Hemostatic Agent For Lifesaving (HEAL)
Final Report
for the
USAF Air Expeditionary Force Battlelab

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PROTOCOL TITLE: Evaluation of hemostatic agents in the pig (Sus scrofa)

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NON-TECHNICAL SYNOPSIS: The purpose of this study is to evaluate agents used to stop bleeding (hemostatic agents) for effectiveness in various types of wounds likely to be seen in combat. The reason for the evaluation is to demonstrate the practicality of these agents on wounds and to develop suggestions for more practical and effective agents, if necessary. The agents chosen are either FDA approved for human use, or are thought to be near approval. At the completion of this study, recommendations for use of these agents in combat will be made, if appropriate.

We propose to evaluate three agents: SeraSeal, a bovine protein preparation; FloSeal, a fibrin glue, and QuikClot, a zeolite mineral preparation. Of these FloSeal and QuikClot are FDA approved, but they have serious drawbacks making them unsuitable for combat use; they have been included for comparison purposes. Because SeraSeal appears to have the most promise for combat use, the bulk of the study will concentrate on this agent. SeraSeal is available in four formulations (liquid, spray, foam, non-absorbable dressings) which will be evaluated in this study. All of these products have been tested in pig models.

This study will be conducted in pigs in two parts. For the first part, six types of wounds will be created in the animals (skin, muscle, liver, spleen, artery, and vein) to simulate those which may be seen in combat. After wound creation, we will apply the five formulations of SeraSeal, FloSeal, and QuikClot to the wounds. Time to stoppage of bleeding and amount of bleeding will be measured for each.

In the second part of this study, the late effects of the hemostatic agents on wound healing will be evaluated.

I. BACKGROUND

A. Background: The problem of bleeding in wounds

Bleeding is till a major cause of morbidity and mortality in wounds. Thirteen percent of patients suffering acute trauma die of bleeding[1]. Eighty-five percent of deaths in Somalia occurred on the battlefield and the majority of these were from blood loss[2]. Particularly vulnerable areas are the face, groin, pelvis and extremities – areas not protected by body armor. In the head and neck trauma, uncontrolled bleeding can cause airway compromise and asphyxiation[3]. In trauma survivors, inability to control bleeding leads to blood transfusion, increased complication rates, immunosuppression, inability to generate red blood cells, and prolonged time in the hospital. The ideal solution to these problems is to control the bleeding in the first place. The limiting factor in performing burn debridement and grafting is bleeding. On a smaller scale, the debridement of chronic wounds results in bleeding which is essentially an end point for debridement because there is no satisfactory way of controlling bleeding in granulation tissue.

This results in repeated debridement. Bleeding is a major cause of skin graft loss and flap necrosis. On the battlefield, the combat medic has only compresses to control bleeding. A quick, effective, and safe device to control bleeding would be extremely beneficial.

Review of literature on hemostatic agents in wounds

Commercially available topical thrombogenic agents include microfibrillar collagen (Avitene), collagen sponges (Gelfoam, Instat), fibrin sealants (FloSeal, Tisseel, AFTA, Vi-Guard), and the hemostatic concentrating agent QuikClot. Topical collagen preparations have been available for more than 25 years and have a limited ability to facilitate clotting. When hemorrhage is excessive or the patient's clotting factors are reduced they are less effective.

Fibrin sealants:

These are prepared from homologous, pooled human or bovine are fibrinogen, Factor XIII, and thrombin, which must be combined to make the fibrin. Other components include calcium to provide activation of Factor XIII, fibrinectin to aid in adhesion, and an inhibitor of fibrinolysis to prolong fibrin clot life. Fibrin sealants have shown great promise in controlling wound hemorrhage. Fibrin sealants have been shown to reduce operating time, blood loss, and do not appear to interfere with healing[4]. When applied to a ballistic wound as a dry dressing the sealant reduced blood loss by 64%[5]. When used after a life threatening porcine liver injury model, bleeding ceased[6].

Problems with current tissue sealants:

Several problems exist with the commercially available fibrin sealants: They are a two-part system of fibrin and thrombin, which must be mixed at operation, adding to the operative time. Previously the FDA withheld approval from European products because they used pooled human plasma as a product source[7]. The fibrin sealants are somewhat difficult to use, as they must be mixed from four components, a process which takes approximately 15 minutes, and used within four hours. If they are to be used endoscopically, they must be placed through a dual lumen catheter and mixed in situ[8]. Their viscosity makes application difficult via catheters and in small areas and limits methods of application such as sprays or foams, which would be useful for large wounds. The time between mixing and hemostasis can be several minutes, typically 3-5 minutes, further slowing operation[9]. The incorporation of fibrin sealants into a dry dressing has eliminated some of these problems. The strength of the clot formation can also be sub-optimal. These shortcomings limit the utility of fibrin sealants for hemostasis in the field and in emergencies.

Bovine thrombin preparations are highly immunogenic and appear to be associated with an increased risk for adverse clinical outcomes during subsequent surgical procedures[10]. Antibody formation and anaphylaxis rates of .5 to 5.8% have also been seen with aprotinin, a polyvalent proteinase inhibitor isolated from bovine lungs used alone to enhance clot stability or in combination with fibrin sealants[11,12]. These antibodies may interfere with the heparin anti-Xa assay, thereby complicating the monitoring of anticoagulant therapy[13]. The commercially available product Tisseel VH fibrin sealant contains bovine aprotinin and has the risk of hypersensitivity.

Characteristics of SeraSeal:

SeraSeal is a bovine protein-derived accelerator of hemostasis, first by creating an agar-platelet sealant over the wound, and reinforcing the sealant with a fibrin clot by acting with Factors VII and IX. It contains clotting factors obtained from pathogen and prion free cows. The formulation is proprietary, but it does not contain fibrin or Factor V or clotting enhancers other than those found in the bovine clotting cascade. SeraSeal can be provided as a solution, spray, foam, or incorporated into a non-absorbable removable dressing. Its time of action is one third to one fiftieth that of fibrin sealants and appears to provide a more secure clot than fibrin sealant. SeraSeal has already proven to be effective in human use. This effort is designed to develop and test the most effective product formulations for battlefield use. SeraSeal has been used in clinical trials in Europe and South America. SeraSeal has been used in more than 2500 patients without side effect or adverse sequelae. In 39 renal patients in Greece, SeraSeal was used 3-4 times each without evidence of allergic reaction. From work in our laboratory and the data from animal and clinical trials provided by the manufacturer, SeraSeal would appear to be much superior to the hemostatic products currently available. SeraSeal is provided with a two-year shelf life when refrigerated, with repetitive freeze-thaw cycles.

Uniqueness of the program: SeraSeal is a unique patented hemostatic sealant agent, derived from bovine proteins available outside the United States. It appears to be more effective in reducing bleeding time and the quantity of hemorrhage than fibrin sealants, is easier to apply, and is available in a variety of formulations and strengths which can be applied on the battlefield. The proposed study evaluates several varieties of SeraSeal which could be used on the battlefield. The liquid, spray, and dressing are easily applied, possible by the victim. The foam can be applied through a bullet or stab wound or similar wound into muscle or abdominal cavity. The end of this program is to produce a formulation of SeraSeal, which is immediately available for battlefield use with minimal training.

Proposed approach:

Part 1 – Initial product trials: SeraSeal, FloSeal, and QuikClot will be compared in the six types of wounds. We plan to test SeraSeal in the following formulations: 1) liquid, 2) spray, 3) foam, 4) dressing. Each agent and formulation will be tested in pigs on wounds of skin, muscle, liver, spleen, artery, and vein. The grid below shows the plan for initial product testing:

Wounds →	Skin	Muscle	Liver	Spleen	Artery	Vein
SeraSeal Liquid	•	•	•	•	•	•
SeraSeal Spray	•	•	•	•	•	•
SeraSeal Foam	•	•	•	•	•	•
SeraSeal dressing	•	•	•	•	•	•
FloSeal control	•	•	•	•	•	•
QuikClot control	•	•	•	•	•	•
No treatment control	•	•	•	•	•	•

Each pig can have 2 wounds of each type or a total of 12 wounds. The experimental approach consists of 5 product trials for each of the 5 SeraSeal formulations on 6 sites per pig. There are 3 controls – FloSeal, QuikClot, and no treatment. Each formulation of SeraSeal will be compared to each of the three controls. There will be a total of fifteen combinations (5 formulations X 3 controls). Each combination will be tried five times. Each pig will receive one formulation of SeraSeal and one control (FloSeal, QuikClot, or no treatment). This will require 75 animals. The application of product or control will be randomized in advance. For each site two end points will be determined: time to stop bleeding, and amount of blood lost.

A total of 75 pigs are required for this part of the experiment.

Part 2 – wound healing studies: After determining the reduction in blood loss and time of bleeding each formulation, trials will be conducted in 3 pigs per product to examine healing of the wound sites after application of the product. These pigs will be selected at random from the 75 pigs used above to recover from surgery and survive 30 days. One type of product formulation or control will be used for each animal. Each animal will serve as its own control with one wound of each type treated with the product formulation, and the other receiving no treatment. The animals will be allowed to recover from wounds. After 30 days the animals will be euthanized and the wound sites examined by tissue fixation and staining. The wounds will be examined histologically and graded for the quality of wound healing. Twenty one (21) animals are required for this part of the protocol.

