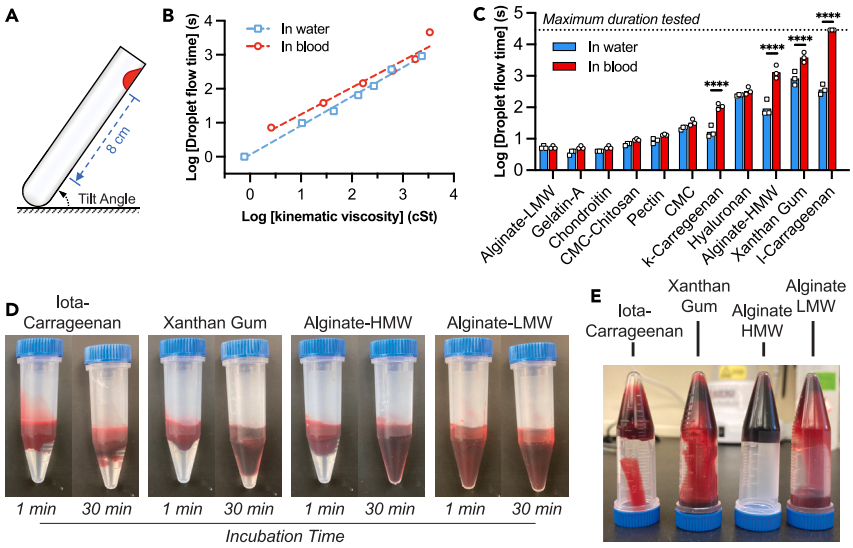

Figure 1. Biopolymers can increase the viscosity of well-mixed solutions of water and defibrinated blood

(A) Setup of the droplet flow test.

(B) Droplet flow time scales proportionally with the kinematic viscosity of the alginate solution.

(C) The screen of flow times for biopolymer solutions well mixed with water or defibrinated blood to a final concentration of 0.5% w/v. Statistical comparisons were performed by two-way ANOVA with a Bonferroni multiple comparisons test. ****$p < 0.0001$.

(D and E) Highly viscous biopolymer solutions can remain phase separated from blood if not well mixed (D), which affects their ability to solidify blood (E). Bars in (D) and (E) correspond to 20 mm.

polysaccharides (Figure 1E). Alginate is well known for hydrogel formation mediated by divalent cations, as shown with calcium-mediated cross-linking,[13] a mechanism also identified in carrageenans.[14] Calcium is present in blood[15] and vaginal fluid throughout the menstrual cycle,[16] so it is likely that calcium-mediated cross-linking contributes to the increased viscosity of alginate mixed with blood. To investigate the properties important for flow time when mixed with blood, we tested several alginates with varied molecular weights and ratios of D-mannuronate to L-guluronate (M/G) as well as comparing water, NaEDTA-treated blood, and defibrinated blood. At low viscosities, M/G ratio and the use of EDTA as an anticoagulant influenced flow time, but these effects were dampened at high alginate viscosities, where polymer length is the dominant factor (Figures S3A and S3B). The viscosity of HMW alginate with different blood samples also revealed an important role for plasma in the gelation process. As shown in Figure S3C, when divalent cations are chelated by EDTA, the resultant alginate-blood mixture has a faster droplet flow time than alginate mixtures with defibrinated blood. Comparable experiments with plasma instead of whole blood showed that plasma from NaEDTA-treated blood flowed faster by an even greater margin than plasma from defibrinated blood. Together, these results indicate that calcium in the plasma is a major driver of gelation with alginate. In addition, we characterized the molecular properties of the LMW and HMW alginates used. LMW and HMW designations were provided by the alginate manufacturers. As one would expect, size-exclusion chromatography (SEC) analysis revealed that the molecular weight of LMW alginate was lower than that of HMW alginate, although the difference is only ~2- to 4-fold. In addition, nuclear magnetic resonance (NMR) characterization revealed M/G ratios of 1.24 and 1.81, respectively (Table S1). Collectively, these results indicate that the molecular weight of alginate plays an important role in the calcium-mediated gelation of defibrinated blood.

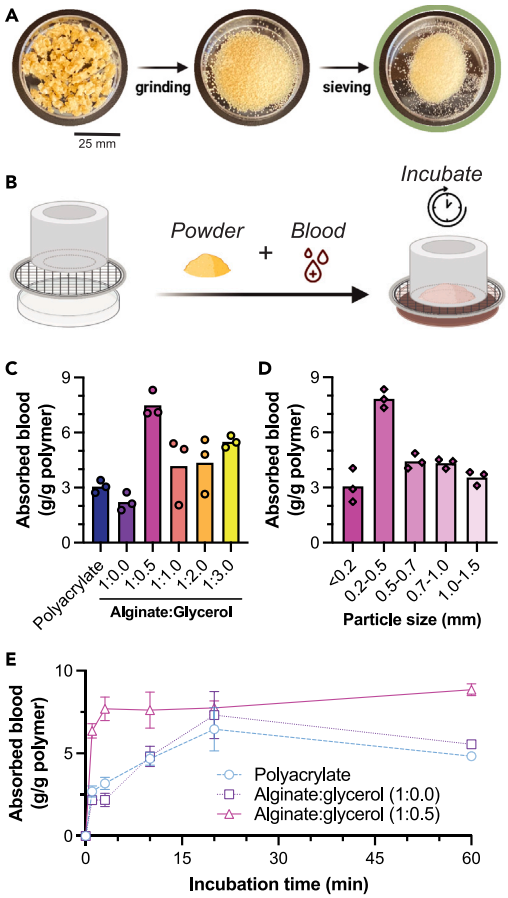

Figure 2. Alginate-based formulations can be tuned to promote maximum blood absorption capacity

(A) Images of the processed alginate-glycerol formulation after drying, grinding, and sieving, with particle sizes ranging from 0.2 to 0.5 mm. Bar corresponds to 25 mm.

(B) Schematic representation of the setup used for testing powder blood retention.

(C) Blood retention for alginate formulations presenting different alginate-to-glycerol ratios (particle size of 0.2–0.5 mm) after 3 min of incubation.

