Preparation of Hydrogels With Herbal Extracts as an Effective Hemostat

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Abstract

Hydrogels have been demonstrated as viable drug delivery matrices. In this study, several hydrogel combinations were explored in conjunction with herbal extracts to develop an effective life-saving hemostatic agent. An alginate-polyacrylate matrix and extracts of common herbs such as shepherd's purse, peony, and yarrow, demonstrated promising activity for a rapid hemostasis in emergency medicine. These applications represent an avenue for future research.

I. General Background

Mineral-based hemostats have been available for over 20 years for emergency and battlefield management of massive hemorrhage. Current hemostatic devices utilize a gauze matrix functionalized with powdered coagulants to absorb blood and promote clotting.

These methods have served well in stopping blood loss, but there have been problems unique to each hemostat type. Quikclot, which utilizes the mineral zeolite, is exothermically absorbent and has a history of causing additional thermal burns to the site of application. HemCon, a chitosan-based trauma gauze developed in collaboration with the US Army, does well as a hemostat but is difficult to apply to irregularly shaped wounds.[1]

In this study, we aim to prepare a hydrogel-based hemostat as a viable alternative to granule or powder-based analogs. An ideal hydrogel would allow ease of application to mitigate improper use by undertrained first responders. The project was divided into two parts: development of the hydrogel, and exploration of naturally derived herbs that possess antioxidant, antibacterial, anti-inflammatory, coagulating, and other reported medicinal properties. Various methods of loading the hydrogels with the natural extracts were explored.

II. Exploring the Hydrogel Matrix

Chitosan, sodium alginate, gelatin, and hydrolyzed collagen powders were selected on the basis of reported biocompatibility and ease of availability as the basis of four initial hydrogels observed.

A series of qualitative tests were performed to analyze viability of the hydrogels, discussed at length in the supplemental information. Furthermore, combinatory studies analyzed potential 1:1 mixtures of hydrogels in an attempt to better understand potential interactions and modifications of the base matrix. Based on the results of these qualitative tests, it was decided that the majority of

Table 1: Matrix Ingredients

Compound	Linkage	Viability	Assessment
Alginate	Ionic	○Can be syringed ○Passes flip test ○Low absorbance	○Good Candidate
Chitosan	Ionic	○Can be syringed ○Somewhat passes flip test	○Good candidate ○Requires modification
Gelatin	Ionic	○Varies in composition (Liquid→Solid)	○Not ideal for current studies
Collagen	Ionic	○Liquid composition ○Fails flip test	○Not ideal for current studies
Polyacrylate	Covalent	○Can be syringed ○Passes flip test ○High absorbance	○Excellent candidate ○Subject for future research

Summary of starting materials, characteristics, and assessment.

the research period would focus on a sodium alginate-chitosan mixture. The molecular and macroscale physical properties of these hydrogels, including ionic cross-linking behavior between hydrogel polymer strands was examined. In the concluding phase of the current research period, sodium polyacrylate was identified as a viable candidate for future research.

Table 2: Dehydration Data

Appearance	Time (min)	Empty Test Tube (g)	Test Tube +Gel (g)	TT After Rotovap (g)	% Loss	Add 1	Add 2
Gel	10	7.61	8.61	8.34	27.00	Pass	
	20	7.56	8.56	8.03	53.00	Fail	
Paper	30	7.51	8.51	7.86	65.00	Fail	Pass
	40	7.62	8.62	7.78	84.00	Fail	
	50	7.60	8.60	7.78	82.00	Fail	
Film	60	7.58	8.58	7.66	92.00	Fail	

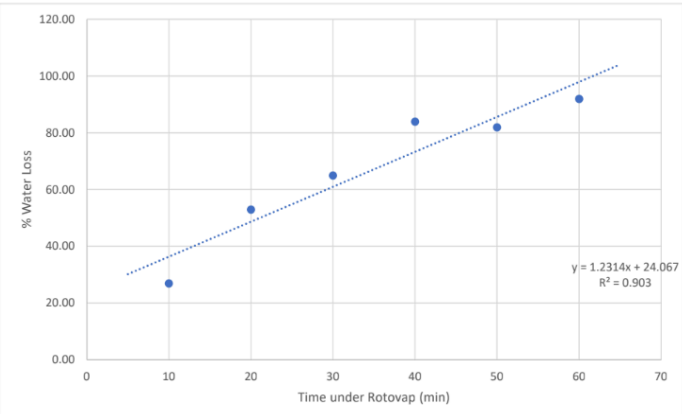

Fig E2: A table and chart of the observed water loss and ability to be rehydrated with 1 and 2 equivalents lost water.

A sodium alginate/chitosan- based hydrogel was subjected to a series of dehydration/rehydration tests to evaluate the ability of the matrix to absorb and discharge aqueous fluids such as blood or potential medicines. Using a horizontal rotary evaporator, the extent of water loss from the hydrogel, resulting changes in physical characteristics, and ability of the gel to be rehydrated was tracked over time. It was discovered that alginate-based hydrogels can tolerate approximately 60% water loss before their ability to rehydrate is compromised.