Wounds →	Skin	Muscle	Liver	Spleen	Artery	Vein
SeraSeal Liquid	•	•	•	•	•	•
SeraSeal Spray	•	•	•	•	•	•
SeraSeal Foam	•	•	•	•	•	•
SeraSeal dressing	•	•	•	•	•	•
FloSeal	•	•	•	•	•	•
QuikClot	•	•	•	•	•	•

The total number of pigs needed is 21.

Technical data and payoff to the Department of Defense:

Upon completion, this proposal will offer the Department of Defense (DoD) a variety of hemostatic products in forms that can easily be used by the injured combatant alone or with the aid of a relatively trained buddy. The prototype formulation of (SeraSeal) has proven to be effective in human use; this proposal refines that effectiveness for combat wounds. This study will also produce practical information on the application of hemostatic agents that will be of immense value on the battlefield. Since bleeding is the major cause of death on the battlefield, a readily available, easy-to-use hemostatic agent, may be capable of significantly reducing mortality and morbidity, may facilitate surgical procedures, and may reduce blood transfusion needs in the field.

B. Literature Search

1. Literature Source(s) Searched: DoD Biomedical Research Database, PubMed, CRISP

2. Key Words of Search: pain, painful procedures, pig, hemostasis, bleeding, wound

3. Results of Search: Five studies of hemostatic agents used in pigs were found, one in rats. The pig is a standardized hemostasis model, particularly for the DoD. Twenty-three studies of fibrin sealants were located, primarily using the pig. No alternative method of studying hemostasis was found other than using anesthetized animals. All of the agents to be used in this protocol have been tested in pigs.

III. OBJECTIVE\HYPOTHESIS: The following hypothesis will be studied: There is no difference in the bleeding time of wounds treated expectantly and wounds treated with SeraSeal.

IV. MILITARY RELEVANCE: This study will determine the effectiveness of SeraSeal in hemostasis on several types of wounds. The utility of the agent for these wounds will be determined and recommendations for its use in combat will be made, if appropriate. If effective, SeraSeal would be an invaluable resource on the battlefield and in military treatment facilities saving lives, reducing morbidity and preserving blood resources.

V. MATERIALS AND METHODS:

A. Experimental Design and General Procedures, Part 1: Initial product trials:

1. Under general anesthesia, two wounds per animal will be created in the following locations:
   a. Skin
   b. Muscle
   c. Liver
   d. Spleen
   e. Femoral artery
   f. Femoral vein

2. The treatment for each wound will be randomized from the following options:
   a) no treatment (control)
   b) SeraSeal liquid
   c) SeraSeal spray
   d) SeraSeal foam
   e) SeraSeal dressing
   f) FloSeal
   g) QuikClot

3. The outcome of each treatment will be assessed by

i. Time from wound creation to hemostasis (Hemostasis is defined as the point in time at which blood ceases to flow from the wound.)

ii. Amount of bleeding determined by weighing gauze absorbing the shed blood

4. The method for the creation of each wound is as follows:

1) Skin: a dermatome will be used to remove 44/1000 of epidermis and dermis to the deep dermal level resulting in a wound like those seen in skin grafting and debrided burns. The size of the wound will be 10 x 3 cm. Two wounds, one on each lower flank will be created.

2) Liver: A laparotomy will be performed and the right lobe of the liver exposed. A 5 cm laceration will be made by amputating a portion of the tip of the lobe. After treatment a second 5 cm laceration will be made by amputating another portion of the lobe.

3) Spleen: the spleen will be exposed and a 3 cm laceration will be made by amputating a portion of the organ. After treatment a second 3 cm wound will be made.

4) Muscle: The semitendinosus muscle will be exposed by reflecting the skin. A 5 cm muscle laceration will be made longitudinal to the fibers and hemostasis allowed to occur. A second laceration will be performed proximal to the first.

5) Femoral artery: Proximal and distal control will be obtained. A 4 mm longitudinal incision will be made in the anterior arterial wall. The hemostasis agent to be tested will be applied and the wound held with gauze under gentle pressure. After hemostasis, a second wound will be made and treated in a similar manner. Our experience with this wound has not let to uncontrolled hemorrhage; if it is encountered the artery will be sutured or ligated.

6) Femoral vein: Proximal and distal control will be achieved. A 0.5 cm laceration will be made in the anterior wall of the femoral vein. Following hemostasis on the first wound a second laceration distal to the first will be made.

7) If the systolic blood pressure falls below 60 mm Hg the study will be stopped until blood pressure can be restored.

8) The animals will be euthanized at the end of this part of the study.

C. Part 2 – wound healing studies: Trials will be conducted in 3 pigs per product to examine healing of the wound sites after application of the product. One type of product formulation or control will be used for each animal. Each animal will serve as its own control with one wound of each type treated with the product formulation or control agent, and the other receiving no treatment. The animals for this part of the trial will be selected at random from the 75 pigs used in the first part of the protocol and will be allowed to recover from the wounds. After 30 days the animals will be euthanized and the wound sites examined by tissue fixation and staining. The wounds will be examined histologically and graded for the quality of wound healing. Twenty one (21) animals are required for this part of the protocol.

1. Under general anesthesia, two wounds per animal will be created in the following locations in the same manner as described above:
    a. Skin
    b. Muscle
    c. Liver
    d. Spleen
    e. Femoral artery
    f. Femoral vein

2. Each animal will receive one of the following treatments on half of the wounds:
    a. no treatment (control)
    b. SeraSeal liquid
    c. SeraSeal spray
    d. SeraSeal foam
    e. SeraSeal dressing
    f. FloSeal
    g. QuikClot

3. The untreated wounds on each animal will serve as controls.

4. The skin wounds will be dressed with Tegaderm, an occlusive dressing. It will be changed as required.

5. The incisions for the muscle and abdominal wounds will be closed in layers with non-absorbable sutures.

6. The animals will be recovered and allowed food and water *ad libitum*.

7. Skin wound dressings will be changed as needed.

8. Thirty days after wounding the animals will be euthanized and necropsied. Histological sections will be made and the wounds assessed for the following:
    a. Infection
    b. Inflammation
    c. Foreign body reaction
    d. Scarring
    e. Extent of healing

D. A total of 75 pigs are required for this protocol.

E. <u>Laboratory Animals Required and Justification:</u>

1. **<u>Non-animal Alternatives Considered:</u>** Bleeding times on various tissues cannot be studied in an in vitro system. For a relevant bleeding time, it is necessary to use a live mammalian animal model. In vitro work has been done extensively with SeraSeal and it cannot substitute for animal work because of the lack of

tissue activators, responses, and platelets which are important parts of the clotting system. Nor can arterial, venous, and capillary beds be evaluated.

2. **Animal Model and Species Justification:** The pig is the only mammal with skin close enough to be human skin to make skin bleeding useful. Lower species have different clotting proteins, which may react differently to SeraSeal. More than 2500 anecdotal human uses are reported but no quantitative evaluation was done. In addition, much data from previous experiments using pigs is available for comparative use. The pig has been established as the standard hemostasis and shock model by the DoD; in order to provide comparative data it is necessary to use pigs in this work. We have used the pig previously in our laboratory in this same model and it has performed satisfactorily.

3. **Laboratory Animals to be Used:**

   a. **Genus & Species:** Sus Scrofa

   b. **Strain/Stock:** Yorkshire Pigs

   c. **Source/Vendor:**

   d. **Age:** Appropriate for weight

   e. **Weight:** approximately 150 pounds

   f. **Sex:** animals of either sex will be used.

   g. **Special Considerations:** Clinically healthy

   h. **Other:** None

4. **Total Number of Animals Required:** 75 pigs

5. **Refinement, Reduction, Replacement:**

   a. **Refinement:** The pigs will be under general anesthesia for surgery; and will be euthanized following the procedure, or allowed to recover for 30 days, and then euthanized. Analgesia will be needed for part 2 of this protocol, and will be provided by injection of Buprenorphine (Buprenex), in the amount of 0.05-0.1 mg/Kg IM q 12 h. The analgesic will be injected regularly q 12 h for 2 weeks after operation in the recovering animals. The animals will be examined twice daily for adequacy of pain relief. If, in the opinion of the attending veterinarian additional pain relief is required, Buprenex will be administered on an as needed basis.

We have considered extensive measurement of vital signs such as cardiac output but rejected this measurement as unnecessarily invasive without providing useful data. Intensive monitoring would have required a large number of animals, longer anesthesia time, and would result in more surgical procedures and complications.

b. **Reduction:** Based on consultation with a statistician the minimum number of animals necessary to answer the research questions and achieve statistical significance will be used. A statistical power analysis indicates that the sample of 12 wounds per animal will provide an 80% chance of detecting differences that are 1 standard deviation in magnitude when testing at the 0.05 alpha level.

c. **Replacement:** Pigs were chosen for this study because they are the standardized hemostasis and shock model for the DoD. It would not be possible to compare this study to data from the US Army or Navy using lower species. Also it is possible to do intensive monitoring of pigs, and pig skin is the closest to human skin for study. We therefore feel that use of lower species would not give useful information.