(D) Impact of the particle size on blood retention for $AG^{0.5}$ after 15 min incubation.

(E) Time series experiment to determine blood retention for superabsorbent PAA, high-molecular-weight alginate reagent, and the $AG^{0.5}$ (particle size 0.2–0.5 mm). Error bars represent standard deviations.

Next, we examined the alginate distribution in static mixtures of alginate solutions with blood by tracking the diffusion of rhodamine-labeled alginate spiked into the initial alginate solutions. We found that, from its initial position in the bottom of the tube, alginate diffused to the top region (Figure S4A). Alginate was confirmed to increase the viscosity of the top, middle, and bottom regions by droplet flow test (Figures S4B and S4C).

Alginate-based powders are highly absorptive for blood

For practical application, we aimed to develop a powder formulation that could absorb and solidify blood. Powders were prepared by solubilization in specific aqueous solutions, dried, mechanically ground, and then sieved by size (Figure 2A). To test blood retention, we added 0.5 g of each test powder to the top of an ~50 mm^2 mesh screen, which was retained within a 25.4 mm inner diameter cylinder (Figure 2B). We tested the absorptivity of these powder formulations by adding 5 mL of blood and incubating for

3 min, after which we removed the cylinder to allow unabsorbed blood to bypass the powder and flow through. Initial tests with non-sieved powders showed that alginate in the absence of glycerol ("alginate/glycerol (1:0.0)") performed similarly to the gold standard superabsorbent polymer, cross-linked polyacrylate (Figure 2C). During the experiments, we found that blood did not completely permeate the alginate/glycerol (1:0.0) powder, leaving a dry core. We then prepared alginate powders containing several concentrations of glycerol, which has previously been used as a plasticizer in films and increases their hydrophilic character.[17,18] Of the several alginate-to-glycerol ratios, we found that a 1:0.5 ratio led to maximal blood absorption (Figure 2C). For this ratio ($AG^{0.5}$), particle sizes ranging from 0.2 to 0.5 mm had superior performance (Figure 2D), possibly due to the surface/volume ratio that enhances contact between polymer and blood with reduced powder aggregation. To determine if blood absorption was maintained over time, we incubated powder-blood mixtures for up to 60 min and found that the alginate-glycerol powders rapidly absorbed blood to levels that were maintained for the duration of the experiment, whereas polyacrylate and alginate-only powders absorbed blood slowly during the first 20 min and then phase-separated by 60 min (Figure 2E). We then measured the release of alginate from these gels using a powder mixture spiked with alginate rhodamine and found that ~13% of alginate was released after 8 h of incubation in blood and phosphate-buffered saline (PBS) (Figure S5).

Material properties of alginate-glycerol formulations

For insight into the performance of the alginate-glycerol material, we examined its material properties. Using contact-angle measurements, we confirmed the greater hydrophilic nature of alginate-glycerol films (i.e., lower water contact angles) compared to alginate films (i.e., higher contact angles), an effect that does not substantially change over 100 s (Figure 3A). Examination of the morphology of these powders by scanning electron microscopy (SEM) at low magnification shows a generally irregular particle shape for pristine alginate, $AG^{0.5}$, and polyacrylate particles (Figures 3B–3D, respectively). Higher magnification shows that alginate has a dense internal structure (Figure 3B, inset), similar to what has been observed previously for alginate powders,[19] whereas the incorporation of glycerol leads to greater texture and a more porous open structure (Figure 3C, inset). By comparison, cross-linked polyacrylate has a smooth surface morphology that is indicative of a crystalline structure previously observed[20] (Figure 3D, inset). Particle sizes determined from the micrographs revealed that pristine alginate had the smallest projected area and diameter, followed by $AG^{0.5}$ and polyacrylate (Figures 3E and 3F). We then determined whether the calcium in blood had a gelation effect similar to that of calcium in buffer by comparing their rheological properties when mixed with the alginate-glycerol powder. Using a strain sweep, we determined the linear viscoelastic range and used 1% for frequency sweep analysis (Figure S6). As shown in Figure 3G, the storage and loss moduli are greater when mixed with blood compared to $CaCl_2$, which suggests that other components of blood (e.g., cells) play a role in the gelation process. In addition, the greater magnitude of storage to loss modulus in both conditions supports a stiffer gel-like behavior that has been previously observed with alginate hydrogels.[21] Inductively coupled plasma mass spectrometry (ICP-MS) analysis of the defibrinated porcine blood we used showed an extracellular Ca^{2+} concentration of 2.47 mM, which is similar to what is found in menstrual fluid (2.5 mM[22]) and similar to the 2.5 mM used in the calcium buffer. Taken together, these results indicate that the inclusion of glycerol into the alginate powders likely improves its dissolution into blood and that these $AG^{0.5}$ mixtures with blood have rheological properties comparable to those of mixtures with aqueous $CaCl_2$ solutions.