With basic knowledge of the behavior of hydrogels at different hydration levels, further modifications to the alginate-chitosan gel were explored. One such modification was incorporation of potassium iodide and concentrated hydrogen peroxide to provide the gel with the qualities of an expanding gas-in-liquid foam. The experiment was successful in expanding the hydrogel to over five times its original volume, but further studies are required in order to find a method of maintaining the structural integrity of the foam over time. Another modulation involved attempts to incorporate kaolin and zeolite mineral powders, the same materials found in commercial gauze-based hemostats, into the hydrogel. These minerals were observed to improve the absorptive qualities of the gels, resulting in a slight enhancement of their performance in the final blood tests discussed later.

Ultimately, the purpose of the hydrogel is to serve only as a carrier for an active ingredient promoting coagulation, and not as the primary hemostat. As research on a viable matrix was slowly concluded, herbal extracts were explored, and various methods of incorporating said extracts were tested.

III. Investigation of Multiple Herbal Extracts for Potential Antioxidant, Anti-Inflammatory, and Other Useful Properties

Herbs were selected based on past iterations of research by this group (peony, lavender) and literature reports of observed hemostatic properties (shepherd's purse, yarrow, plantain, mullein). All herbs were macerated for 48 hours in three different media (methanol, ethanol, and deionized water). The extracts were then used to run a number of qualitative and quantitative analyses to determine which, if any, of the herbs display beneficial properties. Additionally, Infrared Spectroscopic and UV-visual spectrophotometric absorption spectra of the herbal extracts were collected and analyzed to determine the identities of any general functional elements of the herbs by comparative analysis with known antioxidant and antibacterial compounds such as tannic acid.

The herbs were first run through a series of qualitative tests to determine the presence or absence of certain classes of compounds of potential medical significance. These compounds include hydrolyzable and non-hydrolyzable tannins, flavonoids, anthocyanins, quinones, steroids, terpenoids, coumarins, and saponins. The results are summarized below:

Table 3: Qualitative Test Results of Specific Herbs

Tests	Yarrow	Rosemary	Plantain	Mullein
Tannins	Yes	No	Yes	Yes
Flavonoids	Yes	No	No	No
Anthocyanins	No	No	No	No
Quinones	Yes	No	No	No
Terpenoid	Yes	Yes	No	Yes
Steroids	Yes	Yes	No	Yes
Saponins	Mild	Mild	Mild	Mild
Coumarins	Mild	No	Mild	Mild

The antioxidant properties of the herbs were measured with the use of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Methanolic extracts of the herbs were diluted (1:10) and introduced to a solution of DPPH radicals. Absorbance at 517 nm as measured with a spectrophotometer is inversely correlated with the concentration of natural antioxidant(s) present. The percent inhibitions were calculated with a negative control of DPPH solution and methanol. As a result, the data illustrates decreasing antioxidant activity of the herbs as follows:

Table 4: Antioxidant Test Results

Herbs	Peony	Yarrow	Mullein	Plantain
% Inhibitions	91.2	81.2	77.8	61.4

An infrared (IR) spectrometer was used to analyze the extracts for the presence of functional groups correlated with antioxidant activity, reported antibacterial and anti-inflammatory properties, and/or coagulating features. Most of the herbs showed strong C-H and O-H signatures at 3000 to 2500 cm^{-1} and 3500 to 2800 cm^{-1} respectively.

Lastly, UV-Vis spectra of the herbal extracts have been obtained and compared to that of tannic acid. Surprisingly, the UV-Vis of peony extract proved almost identical to that of tannic acid. It is thought that this might explain the high DPPH scavenging activity of peony compared to other herbs. Upon analyzing all the herbs, it was determined that they serve as good candidates for loading in the hydrogel matrix and for testing blood coagulation capabilities.

V. Combining Hydrogels and Herbs

In vitro qualitative assays were done at room temperature (22℃) as an initial test of hemostatic potential by observation of clotting and/or thickening behavior of whole animal (beef and/or pork) blood on application of aqueous herbal extracts. The extracts selected for this series of tests were those of plantain, yarrow, mullein, rose petal, and rosemary. Yarrow, mullein, and plantain mixtures with blood were the first noted to increase in viscosity, suggesting initial formation of a clot. The other extracts tested did not demonstrate notable short-term coagulant activity. All extracts tested demonstrated coagulative properties inferior to commercially available QuikClot Combat Gauze.® This test was repeated with a saturated aqueous solution of the same herb extracts, but yielded similar results with all herbs failing to stop fluidity of blood and failing to substantially coagulate blood as compared to an available positive control.