C. Technical Methods:

1. Pain:

a. USDA (APHIS form 7023) Pain category:

(1) No pain 0 (#) 0 % (Column C)

(2) Alleviated Pain 75 (#) 100 % (Column D)

(3) Unalleviated Pain or Distress 0 (#) 0 % (Column E)

b. Pain Alleviation:

(1) Anesthesia/Analgesia/Tranquilization:

Pre-anesthetic: Thirty to 45 minutes prior to induction of anesthesia, all animals will be sedated with the following:
Telazol 8.0 mg/Kg IM
Atropine 0.04 mg/Kg IM, and
Medetomidine 1 mg IM

Or, alternatively,
Ketamine 10 mg/Kg IM,
Medetomidine 0.08 mg/Kg IM, and
Butorphenol 0.2 mg/Kg IM

The pre-anesthetic will be given in the muscle in the caudal gluteal or distal lateral neck region with a 20-22 ga X 1 ¼ inch needle by a veterinary technologist under the direction of the attending veterinarian. A 20-22 ga 1 ¼ inch intravenous catheter will be placed in the auricular vein for venous access.

Induction: Anesthesia will be induced by a veterinarian or qualified technician under the veterinarian's supervision. The animals will be orally intubated with a 6.0 – 7.5 mm pressure cuff endotracheal tube.

Anesthesia will be maintained by the veterinarian or qualified technician under the veterinarian's supervision, isoflurance 2.5 – 3.0% in 100% oxygen.

(2) Paralytics: none

c. Alternatives to Painful Procedures:

(1) Source(s) Searched: MEDLINE, DoD Biomedical Research Database, CRISP
(2) Key Words of Search: pain, alternative, pig, animal model, hemostatic agent
(3) Results of Search: No non-pain or less painful procedures were found in this search. Poly-N-Acetyl Glucosamine is being evaluated in pigs and humans by the Office of Naval Research. The ONR has also evaluated QuikClot in pigs. Four other studies using swine were found for fibrin sealants. One study using fibrin sealants in rats was found. The fibrin bandage is being tested by the Army in pigs. No study using SeraSeal was found. Twenty two studies using fibrin sealants or N-acetyl Glucosamine were located.

d. Painful Procedure Justification: The attending veterinarian was consulted in the planning of the alleviated painful procedures in the protocol. There are no unalleviated painful procedures. In order to study hemostasis, it is necessary to create a wound. The pig has been established as the standard model for this type of work.

2. Prolonged Restraint: not required

3. Surgery:

Procedure:

a. After satisfactory general anesthesia is obtained, the animals will be placed in the prone (ventral recumbent) position.
b. The **skin wounds** will be created as described below: a dermatome will be used to remove 44/1000 of epidermis and dermis to the mid dermal level resulting in a wound like those seen in skin grafting and debrided burns. The size of the wound will be 2 X 4 in. Two wounds, one on each lower flank will be created.

c. The animals will be repositioned in the supine position and the following wounds will be created:
d. **Liver:** A laparotomy will be performed and the right lobe of the liver exposed. A 5 cm laceration will be made by amputating a portion of the tip of the lobe. After treatment a second 5 cm laceration will be made by amputating another portion of the lobe.
e. **Spleen:** the spleen will be exposed and a 3 cm laceration will be made by amputating a portion of the organ. After treatment a second 3 cm will be made and treated.
f. **Muscle:** The semitendinosus muscle will be exposed by reflecting the skin. A 5 cm muscle laceration will be made longitudinal to the fibers and hemostasis allowed to occur. A second laceration will be performed adjacent to the first.
g. **Femoral artery:** Proximal and distal control will be obtained. A 4 mm incision will be made in the anterior wall of the artery. In this wound the hemostatic agent will be applied first and gentle pressure applied with gauze. After hemostasis, a second wound will be performed proximally and treated similarly. If bleeding has not stopped after 3 minutes of gentle pressure with gauze for either wound, the wound will be sutured with 3-0 sutures.
h. **Femoral vein:** Proximal and distal control will be achieved. A 0.5 cm incision will be made in the anterior wall of the vein. Following hemostasis on the first wound a second incision distal to the first will be made.
    5. If the systolic blood pressure falls below 60 mm Hg the study will be stopped until blood pressure can be restored.
    6. If the animals will not be recovered, they will be euthanized at this point.
    7. Animals to be recovered will have their abdominal and femoral wounds closed in layers with non-absorbable sutures. The skin wounds will be dressed with Tegaderm, which will be changed as needed.

b. <u>Pre- and Postoperative Provisions:</u>

1. **Pre-Operative Provisions:** Pre-operatively the animals will be housed at the animal care facility in Building 125 or 185. The pigs will be group housed in pens in a climate-controlled building pre-operatively. The pigs will undergo a seven-day acclimatization period before initiation of the study. The animals will be NPO after midnight except for water on the morning of the procedure.

2. **Post-Operative Provisions:** Post-operatively the animals will be individually housed at the animal care facility in Building 125 or 185. The pigs will be individually housed in pens in a climate-controlled building. They will be examined twice daily by the veterinary staff and assessed for pain relief and any sign of illness.

Analgesia will be needed for part 2 of this protocol, and will be provided by injection of Buprenorphine (Buprenex), in the amount of 0.05-0.1 mg/Kg IM q 12 h. The anesthetic will be injected regularly q 12 h for 2 weeks after operation in the recovering animals. The animals will be examined twice daily for adequacy of pain relief. If, in the opinion of the attending veterinarian additional pain relief is required, Buprenex will be administered on an as needed basis.

Animals exhibiting signs of illness will be examined by the attending veterinarian. He will determine whether the animal should be treated or euthanized. The appropriate treatment will be at the discretion of the attending veterinarian.

Location: Surgical/Procedure Suite, Building 125, 4th floor, Brooks AFB, TX.

Multiple Survival Surgery Procedures: not required

Animal Manipulations:

a. Injections: Buprenex for pain relief

b. Biosamples: none

c. Animal Identification: ear tags and cage cards applied by the veterinary technologists.

d. Behavioral Studies: none

e. Other procedures: none

5. Adjuvants: none

6. Study Endpoint: The endpoint of the study will be completion of the operative procedure for part 1, and the end of the 30 day recovery period for part 2. The animals will be euthanized following completion of the study.

7. Euthanasia: The animals will be euthanized after the study endpoint is reached. Euthanasia will be by overdose of sodium pentobarbital 100 mg/Kg (Euthanasia-5 solution) given IV in an auricular vein using an 18 or 20 ga 1 ¼ inch needle. Euthanasia will be performed by the attending veterinarian, veterinary technologist or qualified surgical technician under the direction of the veterinarian.

D. Veterinary Care:

1. Husbandry Considerations

a. General Husbandry: The pigs will be housed at Animal Care Facility in accordance with the Ols on quarantine and stabilization of animals and fed and watered in accordance with established Old. The pigs will be individually housed.

b. Special Husbandry Provisions: NA

2. **Attending Veterinary Care:** Provided by the veterinarian as deemed appropriate and in accordance with applicable Ols. Administration of anesthetics and analgesics at the discretion of the attending veterinarian is authorized.

3. **Enrichment Strategy:** Enrichment of the pigs will be in accordance with standard practice at the animal care facility. The pigs will be individually housed in pens and will be provided hard rubber balls for entertainment.

E. Data Analysis:

Part 1: Two end points will be available for each wound: time to hemostasis and amount of bleeding. The wounds are:

Skin
Muscle
Liver
Spleen
Femoral artery
Femoral vein

Part 2: Time to hemostasis and amount of bleeding are available for these wounds also. In addition, qualitative assessment of the wounds at 30 days for infection, inflammation, foreign body reaction, scarring, and extent of healing will be made.

Statistical analysis of data:

Part 1: There are two outcome variable for statistical analysis for each of six wounds per treatment time to hemostasis, and amount of bleeding. With an estimated effect size of .90 (one tenth the time) the power with 75 pigs would be 80%. This assumes the standard error were .21 with a confidence interval of .00 - .72.

VI. Enclosures:

A. References/Citations/Bibliography:

Enclosure A

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B. Literature Search Summary

The search revealed that the pig is an established model for evaluation of hemostasis. Most topical hemostatic agents have been either fibrin sealant or microfibrillar collagen. The review of the literature found no duplication of effort.

Results

Ease of Use

FloSeal The process to mix FloSeal became cumbersome, time consuming (~25 minutes), and probably technically impossible to use in a combat setting. The syringe applicators were bulky and somewhat difficult to handle. We recommend the manufacturer produce a one step process for application of FloSeal.

QuikClot Originally, QuikClot was used by dumping the contents of the package into the wound, leading to easy usage. This was effective, but led to full thickness burns on the skin and muscle. In addition, the granules adhered to areas surrounding the wound during application that was hard to clean. The manufacturer then revised the instructions, recommending that the product be added to the wound a little at a time. We found that doing this did not increase effectiveness, and still produced burns. This finding is discussed in detail in the section "thermal injury resulting from Application of a Granular Hemostatic Agent."

The granular nature of QuikClot was also problematic. It was very difficult to remove the granules from the wound after application. Irrigation was minimally effective. Around a bleeding site QuikClot® formed a solid granule-clot complex which could not be removed without provoking further bleeding. Clinically anecdotal reports have stated that patients were closed surgically leaving the agent in place to be removed at a secondary operation some time later. In our groin wound model the QuikClot produced abscesses when the wound was closed.

SeraSeal-Liquid The liquid was easy to use and effective to control deep wounds and arterial bleeds. It is impractical to apply SeraSeal-Liquid drop wise from a syringe for large surface wounds unless the wound is flooded.