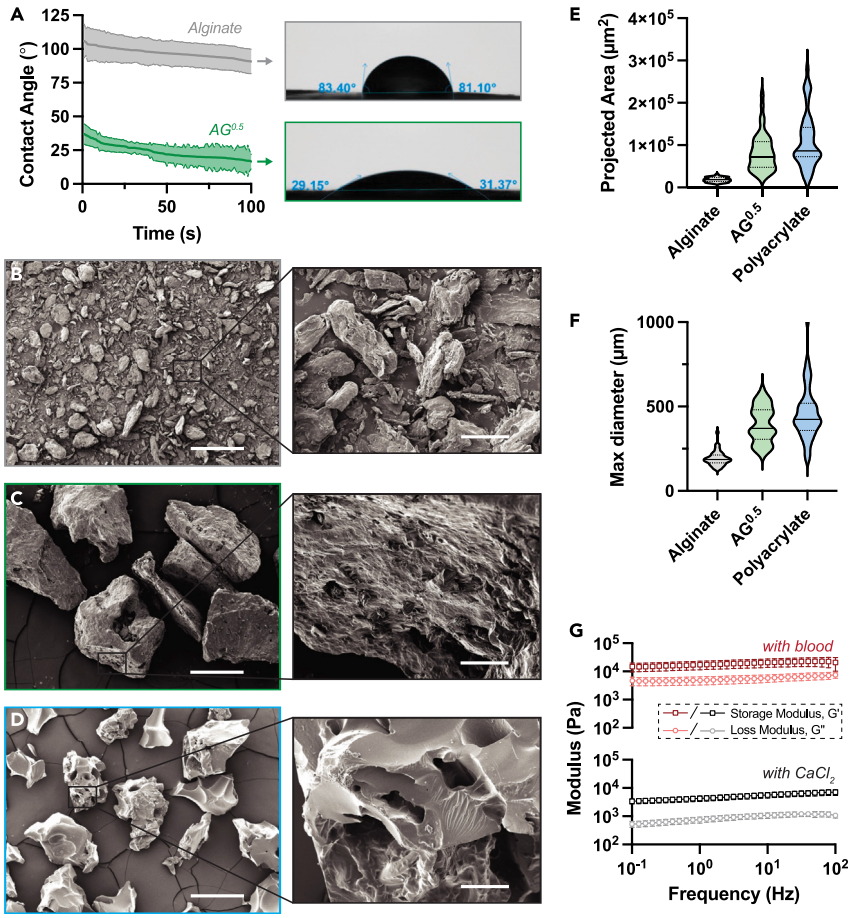

Figure 3. Material properties of alginate formulations

(A) Water contact-angle measurements of alginate or $AG^{0.5}$ films over time with representative images. Bold lines represent means and shaded areas represent standard deviations.

(B–D) SEM micrographs of alginate (B), $AG^{0.5}$ (C), and cross-linked polyacrylate particles (D). Scale bars represent 500 μm for the low-magnification images or 50 μm for the higher-magnification insets.

(E and F) Projected particle area (E) and particle diameter (F) were calculated from the micrographs using ImageJ. (G) Frequency sweep of 0.1 g/mL of pristine alginate or $AG^{0.5}$ powders mixed with blood or calcium chloride solutions at a final concentration of 2.5 mM. Error bars represent standard errors.

Alginate-glycerol powders absorb blood with high retention as menstrual pads

To test the absorptivity of our alginate-glycerol powders, we simulated their application as a menstrual pad, but without topsheets or backsheets to enable the measurement of blood leakage. As shown in Figure 4A, various fillers (nothing, cross-linked polyacrylate, $AG^{0.5}$, or the commercial cellulose-based filler) were spread as a single layer within a folded sheet of cotton gauze and then taped to the opening of a silicone-based artificial vagina (Figure 4B). We used this device to simulate the channeling of menstrual fluid by the vaginal canal onto an irregular but focused area, testing the effect of overloading a small area. We also did not observe significant differences between the absorptive capacities of commercial tampons tested with defibrinated blood or an FDA-recommended Syngina fluid (Figure S7). We tested for blood permeation by rapidly (<30 s) adding 8 mL of blood to the top of the artificial vagina. This volume within a short time frame simulates prolonged accumulation