Table 5: Blood Coagulant Data

Extract	1g/10mL	Concentrated
Plantain	Negative *(slight change in viscosity)*	Negative *(slight change in viscosity)*
Rosemary	Negative	Negative
Yarrow	Negative *(slight change in viscosity)*	Negative *(slight change in viscosity)*
Mullein	Negative *(slight change in viscosity)*	Negative *(slight change in viscosity)*
Rose	N/A	Negative

Observance of blood coagulation in mixing standard and concentrated herb extracts with blood.

In an attempt to encourage absorbance and coagulative properties, hydrogels were later made with both the target herb aqueous solutions and the powdered herbs used to make the extract itself. Hydrogels of hydrolyzed collagen, chitosan-sodium alginate, sodium alginate alone, yarrow-infused chitosan- sodium alginate, plantain-infused chitosan- sodium alginate, sodium alginate with yarrow, and sodium alginate with plantain were applied to whole animal blood and any resulting effects noted. Alginate-containing hydrogels were observed to absorb blood over the course of minutes. The presence of yarrow appeared to be weakly correlated with increased blood viscosity after several minutes.

Tests for blood coagulation continued in the form of addition tests of the following combinative matrices to whole animal blood: alginate with kaolin powder, alginate with peony powder, alginate with shepherd's purse powder, and alginate with zeolite-kaolin powder. Alginate and kaolin powder hydrogels demonstrated substantial blood absorption and promising results as a coagulant. Peony, shepherd's purse, kaolin, and zeolite powders on their own all absorbed blood, appeared to cause coagulation, and uniformly resulted in higher overall blood viscosity. Separate tests of calcium carbonate powder in blood and sawdust in blood were negative for coagulative behavior, suggesting that the observed increases in viscosity were not due merely to the physical presence of a powder in the blood as a "seed crystal" for clot formation. Kaolin and zeolite appeared to help with clotting of blood, while peony extract was eliminated as a candidate hemostat.

Table 6: Blood Coagulant Test

Matrix	Standard Mixture
Collagen	Negative
Alginate	Negative
CHI-ALG	Negative
CHI-ALG w/ Yarrow	Negative
CHI-ALG w/ Plantain	Negative
ALG w/ Yarrow	Negative
ALG w/ Plantain	Negative

Observance of blood coagulation in combination of hydrogel and extract with blood.

Potential hemostatic properties of Vitamin K along with established accounts of the ancient use of dried yarrow and shepherd's purse as a treatment for traumatic hemorrhage. Flip tests (in which a 1mL conical vial containing 0.2mL of whole animal blood and 0.2mL of test material was periodically inverted and physically tapped to break any confounding surface tension) were conducted with blood and yarrow extract, shepherd's purse extract, both yarrow and shepherd's purse extract, and vitamin K solution at both room temperature ("RT," 22℃) and body temperature ("BT," 37℃). At each interval, any evidence of viscosity changes or clot formation was recorded. At RT, the sample containing shepherd's purse showed the most evidence of sustained coagulation. Surprisingly, the best results were obtained at RT rather than BT, confounding the notion that increasing temperature necessarily increases rate of coagulation.

Figure 1: Flip Test

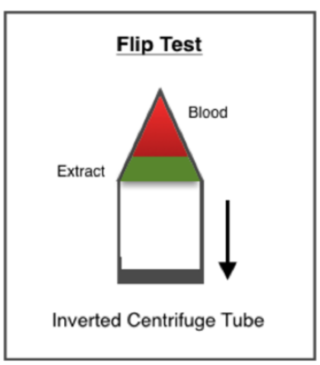

Illustration of flip test conducted at room and body temperature

VI. Conclusion and Future Work:

While sodium alginate and chitosan proved to be a promising hybrid hydrogel, sodium polyacrylate could potentially provide all of the necessary requirements of a hemostatic matrix material, provided that it is bioavailable and non-toxic in open wounds. There is certainly promise in utilizing shepherd's purse for coagulant properties as a potential admixture to a hemostatic hydrogel, but current research is limited by extraction and loading techniques.

There are still multiple individual studies to be conducted on hydrogels, including definitive research on absorbency, mesh size, and functionalization.Other hydrogels will be explored, with special emphasis sodium polyacrylate-derived gels.

Furthermore, further studies on herbs will be conducted to explore wound healing and blood clotting abilities. Alternative methods of extraction will be explored, and the chemical structure of potential herbal coagulant compounds will be assessed to determine the underlying mechanism of any observed action on the clotting of blood. There is interest in exploring the structure of shepherd's purse and vitamin K in particular due to their observed positive effect on coagulation rate.

Finally, non-hydrogel forms of hemostats will also be explored. Incipient experiments were performed to analyze the viability of a foam-based hemostat. Delivery of hydrogels is an area of potential research, especially in the future development and use of more effective multi-step delivery systems.

Acknowledgment:

This would not have been without the help of Title V and ASGCC. We offer our sincerest thanks to Professor Oganesyan for her many sacrifices and tireless work in support of this unique research program.

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