SeraSeal-Spray The spray was very easy to use. Over spraying the wound diluted the blood making it hard to assess how much blood was lost. SeraSeal-Spray was ineffectual in arterial wounds because the nebulizing effect of the spray had diffused the product over the wound, limiting the amount of the hemostatic agent to reach directly the site of bleeding.

SeraSeal-Dressing The dressing was effective and easy to use. It had to be held in direct contact with the bleeding source to be consistently effective. For brisk bleeding it was easy to assess the blood lost from the bleeding through the dressings, but for minimally bleeding wounds the assessment of bleeding through the dressing was difficult. As with all hemostatic dressings were only effective when placed in direct contiguity with the bleeding source. Thus, the dressing was effective only in wounds palpable by hand at the bleeding site.

SeraSeal-Foam The foam was easy to use and reached all parts of the wounds, including deep wounds. Of all the agents, SeraSeal-Foam was the easiest to use. The foam was very effective in wounds where the hemostatic agent could be contained.

Effectiveness in Achieving Hemostasis

FloSeal All wounds stopped bleeding in less than 5 minutes (300 sec). (**Table 1 and 2**) None of the wounds met the desirable standard of hemostasis within 60 seconds of application. Blood loss results were evaluated as the percent of bleeding seen in control wounds. FloSeal worked best on venous bleeding and achieved an overall rating of 46% of the amount of control bleeding. **(Table 3)**

QuikClot All wounds stopped bleeding in less than 5 minutes (300 sec). None of the wounds met the desirable standard of hemostasis within 60 seconds of application. Blood loss results were evaluated as the percent of bleeding seen in control wounds. It was difficult to estimate the true amount of bleed loss in wounds treated with QuikClot, because the agent formed a hard compound with bleed and absorbed blood, tissue fluid, and serum when applied. QuikClot worked best on muscular and hepatic bleeding and achieved an overall rating of 43% of control bleeding.

SeraSeal-Liquid All wounds stopped bleeding within the desirable standard of hemostasis of 60 seconds from the time of application, and in the overall assessment of time to hemostasis. Blood loss results were evaluated as the percent of bleeding seen in control wounds. SeraSeal-Liquid achieved an overall rating of 4% of the amount of control bleeding.

SeraSeal-Spray Other than in arterial wounds, SeraSeal-Spray was consistently effective to stopped bleeding within the desirable standard of 60 seconds after application. The failure to achieve hemostasis in the arterial wounds was due to the nebulizing effect of the spray. Blood loss results were evaluated as the percent of bleeding seen in control wounds. SeraSeal-Spray achieved an overall rating of 29% of the amount of control bleeding.

SeraSeal-Dressing SeraSeal-Dressing brought wounds to hemostasis within 60 seconds, and meeting the desirable standard of hemostasis. Blood loss results were evaluated as the percent of bleeding seen in control wounds. SeraSeal-Dressing achieved an overall rating of 6% of the amount of control bleeding.

SeraSeal-Foam All wounds stopped bleeding within the desirable standard of hemostasis of 60 seconds from the time of application. Blood loss results were evaluated as the percent of bleeding seen in control wounds. SeraSeal-Foam achieved an overall rating of 14% of the amount of control bleeding.

Table 1: Time to Hemostasis

No Hemostatic Agent (n=12)					
Hemostasis (sec)					
skin	muscle	liver	spleen	vein	artery
90	178	300	300	300	300
300	67	300	300	300	300
300	300	300	300	300	300
300	240	300	300	300	300
300	165	300	300	300	300
300	240	300	300	255	260
300	300	300	300	175	158
300	60	300	210	300	300
243	199	300	300	300	300
300	240	150	300	300	300
300	185	300	300	300	300
300	247	163	180	300	300
X 277.75	201.75	276.08	282.50	285.83	284.83
SD 61.35	77.57	55.93	41.37	37.22	41.56

FloSeal (n=9)					
Hemostasis (sec)					
skin	muscle	liver	spleen	vein	artery
65	13	300	132	195	300
46	80	100	300	65	300
120	30	80	190	300	300
120	40	300	174	158	282
120	38	240	45	120	250
44	120	180	180	150	80
59	80	120	300	72	120
210	30	90	80	300	300
130	135	285	255	300	300
X 101.56	62.89	188.33	184	184.44	248
SD 53.78	43.07	94.14	90.10	95.60	86.09

QuikClot (n=7)						
Hemostasis (sec)						
	skin	muscle	liver	spleen	vein	artery
	180	49	62	96	300	300
	240	60	192	200	170	195
	300	300	120	240	120	300
	300	70	300	300	140	300
	300	80	80	300	90	300
	300	70	300	300	140	300
	300	30	30	240	64	270
X	274.28	94.14	154.86	239.43	146.29	280.71
SD	47.21	92.25	111.46	74.31	76.27	39.42

SeraSeal liquid (n=5)						
Hemostasis (sec)						
	skin	muscle	liver	spleen	vein	artery
	4	2	2	2	4	8
	3	2	3	2	5	10
	3	2	2	3	3	7
	5	2	2	3	5	8
	3	2	2	2	4	11
X	3.60	2.00	2.20	2.40	4.20	8.80
SD	0.89	0.00	0.45	0.55	0.84	1.64

SeraSeal spray (n=6)						
Hemostasis (sec)						
	skin	muscle	liver	spleen	vein	artery
	2	4	3	2	85	300
	3	5	5	3	93	300
	2	3	4	4	90	270
	2	2	6	2	96	300
	2	4	3	2	88	300
	4	2	4	2	75	300
X	2.50	3.33	4.17	2.50	87.83	295
SD	0.84	1.21	1.17	0.84	7.36	12.25

SeraSeal dressing (n=15)					
Hemostasis (sec)					
skin	muscle	liver	spleen	vein	artery
20	20	40	30	40	50
30	20	40	20	40	60
20	20	30	20	30	50
20	40	50	20	40	60
30	20	30	30	50	60
30	30	40	20	30	40
30	20	40	30	30	60
20	20	40	30	40	50
20	30	30	30	40	50
20	30	0	20	40	40
30	20	50	30	30	60
20	20	40	20	40	60
20	20	40	20	40	60
20	20	30	30	50	50
30	20	40	30	40	50
X 24.00	23.33	39.33	25.33	38.67	53.33
SD 5.07	6.17	7.04	5.16	6.40	7.24

SeraSeal foam (n=25)					
Hemostasis (sec)					
skin	muscle	liver	spleen	vein	artery
3	2	4	3	8	14
2	3	5	3	9	18
3	2	4	3	8	12
3	3	4	3	7	13
4	3	4	2	8	15
2	2	4	4	8	14
3	3	5	3	9	14
3	3	3	3	8	11
2	2	4	3	10	15
2	3	4	3	8	14
2	3	4	2	7	14
3	2	5	3	8	13
3	3	4	3	8	16
3	3	4	3	8	14
4	2	4	4	8	13
3	3	4	4	8	11
3	3	4	3	7	12
3	2	5	3	8	13
2	2	4	3	9	11
2	3	6	3	8	14
3	3	4	3	8	15
3	3	4	2	8	13
3	2	4	3	9	14
3	2	5	2	8	12
4	2	4	3	8	13
X 2.84	2.56	4.24	2.96	8.12	13.52
SD 0.62	0.51	0.60	0.54	0.67	1.61

Table 2: Blood Loss

No Hemostatic Agent (n=12)					
Blood Loss (ml)					
skin	muscle	liver	spleen	vein	artery
0.5	6.5	42.4	67.2	235.5	143.8
3.2	2.2	8.6	48.3	411.7	282.1
28.6	6.3	47.3	75.4	271.8	425
6.8	16.6	33.6	22.2	339.2	87.1
1.8	6.1	32.8	35	136.9	163.8
3	14.1	43.2	12	8.6	39.9
15.2	7.2	104.9	74.7	115.8	490.8
11.1	2.5	265.8	42.8	186.8	51.8
2.3	10.2	116.9	91.7	164.2	320.8
12.4	4.5	13.1	20.9	74.2	25.4
4.2	5.9	85.8	111.8	252.5	152.7
20	17	48.8	11.8	258	158.3
X 9.09	8.26	70.27	51.15	204.6	195.13
SD 8.67	5.10	70.17	32.89	113.40	152.41

FloSeal (n=9)					
Blood Loss (ml)					
skin	muscle	liver	spleen	vein	artery
1.2	0.2	60.2	27.7	43.5	20.2
19.6	19.2	18.6	25.2	21.3	6.8
6.8	5.4	1.1	5.2	84.2	326.3
5.2	8.2	84.8	15.2	49.1	94.9
7	1.7	30.5	18.5	3.4	30.5
3.2	2.3	62.5	21.6	41.5	289.2
2.1	2.2	8.1	32.3	10.8	19
3.2	4	23.3	7.8	49.7	26.9
1.7	1.4	62.8	53.9	19.8	53.6
X 5.56	4.96	39.10	23.04	35.92	96.38
SD 5.68	5.86	29.14	14.57	24.86	122.91