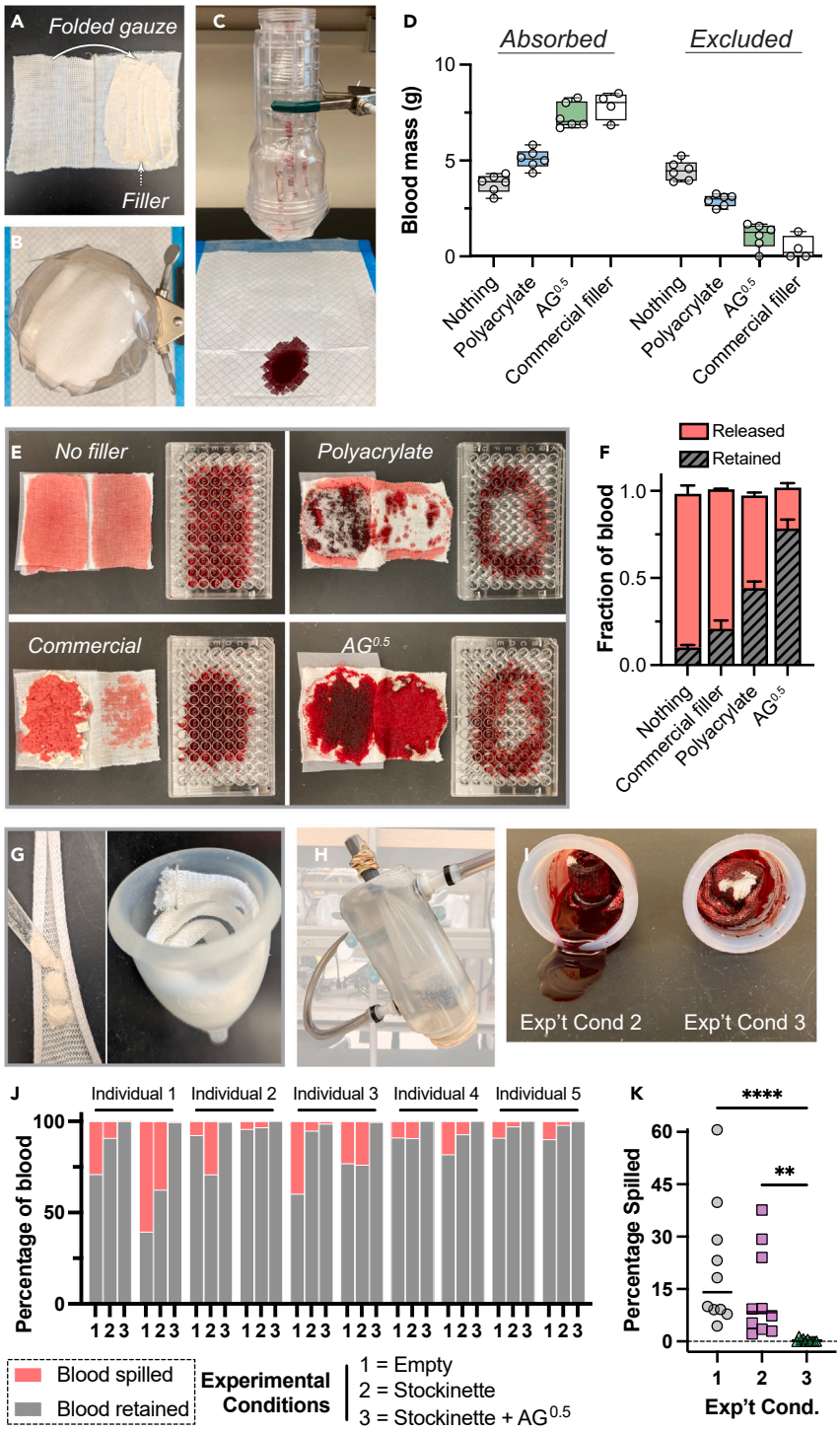

Figure 4. Application of alginate-glycerol formulations for menstrual care

(A and B) Scheme pad assembly (A) and attachment to a silicone-based artificial vagina (B).

(C) Representative experiment showing the result of blood application to the device and permeation through a pad.

(D) Blood retained within the pad was measured, as well as blood that permeated the pad or remained unabsorbed but pooled above the pad.

Figure 4. *Continued*
(E and F) Representative results of testing for blood retention in the used pads (E) and the measured results (F). Error bars represent standard deviations.
(G) For testing alginate-glycerol formulations to solidify blood in menstrual cups, powders were spread in the stockinette and then coiled into the cups.
(H) Cups were inserted into a Syngina, received blood, and incubated for 4 h at 37°C.
(I) Representative images show the impact of alginate-glycerol formulations on blood consistency within menstrual cups.
(J) After individuals removed the incubated menstrual cups, the fraction of blood spilled was measured.
(K) Compiled results show significantly less blood is spilled when a stockinette containing the alginate-glycerol formulation is used. Lines represent the median. Statistical analysis was performed by Kruskal-Wallis test with Dunn's multiple comparisons test. $^{**}p < 0.005$, $^{****}p < 0.0001$.

(e.g., sleeping or a change in body position) or spontaneous events (e.g., laughing, sneezing, coughing). After 1 h, the pad was removed, and the amount of blood that permeated the pads or was not absorbed into the pad (i.e., retained above) was measured, as shown in Figure 4C. Our alginate-glycerol powder and the commercial cellulose filler had similarly high levels of blood absorption and low levels of excluded (i.e., permeated or retained) blood compared to the absence of a filler and the polyacrylate filler (Figure 4D). While absorptivity is an important parameter, the transfer of moisture can be a source of discomfort that can lead to rashes. To mimic mild compression and contact that might lead to moisture transfer, we placed blood-absorbed pads on top of 96-well microtiter plates, centrifuged them at low speed, and measured the quantity of blood that was discharged (Figure 4E). We found that the commercial filler released nearly all of the absorbed blood, comparable to the empty gauze, whereas the polyacrylate retained an intermediate quantity of blood, and the $AG^{0.5}$ filler retained the maximum amount of blood (Figure 4F). This effect can also be observed by gently squeezing these materials (Video S1). Collectively, these experiments show that the alginate-glycerol powder rapidly absorbs blood with high retention.

Alginate-glycerol powders eliminate spillage when used in menstrual cups

Menstrual cups are silicone cups that are inserted into the vaginal canal to create a seal against the vaginal wall, retaining menstrual fluid until it can be removed and cleaned. These menstrual products are favored over disposable products because they are reusable and have a low likelihood of leakage during use. However, poor placement and/or manipulation during removal can lead to leakage and spillage of fluid. To test our formulation as a sustainable material that can be used as a complement to environmentally friendly menstrual cups, we spread 1.5 g of the $AG^{0.5}$ powder in a 25.4 mm cotton tube ("stockinette") and coiled it within a menstrual cup (Figure 4G). Cups alone or those with stockinettes filled with nothing or with $AG^{0.5}$ powder were placed in the Syngina, a device used by the FDA to test the absorptive capacity of menstrual care products[23] (Figure 4H). To evaluate the retentive capacity of the menstrual cups, 15 mL of blood was added to the top to simulate a daily average of menstrual fluid.[24] After incubation for 4 h at 37°C, five women were blinded to the content of the menstrual cups and asked to remove them with care to minimize spillage. As shown in representative experiments (Figure 4I), the alginate-glycerol powder improved blood retention. When examining individual attempts, each woman spilled blood when removing menstrual cups that contained nothing or the empty stockinette, which could be highly variable between attempts. By contrast, spillage was consistently rare when the $AG^{0.5}$ powder was used (equivalent to a few drops, if occurring at all) (Figures 4J and S8). Outside of the Syngina, inversion of these menstrual cups showed that, unlike the empty stockinette, the

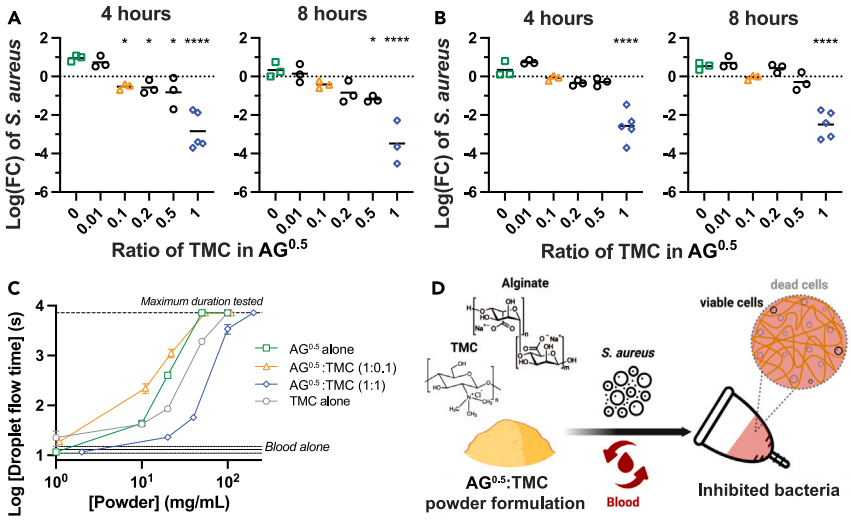

Figure 5. Antimicrobial activity of alginate-glycerol formulations processed with TMC

(A–C) Formulations containing 20 mg of $AG^{0.5}$ and varying amounts of TMC were mixed with blood inoculated with (A) 5×10^5 CFU/mL or (B) 5×10^6 CFU/mL of *S. aureus* and incubated for 4 and 8 h at 37°C. (C) Flow tests of these powders mixed with blood. Error bars represent the standard error. (D) A schematic representation of powder function when mixed with blood and *S. aureus*. Statistical analysis was performed by one-way ANOVA compared to a TMC ratio of 0 with Dunnett's post test. ****$p < 0.0001$.