QuikClot (n=7)					
Blood Loss (ml)					
skin	muscle	liver	spleen	vein	artery
1.7	2	3.2	8.4	151.2	23.8
6.3	2.4	12.4	22.4	131.7	121.5
1.8	5.4	39.5	43.9	52.5	22
1.2	3.8	5.3	44.2	5.5	316
5.2	3.6	39.2	21.8	63.3	222
1.2	0.2	23	75.8	11.1	206.6
16.1	2.8	5.3	30.1	6.2	36.5
X 4.79	2.89	18.27	35.23	60.21	135.49
SD 5.39	1.63	15.86	21.96	60.25	115.81

SeraSeal liquid (n=5)					
Blood Loss (ml)					
skin	muscle	liver	spleen	vein	artery
1.1	0.3	2.4	1.3	4.2	8.9
0.8	0.4	1.8	0.9	4.5	12.2
0.5	0.2	2.6	1.7	3.8	8.4
0.9	0.5	2.2	1.3	5.2	9.2
0.6	0.2	2.5	1.1	4.7	11.6
X 0.78	0.32	2.30	1.26	4.48	10.06
SD 0.24	0.13	0.32	0.30	0.53	1.72

SeraSeal spray (n=6)					
Blood Loss (ml)					
skin	muscle	liver	spleen	vein	artery
0.2	0.6	1.1	0.5	96.4	321.2
0.2	0.8	0.9	0.6	108.2	281.9
0.1	0.5	1.4	0.3	103.5	314.8
0.2	0.4	1.8	0.7	124	296.3
0.3	0.7	1.3	0.4	156.8	352.2
0.3	0.9	2	0.6	163.4	306.4
X 0.22	0.65	1.42	0.52	125.38	312.13
SD 0.08	0.19	0.42	0.15	28.45	24.05

SeraSeal dressing (n=15)					
Blood Loss (ml)					
skin	muscle	liver	spleen	vein	artery
0.4	0.8	3.2	1.6	5.7	10.3
0.6	1.1	3.5	1.2	4.8	11.7
0.3	0.6	2.7	1.5	5.6	10.5
0.7	2.1	4.1	0.7	4.8	12.2
0.5	0.8	3	1.3	5.2	10.8
0.4	0.7	3.7	0.8	5.5	13.2
0.3	1.3	4.3	0.6	6.1	10.6
0.4	0.9	4.5	1.4	5.7	11.4
0.5	1.4	3.8	1.2	5.5	12.8
0.7	1.1	3.2	0.8	4.8	11.7
0.8	0.7	4.4	1.5	5.2	11.2
0.5	0.9	4.2	1.2	5.4	11.5
0.4	0.5	3.9	0.7	5.9	12.3
0.6	0.9	4.2	0.8	5.6	10.8
0.7	0.5	4.7	0.5	5.3	11.3
X 0.52	0.95	3.83	1.05	5.41	11.49
SD 0.16	0.41	0.60	0.37	0.40	0.85

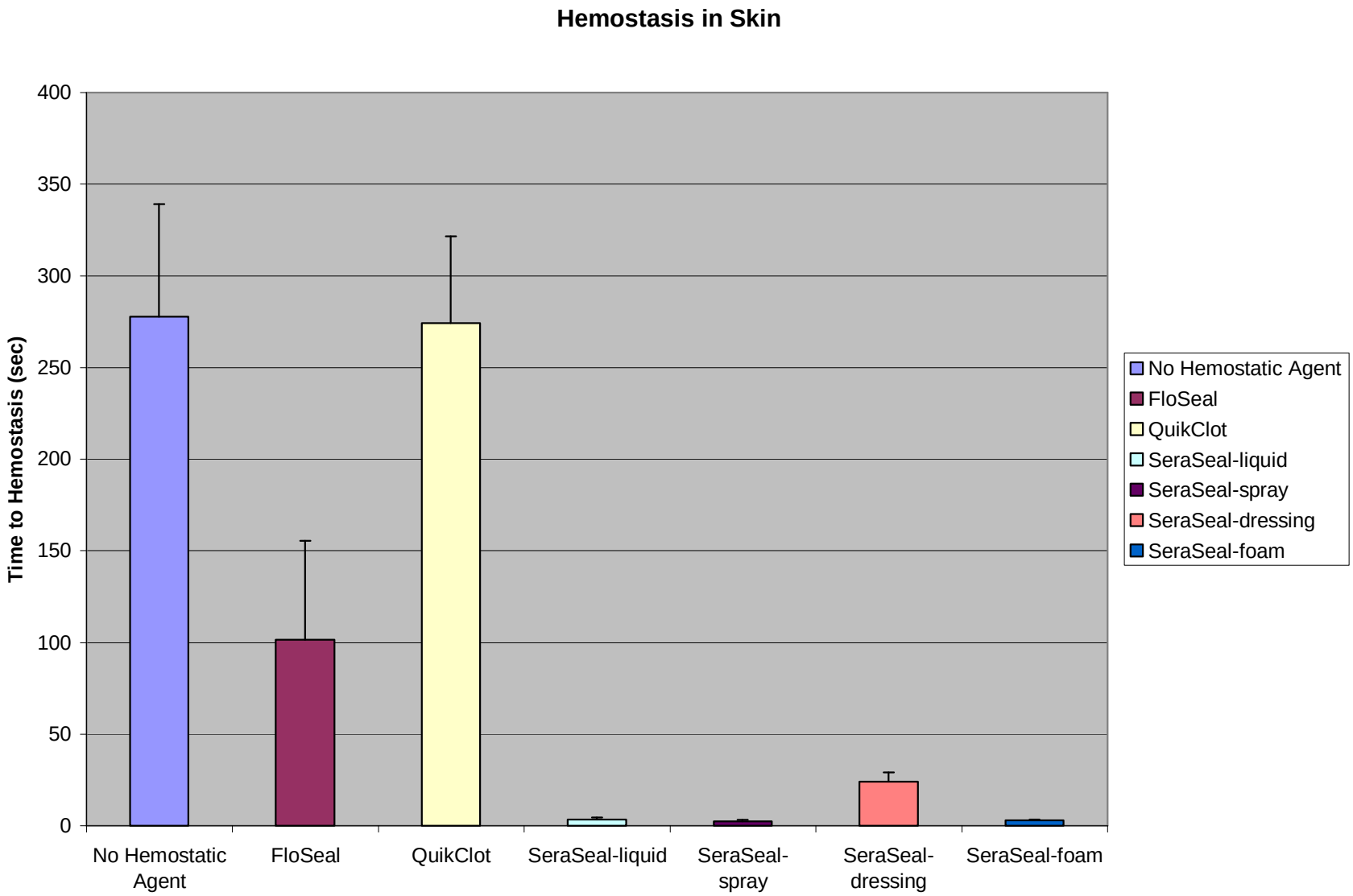
SeraSeal foam (n=25)					
Blood Loss (ml)					
skin	muscle	liver	spleen	vein	artery
1.9	2.3	4.5	3.2	12.1	26.8
1.3	3.1	4.2	2.8	10.8	27.6
2.2	2.5	4.8	3.4	14.2	23.4
1.8	2.4	5.2	4.3	11.5	32.3
1.3	2.5	4.8	3.4	12.4	29.5
1.4	1.9	4.5	3.1	12.2	31.7
1.5	2.4	4.6	3.5	14.1	25.8
2.2	2	6.1	3.6	13.5	40.1
2.3	3.8	4.4	2.8	12.8	28.7
1.8	2.7	4.8	4.2	12.4	25.9
1.8	2.5	5.2	3.9	12.1	26.8
1.9	2.2	5.5	3.5	11.2	32.4
0.8	2.8	4.9	3.5	13.3	27.6
1.5	3.2	5.2	3.2	12.7	28.7
1.6	2.9	4.8	3.7	15.2	24.5
1.2	2.4	4.7	4.4	13.4	22.8
2.4	2.6	4.6	4.2	14.6	29.4
1.8	2.1	4.3	3.8	14.3	28.7
1.7	1.9	5.2	3.5	15.2	33.2
1.9	2.2	7	2.6	14.7	25.9
1.5	2.8	5.8	3.4	13.5	28.6
2.3	2.5	4.2	3.7	12.4	31.3
1.4	3.3	4.8	3.9	15.2	28.1
1.8	2.4	7.2	2.8	14.8	27.8
1.3	2.8	4.4	4.1	11.9	27.3
X 1.70	2.57	5.03	3.54	13.22	28.60
SD 0.39	0.45	0.78	0.50	1.32	3.60