$AG^{0.5}$ powder improves blood retention (Video S2), this blood is not readily exuded (Video S3), and little blood remains in the menstrual cup (Video S4). The summation of results shows that the use of the stockinette with powder eliminates spillage of blood to levels significantly reduced compared to empty or stockinette conditions (Figure 4K).

Suspension of bacterial growth

Highly absorptive menstrual products have been correlated with toxic shock syndrome, where toxigenic *S. aureus* from the vaginal tract is able to proliferate and produce toxins. This is predominantly observed with tampons,[25] but is possible with other menstrual hygiene products, including menstrual cups.[26] Alginate can potentially act as a nutrient source for some microbes, so including a strategy to inhibit bacterial growth would be important to minimize the risk of toxic shock syndrome. Because of the significance of the endogenous vaginal microflora,[27] we aimed to use a minimally leeching formulation to inhibit bacterial growth within the alginate-glycerol powder without complete bacterial eradication because of potential collateral effects to the vaginal microbiota. We used trimethyl chitosan (TMC) because it has antimicrobial activity and will anchor in the alginate via ionic cross-linking and polymer entanglement to minimize leaching. When alginate and TMC were dissolved in solution in the first steps of preparation, we found significant inhibition of bacterial growth when mixed with blood spiked with 5×10^5 (Figure 5A) or 5×10^6 colony-forming units (CFU)/mL of *S. aureus* (Figure 5B). This effect was observed after 4 and 8 h of incubation. Mixing alginate-glycerol with TMC in powder form, without co-solubilization, led to less consistent antimicrobial activity, which is likely due to heterogeneity in the TMC distribution (Figure S9). To test whether including TMC altered the alginate-glycerol powder function, we measured the flow of these powders mixed with blood. As shown in Figure 5C, the addition of a lower weight fraction of TMC led to slight increases in flow time at low powder concentrations. TMC alone can increase droplet flow times, although not to the same

levels as the alginate-glycerol formulations. Interestingly, equal amounts of TMC and alginate-glycerol dramatically reduce droplet flow times, which we suspect is due to ionic cross-linking between the polyanionic alginate and the polycationic TMC, sequestering alginate from calcium-mediated cross-linking and gel formation. As schematically shown in Figure 5D, the inclusion of TMC can impart antimicrobial properties while retaining the ability to solidify blood.

DISCUSSION

Here, we describe the design of a biodegradable, blood-absorbent biomaterial based on a natural polysaccharide that improves the performance of menstrual products by minimizing blood leakage and spillage. First, we developed a screening method to quantify the gelation of blood by testing mixtures of several polysaccharides. This strategy identified HMW alginate as an optimal candidate because of the dramatic increase in viscosity and spontaneous miscibility with blood when tested in liquid form. For application as an absorptive powder and to maximize the dissolution of alginate powders in blood, we developed a glycerol-supplemented formulation that both accelerated the stiffening of blood into a gel-like consistency and increased the capacity for blood absorption, an effect that depended on particle size. We then tested the practicality of this material to improve menstrual care by using it as the absorptive component of a menstrual pad and as a complementary component of menstrual cups. In both cases, the use of our alginate-glycerol powder formulation minimized blood leakage and spillage. Finally, to inhibit bacterial growth, we included the quaternary ammonium polysaccharide TMC, which inhibited the growth of *S. aureus*, a bacterium responsible for toxic shock syndrome.

Traditional menstrual hygiene products manage menstrual fluids by their absorption or collection. While the principles can be traced to antiquity, their early modernized designs can be identified in products as early as the tampon patented in the United States in 1933,[28] the Kotex menstrual ("sanitary pad") marketed in 1921,[29] and the menstrual cup patented in the United States in 1937.[30] Since their inception, menstrual products have focused on managing menstrual fluid in its liquid state, which faces the same challenges of any other liquid: leakage and spillage/dripping during changing or replacing the menstrual product. Although menstruation is a natural biological process and its healthy progression is not traditionally considered a disease or disorder, its poor management has a major global impact for women.[31] In many settings, including workplaces in LMICs, the lack of access to facilities with privacy, clean water, and discreet disposal leads to anxiety and stress. This negatively impacts the productivity of women, their ability to remain in the workforce, their income, and their quality of life.[32] By increasing the stiffness of blood, producing a more gel-like material, we show greater performance compared to traditional menstrual products.

Alginate has been explored in various biological applications, including wound dressings, hemostatic products, and implantable devices,[13] but has not been demonstrated as a bulk absorbent material for biological fluids, especially menstrual fluid, which does not coagulate. Notably, in our use of alginate, we rely on blood-derived divalent cations to mediate the ionic cross-linking that leads to gelation,[33] enabling the gelation of a non-clotting defibrinated blood that mimics the lack of coagulation in menstrual fluid.[11] This contrasts with other materials that leverage the presence of clotting factors within blood to mediate coagulation.[34] Building on its broad applications in biotechnology, our use of alginate to increase the viscosity of non-coagulating blood to improve menstrual care represents an advancement toward women's health, which is traditionally understudied.

Table 1. Alginate-based formulations for the production of blood-absorbent powder

Alginate/glycerol (w/w)	Alginate (g)	Glycerol (g)	Water (mL)
1:0 (AG^0)	2.5	0	10
1:0.5 ($AG^{0.5}$)	2.5	1.25	10
1:1 (AG^1)	2.5	2.5	10
1:2 (AG^2)	2.5	5.0	10
1:3 (AG^s)	2.5	7.5	10

The use of a biodegradable formulation based on renewable resources is an important consideration for menstrual care products. Current commercial products largely utilize non-degradable materials derived from non-renewable sources, such as plastics in the packaging or cross-linked polyacrylate in the absorptive material. Given the biodegradability of alginate,[33] the formulation presented in this work allows for convenient disposal of the solidified blood mixture. The established large-scale manufacturing of alginate from natural sources[33] and its approval for use in several products by the Food and Drug Administration[35] also simplifies its safe and scalable implementation. An additional benefit of using a known biopolymer is the option for further functionalization. With toxic shock syndrome as an important concern, we included TMC and showed that, at low concentrations, it inhibits the growth of *S. aureus* without impairing the blood-gelation function of alginate, illustrating the potential versatility of alginate-based menstrual products.

While we believe our work represents exploratory but important progress in improving the quality of life for women, there remains several questions that would benefit from further investigation. For example, the large-scale processing and manufacturing of alginate-glycerol materials may require additional adjustments that maintain batch-to-batch performance and homogeneity. Additional considerations should monitor several parameters, including water content and particle size distribution, especially with regard to blood absorptivity. When considering the use of the alginate-glycerol material as an additive to current menstrual products, understanding how powder parameters (i.e., particle size) impact the robustness of function in conjunction with menstrual pads or cups, as well as its compatibility with existing waste streams,[36] would be important factors. In LMICs, where improved menstrual care products would have a strong impact, cost will be a major factor. Although a more comprehensive and detailed cost analysis would be necessary, based on the amount of purified, commercially obtained materials used in this study, we estimate the amount per menstrual cup would cost ~$0.20. To meet regulatory approval for commercialization, several additional tests would be required, including the assessment of cytotoxicity and allergenicity. Finally, to improve our understanding of the performance of menstrual care products, the development of established *in vivo* models would help address potential gaps and limitations in the evaluation of materials.

In conclusion, our work addresses a major factor in the quality of life for women globally. In addition to developing new materials for menstrual care, we hope our work will stimulate greater technological investment and scientific interest in women's health.

EXPERIMENTAL PROCEDURES

Resource availability

Lead contact

Requests for further information or data should be directed to the lead contact, Bryan Hsu (bhsu@vt.edu).

Materials availability

Raw materials are available from the commercial suppliers indicated in this section.

Data and code availability

All data reported in this paper are available from the lead contact upon request.

Materials

LMW sodium alginic salt, iota-carrageenan, kappa-carrageenan, pectin, and the superabsorbent cross-linked sodium polyacrylate were purchased from Sigma-Aldrich (USA). HMW sodium alginic salt, carboxymethylcellulose, chondroitin sulfate, gelatin type A, TMC (LMW, ~85% deacetylated, degree of quaternization >50%, no. 912700), and sodium hyaluronan were obtained from Thermo Scientific (USA). High-grade alginate samples with different molecular ranges and M/G ratios were ordered from Promega (USA), chitosan was purchased from Polysciences (USA), carboxymethyl chitosan was purchased from Santa Cruz Biotechnology (USA), and xanthan gum was purchased from TCI Chemicals (USA). Alginate rhodamine (high viscosity, 1,000–1,500 cP, AL-512) was purchased from Creative PEGWorks. Aseptically confirmed porcine blood (defibrinated or NaEDTA supplemented) was purchased from Lampire (USA). Fuchsin acid-certified was ordered from Neta Scientific (CMX-01606-25G). Aqueous polymer solutions were prepared with ultrapure water (18.2 MΩ cm at 25°C) from a Milli-Q IQ-700 ultrapure water purification system (Millipore Sigma, USA).

SEC

The molecular weight and the polydispersity index (PDI) for the alginates and TMC were determined by SEC. Samples were prepared by dissolving 0.1%–0.5% of sample in 100 mM sodium nitrate buffer and filtering through a 0.45 μm syringe filter. Twenty microliters of the sample was loaded onto a Shodex LB-806M SEC column at a flow rate of 1 mL/min. Molecular weight determination and concentration were measured by multiangle light scattering (MALS) and refractive index (RI), respectively, via in-line Wyatt detectors. All data were analyzed in Astra 8 software and normalized to commercial dextran standards (Wyatt).

NMR spectroscopy

Commercial alginate samples (HMW and LMW) were first depolymerized by partial hydrolysis with HCl and resuspension in 99.9% D_2O (5–10 mg/mL).[37] The probe temperature was set to 353 K, followed by the insertion of the NMR tube with the hydrolyzed sample and equilibration for 15 min. Afterward, we set the relaxation delay to 2 s and locked the solvent to D_2O and the number of scans to 128. Additional details for recording the spectra and determining the sample M/G ratio are provided elsewhere.[37]

Biopolymer solutions

Polymers weighed in an analytical scale were dissolved at concentrations ranging from 0.5% to 6.0% w/v in ultrapure water, vortexed, and heated in a water bath at 50°C until complete solubilization. For measurements in polymer/blood mixtures, aqueous polymer solutions were mixed with equal volumes of aqueous polymer and blood at room temperature and were homogenized by gently pipetting the mixture up and down. Solutions were left on the bench at room temperature for 15 to 30 min to eliminate bubbles before conducting any test.

Blood-absorbent powder formulation

Alginate-based powder formulations were prepared by mixing HMW alginate in glycerol aqueous solution to test various alginate to glycerol solution ratios (Table 1).

For powder solubilization, the aqueous glycerol solution was spread in a 101.6 mm square weighing boat, and the powder alginate reagent was evenly dispersed on the liquid surface, followed by gentle mixing at the liquid surface that formed a solid mixture. The homogeneous solid mixture was cut into small pieces with a disposable spatula and dried at 50°C for 48 h, followed by manual grinding with liquid nitrogen to obtain millimeter-sized particles. The ground mixture was manually sized with a set of sieves with openings that ranged from 0.2 to 1.5 mm (US Standard Sieve Series, USA, ASTM E11) to collect particles within different size ranges (<0.2, 0.2–0.5, 0.5–0.7, 0.7–1.0, and 1.0–1.5 mm ranges; for example, particles in the 0.2–0.5 mm size range passed through the sieve with 0.5 mm and were retained on top of the sieve with 0.2 mm opening size). The processed samples were stored in at 4°C–8°C before use to minimize product degradation and contamination.

Test-tube flow test

Regular glass tubes were first washed with a commercial detergent and rinsed with deionized (DI) water five times. These tubes were soaked in hydrochloric acid solution (1.0 M) for 1 h, followed by copious rinsing with DI water and three final rinses with ultrapure water and drying at 70°C. Aqueous polymer solutions were mixed with equal volumes of ultrapure water or porcine blood (defibrinated or NaEDTA treated) by pipetting the mixture up and down with low-retention tips (VWR, USA). For tests with blood supernatant, defibrinated or NaEDTA-treated blood was centrifuged at 2,000 × *g* for 15 min at 4°C, and the supernatant was collected for blood mixing. After complete removal of bubbles, aqueous or blood polymer mixtures (80 μL) were pipetted ~10 mm below the top of the pre-cleaned test tubes, which were positioned at an angle of 45° at room temperature, to track the time interval for the droplet to travel 80 mm toward the bottom of the tube. Measurements were conducted in at least triplicate (Figure 1A). The maximum flow time interval observed was 8 h for mixtures that did not flow.

Flow viscometer measurements

Kinematic viscosity measurements were performed in pre-calibrated Cannon-Manning glass viscometers (viscometer sizes 75, 150, 200, 300, 400, 500, and 600) with pre-mixed aqueous or blood polymer solutions. After sample loading, the viscometer was placed in a water bath at 40°C and kept for 15 min to reach equilibrium before measuring the mixture flow time.[38] The kinematic viscosity was calculated by converting the efflux time in seconds by the viscometer constant (Table S2) at 40°C for measurements in triplicate.

Spontaneous alginate/blood mixing

The HMW alginate solution (1% w/v) was first prepared with 1:100 alginate rhodamine by mixing the fluorescent polymer into the HMW alginate solution protected from light. The polymer (1 mL) was transferred to a 5 mL conical tube, and 1 mL of blood was carefully added on top of the polymer phase and at room temperature without shaking. Fluorescence was measured from sample aliquots (80 μL) by resuspension in NaEDTA solution to a final concentration of 100 mM NaEDTA, followed by centrifugation at 2,000 × *g* for 15 min at 4°C. Fluorescence was measured from the supernatant in an Agilent Biotek Cytation plate reader with emission and excitation wavelengths of 520 and 583 nm, respectively, in black 96 multiwell plates. The total alginate concentration was determined based on the concentration of fluorescent alginate and the ratio of HMW alginate to alginate rhodamine.

Blood absorption capacity

Powder formulations (PAA or the formulated alginate-based mixtures with different alginate-to-glycerol ratios, 0.5 g) were added to a 25.4 mm cross-sectioned acrylic

tube (~25.4 mm height) on top of a pre-weighed high-density polyethylene screen (46 mesh, ~50 mm^2), above a pre-weighed Petri dish (101.6 mm diameter) (Figure 2A). Defibrinated blood (5 mL) was transferred at a rate of 5 mL/min using a syringe pump (model 300, New Era Pump System, USA) at the top of the powder, and the system was maintained on the bench to measure blood absorption over time (static, room temperature). At the end of the experiment, the cross-sectioned tube was removed, and the contents collected in the Petri dish and retained at the top of the screen were weighed to determine the amount of blood that leaked from or was retained in the powder for measurements in triplicate.

External tamponade test (menstrual pads)

The powder formulation was first tested as a menstrual pad filler and challenged for blood retention in a silicone-based, anatomically similar vaginal model (Ice Lady-Clear, 247.65 × 98.43 mm [L × W], Interactive Life Forms, USA) (Figures 4A–4C). Powder formulations (1.5 g) were added to a cotton-based gauze (~1.5 g, 50.8 × 76.2 mm) for a simple yet practical design of the external tamponade element in a pad format for testing blood retention and loss during the pad transfer. The pads filled with different materials ($AG^{0.5}$, PAA, or the commercial absorbent element from pads) were taped underneath the vaginal model, and the defibrinated porcine blood (8 mL) was added from an opening at the top using a transfer pipette in an ~30 s interval. After 1 h of incubation at room temperature, blood leakage and retention were determined by weighing the blood that flowed through and the blood was retained in the tamponade element. To determine blood release due to mechanical force, pads were detached from the vaginal model and placed on top of pre-weighed 96 well plates, followed by spinning in a swing bucket centrifuge (centrifuge 5810-R Eppendorf, rotor A-2-DWP) at 1,000 × *g* for 5 min at 25°C. The pad and the plate were weighed to determine the fractions of blood retained and released in the pad, respectively.

Internal tamponade test (menstrual cups)

The alginate powder formulation ($AG^{0.5}$) was also assessed as a filler in menstrual cups as a method to minimize spillage and blood loss during cup removal. This test was conducted with an in-house designed Syngina (synthetic vagina) adapted from the FDA-approved methodology for testing tampon absorbency,[23] using a bottle with a larger opening (~35.9 mm inner diameter) and a circulating water-heating system kept at 37°C (Figures 4G and 4H). Powder formulations (~1.5 g) were dispersed in a 25.4 mm tube-shaped stockinette (~1.0 g, 152.4 mm length) and the tube was arranged in a helical shape inside of a regular menstrual cup (CVS Health, cup size A, maximum working volume 15 mL, medical-grade silicon, no. 722537). The menstrual cup was placed inside the Syngina, and defibrinated blood (15 mL) was transferred to the cup with a transfer pipette (~5 mL/min) from the top opening. After the incubation period (1 h at 37°C), the menstrual cup was removed using a pre-weighed white superabsorbent glove with a pre-weighed white absorbent pad underneath the experimental setup, and both the glove and the pad were weighed and imaged to determine the blood messiness during menstrual cup removal. A blinded, randomized test was conducted in duplicate for each sample (empty cup, filled with the empty stockinette, and filled with the stockinette containing the alginate powder) by different female co-workers from the Hsu lab.

Absorptivity test of commercial tampons

The tampon absorptivity test was conducted with pre-weighed regular-size tampons (o.b. Original regular tampons, Playtex Manufacturing) placed in the center

of the Syngina at 37°C. The tested fluid was pumped from the top of the Syngina at 50 mL/h using a syringe pump (model 300, New Era Pump System, USA) and stopped when the tampon was visually saturated and the fluid started leaking from the tampon.[23] The tampon was weighed again on an analytical scale, and the amount of fluid absorbed was determined by the weight difference before and after fluid absorption.

Alginate release test

To study the release of alginate from the polymer/blood mixture, the blood-absorbent powder was prepared with an aqueous glycerol solution containing alginate rhodamine (0.2 mg/mL), leading to a mixture with 1:100 alginate rhodamine to HMW alginate. The powder mixture was processed as described above (see "Blood-absorbent powder formulation"), protected from light. The processed powder was mixed with defibrinated blood (1 g powder per 10 mL of blood), and 300 μL of gel was transferred to the bottom of a 24-multiwell plate. PBS or defibrinated porcine blood (2 mL) was added on top of the gel, and the plate was incubated at 37°C on a rocker with orbital shaking (50 rpm) for 24 h. Sample aliquots (80 μL) were collected and processed as described under "Spontaneous alginate/blood mixing." The total amount of alginate released was based on the alginate rhodamine signal, the ratio of alginate rhodamine to alginate, and the initial concentration of alginate in the gel.

Antimicrobial assay

The $AG^{0.5}$ formulation was supplemented with the antimicrobial agent TMC (0.2–20 mg per 20 mg of alginate) to investigate the antimicrobial performance of blood-absorbent powder against *S. aureus*. The homogeneous formulation was prepared by adding TMC during the alginate solubilization in the glycerol aqueous solution, followed by snap freezing at −80°C and lyophilization for at least 24 h. After drying, the powder was crushed and used for the antimicrobial test. The heterogeneous formulation was prepared by vigorously mixing the pre-processed $AG^{0.5}$ formulation with TMC prior to the antimicrobial test. For the bacterial inoculum preparation, *S. aureus* was streaked from the frozen bacterial stock in a Luria broth (LB) agar plate and incubated at 37°C overnight. One single colony was used to inoculate 5 mL of LB in a test tube, followed by overnight incubation at 37°C and 200 rpm. The overnight cell culture was back-diluted to an OD_{600} of 0.125 ($\sim 10^8$ CFU/mL) and spiked in aseptically tested defibrinated porcine blood to concentrations of 5×10^5 and 5×10^6 CFU/mL of *S. aureus*. The TMC-supplemented powder (20 mg) was transferred to the bottom of 24 well plates, followed by the addition of 1 mL of blood with different bacterial loads (tests performed, at least, in triplicate for each powder formulation) and incubation at 37°C and 200 rpm in an incubator with orbital shaking. To test bacterial inactivation, blood-mixture aliquots (100 μL) were collected after 4 and 8 h, serial diluted in LB, and plated in LB agar plates. After overnight incubation at 37°C, we determined the number of colony-forming units and converted it to the final bacterial concentration in the gelled blood mixture. The antimicrobial performance was determined as the fold change (FC) in the bacterial concentration of polymer-containing samples compared to samples with no polymer added.

Morphological analysis

For the morphological analysis using SEM, powder samples of pristine alginate, superabsorbent polymer, or $AG^{0.5}$ were placed on top of copper tape. The samples were then coated with 10 nm Pt/Ir coating using the sputter for a few min and probed using a JEOL IT500 (JEOL) with an accelerating voltage of 5 kV and magnifications of 50× and 500×. The minimum and maximum particle

dimensions, as well as the particle area, were calculated from the 50× images using the ImageJ software.

Contact-angle measurements

The surface hydrophobicity of the pristine alginate and $AG^{0.5}$ powder was investigated through water contact angle by using an optical tensiometer (Biolin Scientific Theta Flow). A thin film of alginate and alginate glycerol powder was prepared over the glass slide. The film was prepared with a 1% solution of alginate and $AG^{0.5}$ in water, which was then poured over the glass slide and dried in the oven at 50°C. The sessile drop mode of the OneAttension software was used for the static contact-angle measurement. A drop of water (5 μL) was placed on the thin layer of the pristine alginate and $AG^{0.5}$ film using the automatic dispenser and imaged with a 5 MP resolution against monochromatic light for 100 s in the equipment software. The angles at the left and right sides of the droplet were automatically determined by the software, and their average was used as the contact angle of the sample (measurements in triplicate).

Rheology

The strength of the $AG^{0.5}$ gels with $CaCl_2$ and defibrinated porcine blood was assessed through the oscillatory rheological technique using an Anton Paar MCR302 rheometer (Anton Paar). The strain and frequency sweeps were conducted with a 50 mm parallel plate geometry of the rheometer. The gels were prepared by mixing 1 g of $AG^{0.5}$ powder with 10 mL of blood or $CaCl_2$ (2.50 mM) solution and leaving them on the bench to remove bubbles. The porcine-defibrinated blood use presents 2.47 mM Ca^{2+} as measured by the ICP-MS method. The strain sweep was performed to determine the optimum strain value from the linear viscoelastic region for each gel at 37°C. The storage (G′) and loss (G″) moduli were recorded as a function of frequency between 0.1 and 100 Hz.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.matt.2024.06.028.

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AUTHOR CONTRIBUTIONS

Conceptualization, B.B.H.; investigation, B.B.H., R.A.B., H.K., J.M., E.G., and C.C.; resources, B.B.H.; writing – original draft, B.B.H., R.A.B., and H.K.; writing – review & editing, B.B.H., R.A.B., and H.K.; funding acquisition, B.B.H.

DECLARATION OF INTERESTS

R.B. and B.B.H. are inventors on a pending patent application related to the materials described in this paper.

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