Table 3: Effectiveness in Achieving Hemostasis

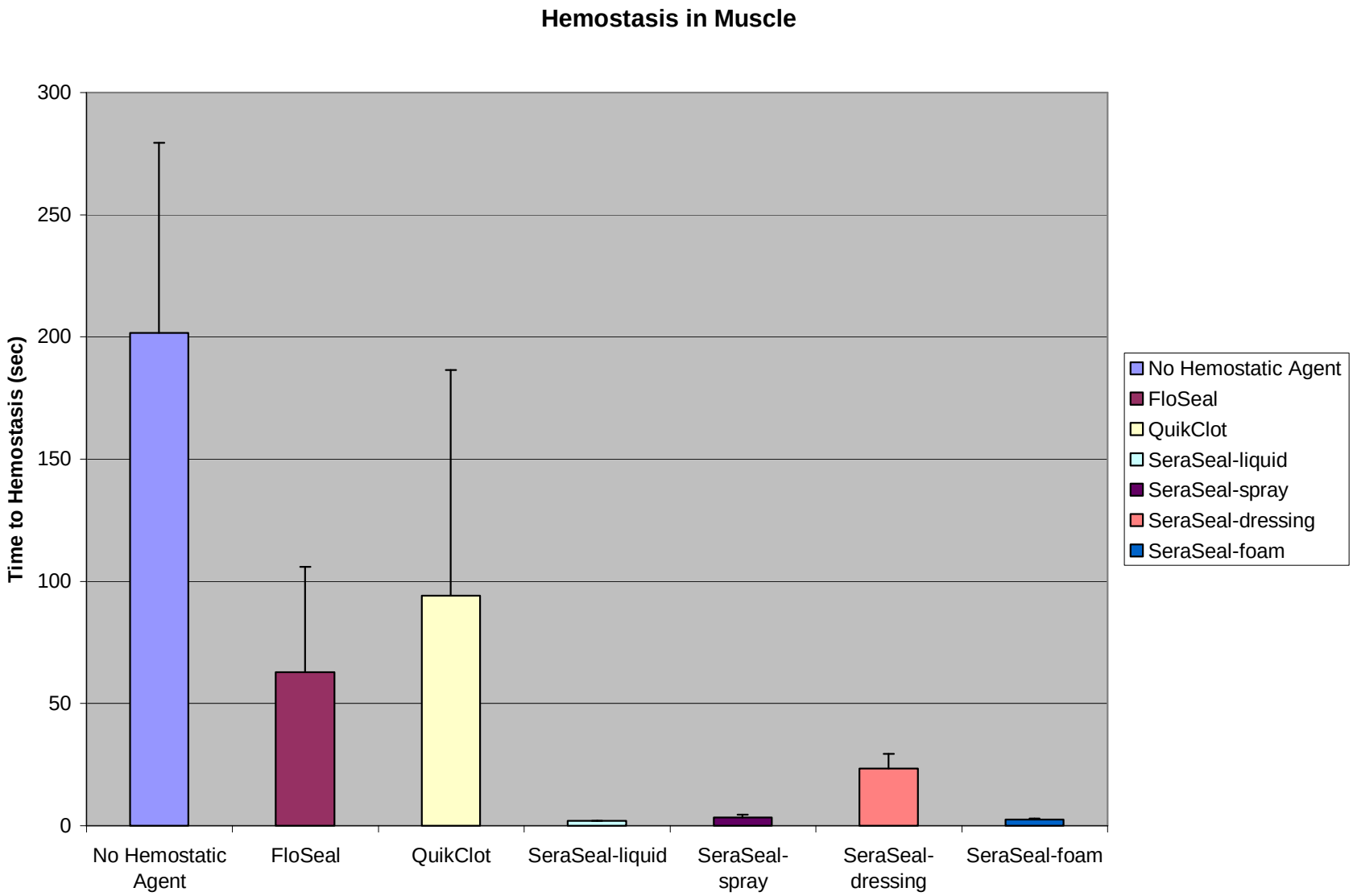
Time to Hemostasis (sec)							
	Skin	**Muscle**	**Liver**	**Spleen**	**Vein**	**Artery**	**Overall**
No Hemostatic Agent	278	202	276	282	286	285	268
FloSeal	102	63	188	184	184	248	166
QuikClot	274	94	155	239	146	281	198
SeraSeal-liquid	4	2	2	2	4	9	4
SeraSeal-spray	2	3	4	2	88	295	66
SeraSeal-dressing	24	23	39	25	39	53	34
SeraSeal-foam	3	3	4	3	8	14	6

Blood Loss (% of control)							
	Skin	**Muscle**	**Liver**	**Spleen**	**Vein**	**Artery**	**Overall**
No Hemostatic Agent	100	100	100	100	100	100	100
FloSeal	61	60	56	29	18	49	46
QuikClot	53	35	26	45	29	69	43
SeraSeal-liquid	8	4	3	2	2	5	4
SeraSeal-spray	2	8	2	1	61	100	29
SeraSeal-dressing	6	12	5	1	3	6	6
SeraSeal-foam	19	31	7	4	6	15	14

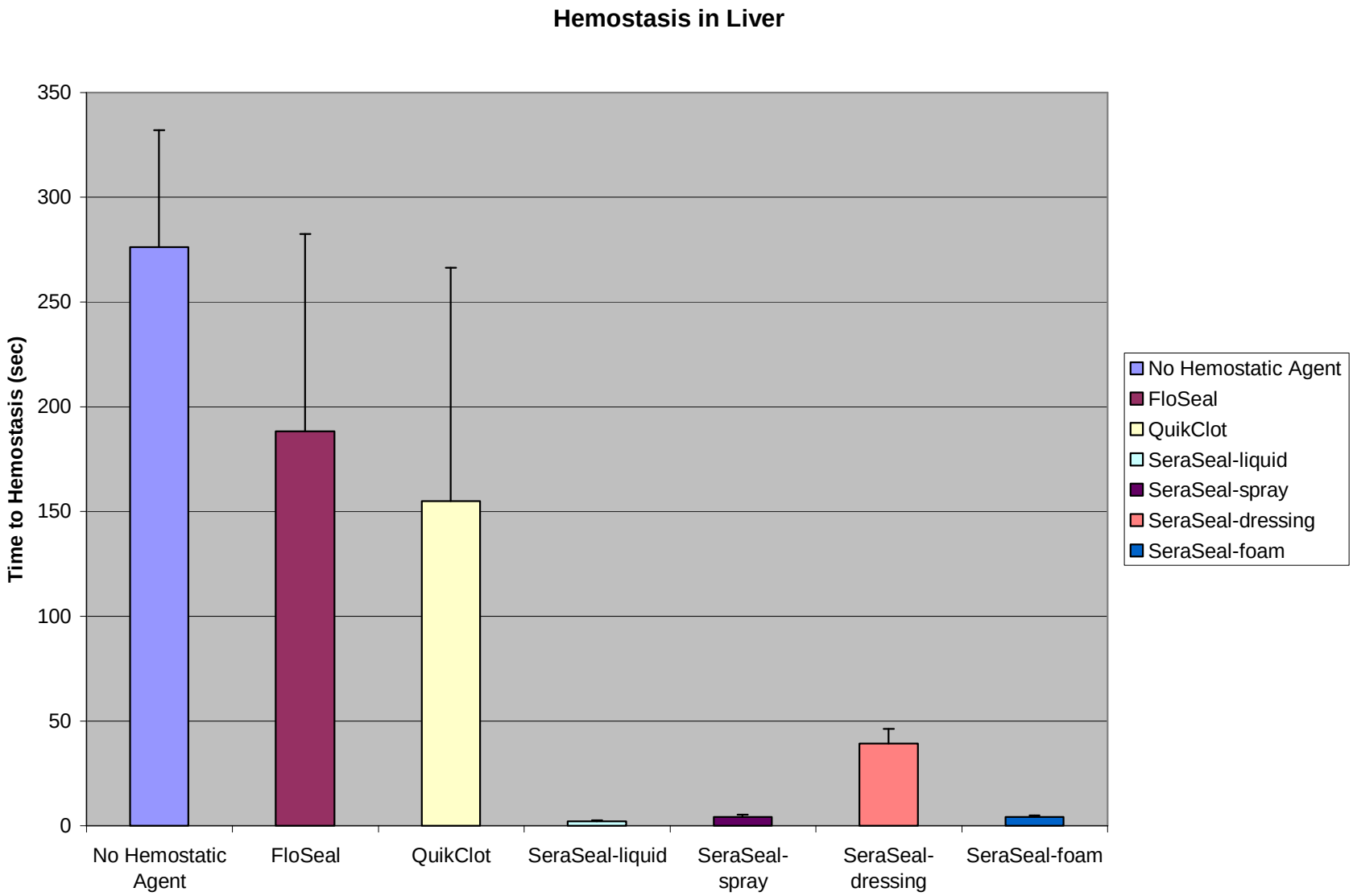
Graph 1:


Hemostasis in Skin
Time to Hemostasis (sec)
400
350
300
250
200
150
100
50
0
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam

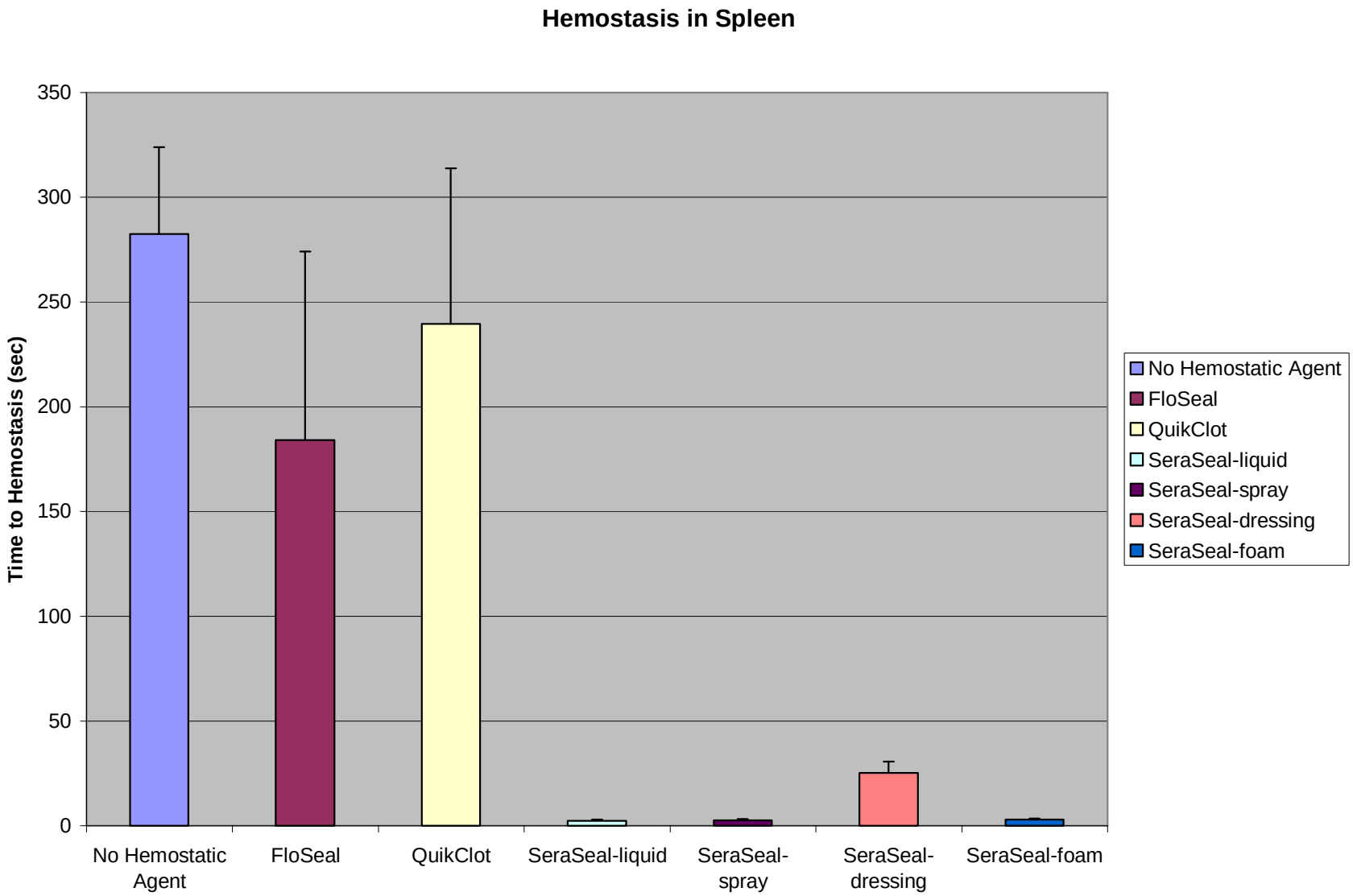
Graph 2:


Hemostasis in Muscle
Time to Hemostasis (sec)
300
250
200
150
100
50
0
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam

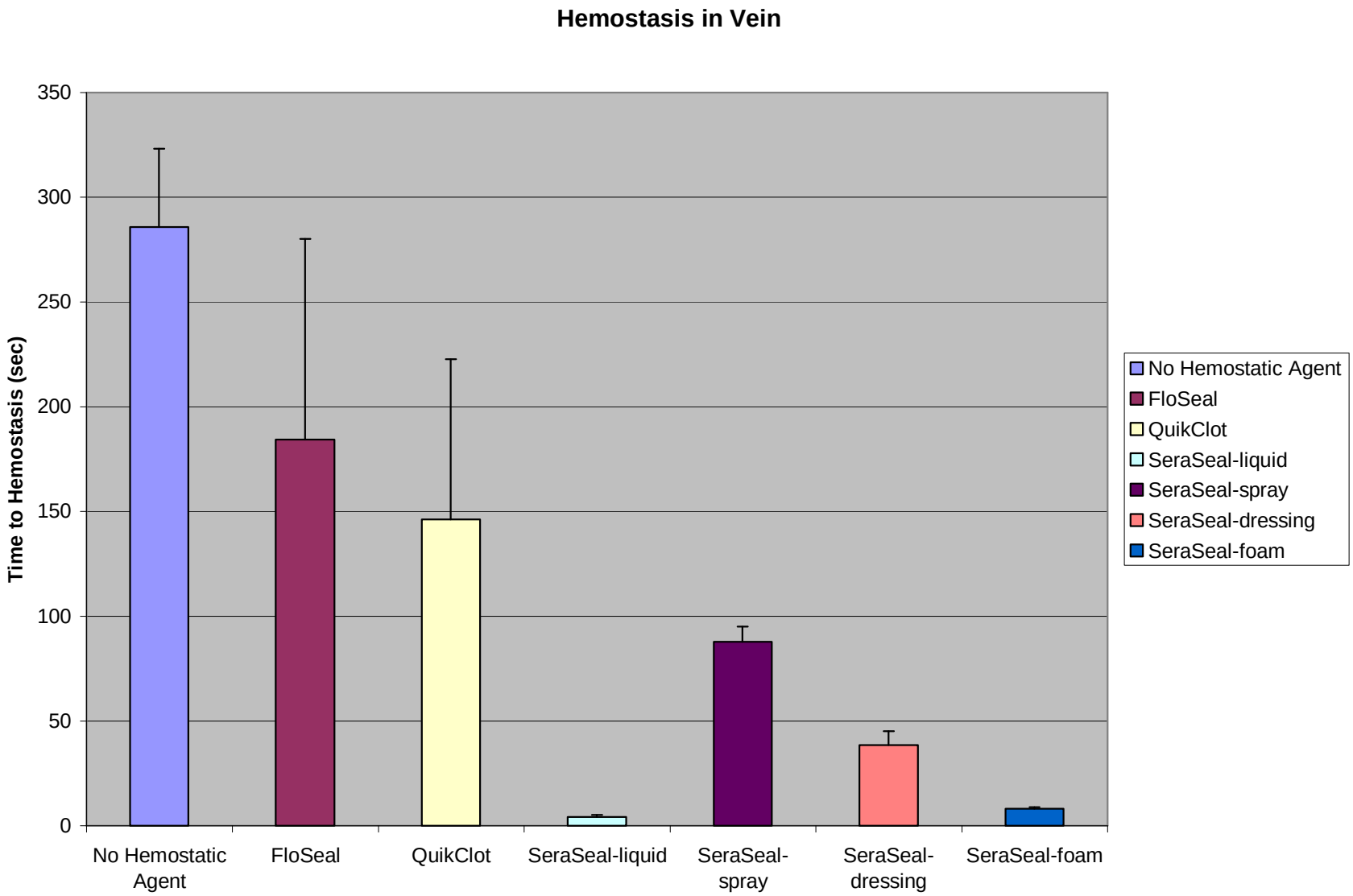
Graph 3:


Hemostasis in Liver
Time to Hemostasis (sec)
350
300
250
200
150
100
50
0
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam

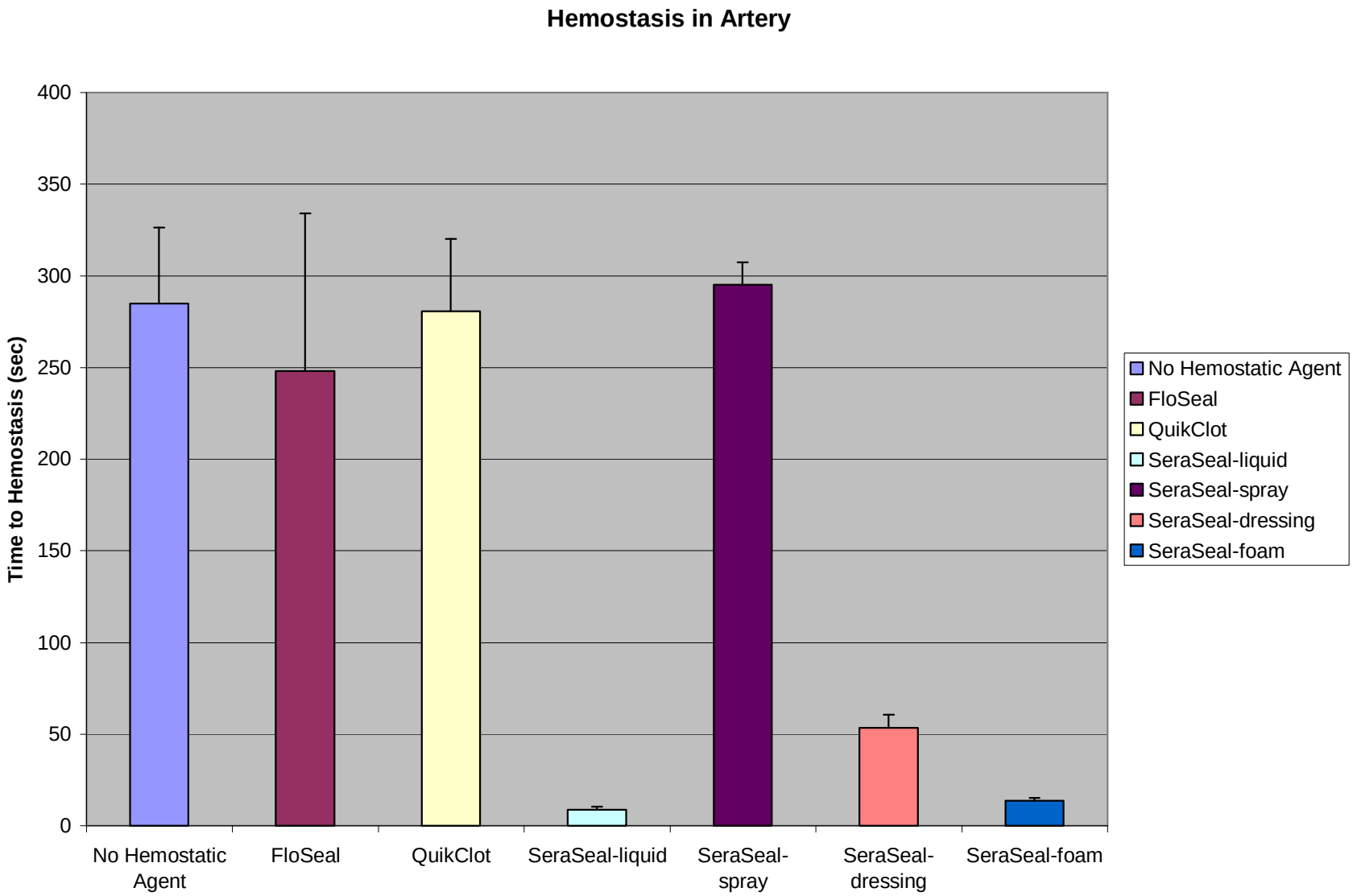
Graph 4:


Hemostasis in Spleen
Time to Hemostasis (sec)
350
300
250
200
150
100
50
0
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam

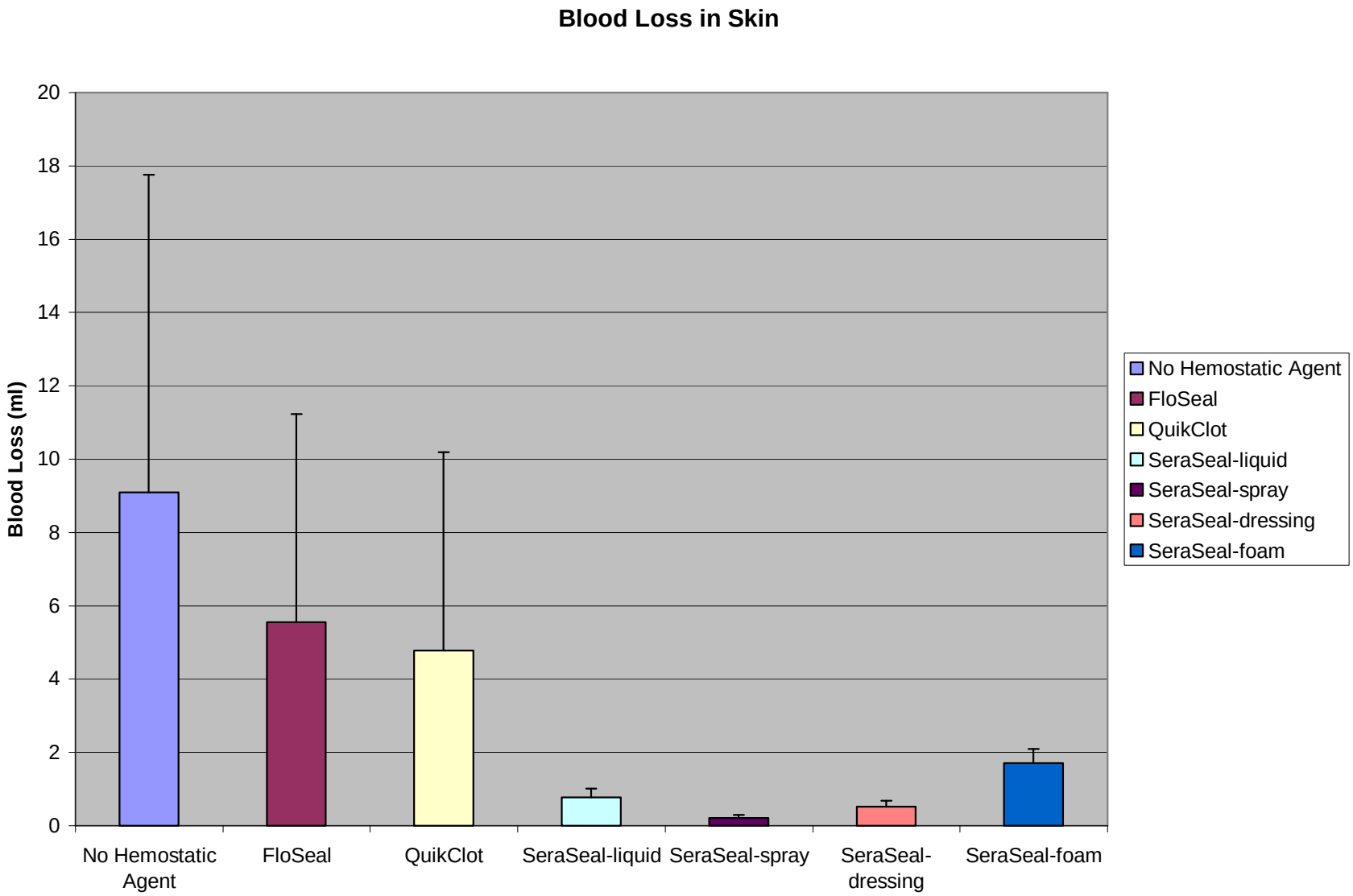
Graph 5:


Hemostasis in Vein
Time to Hemostasis (sec)
350
300
250
200
150
100
50
0
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam

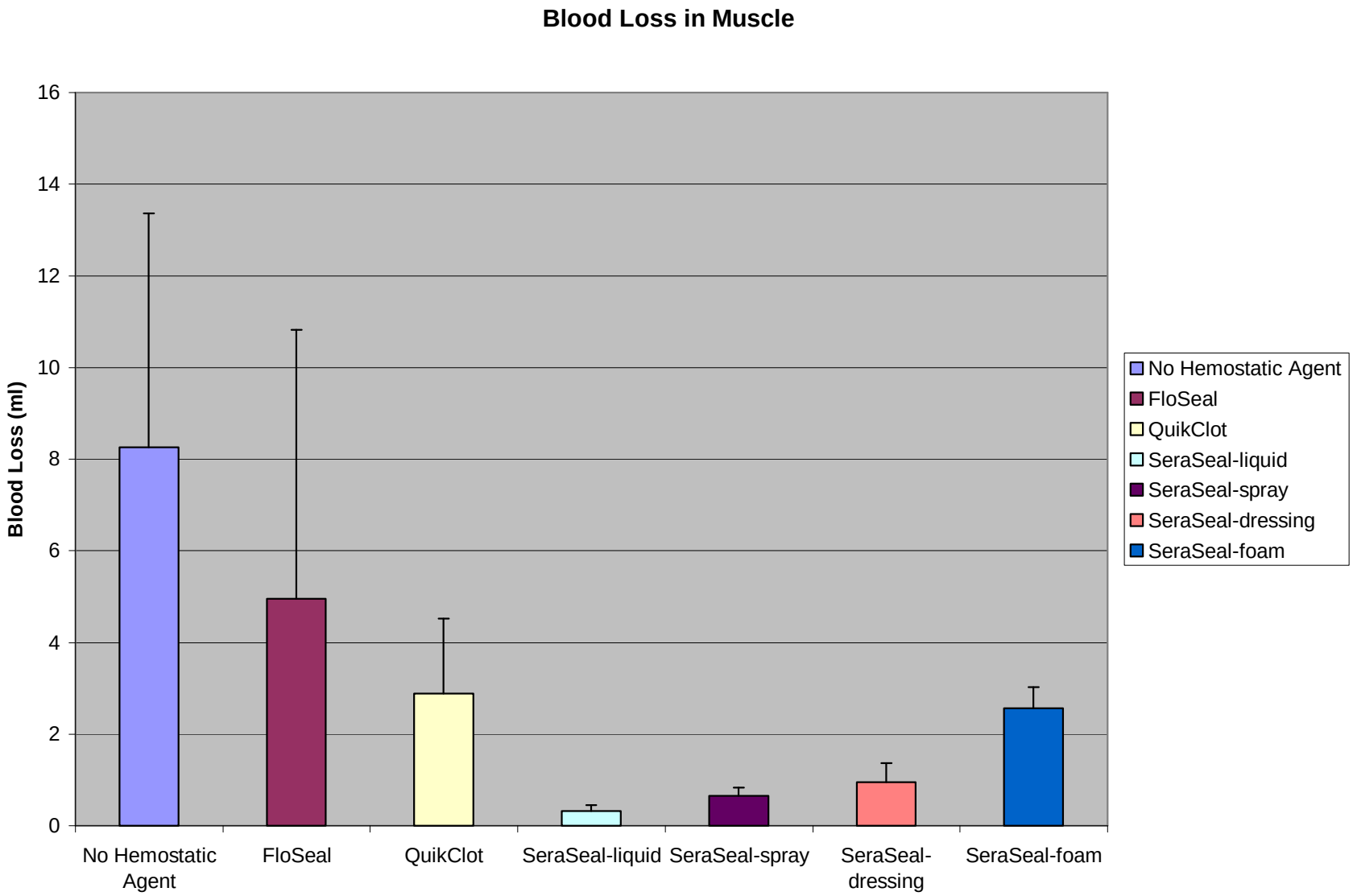
Graph 6:


Hemostasis in Artery
Time to Hemostasis (sec)
400
350
300
250
200
150
100
50
0
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam

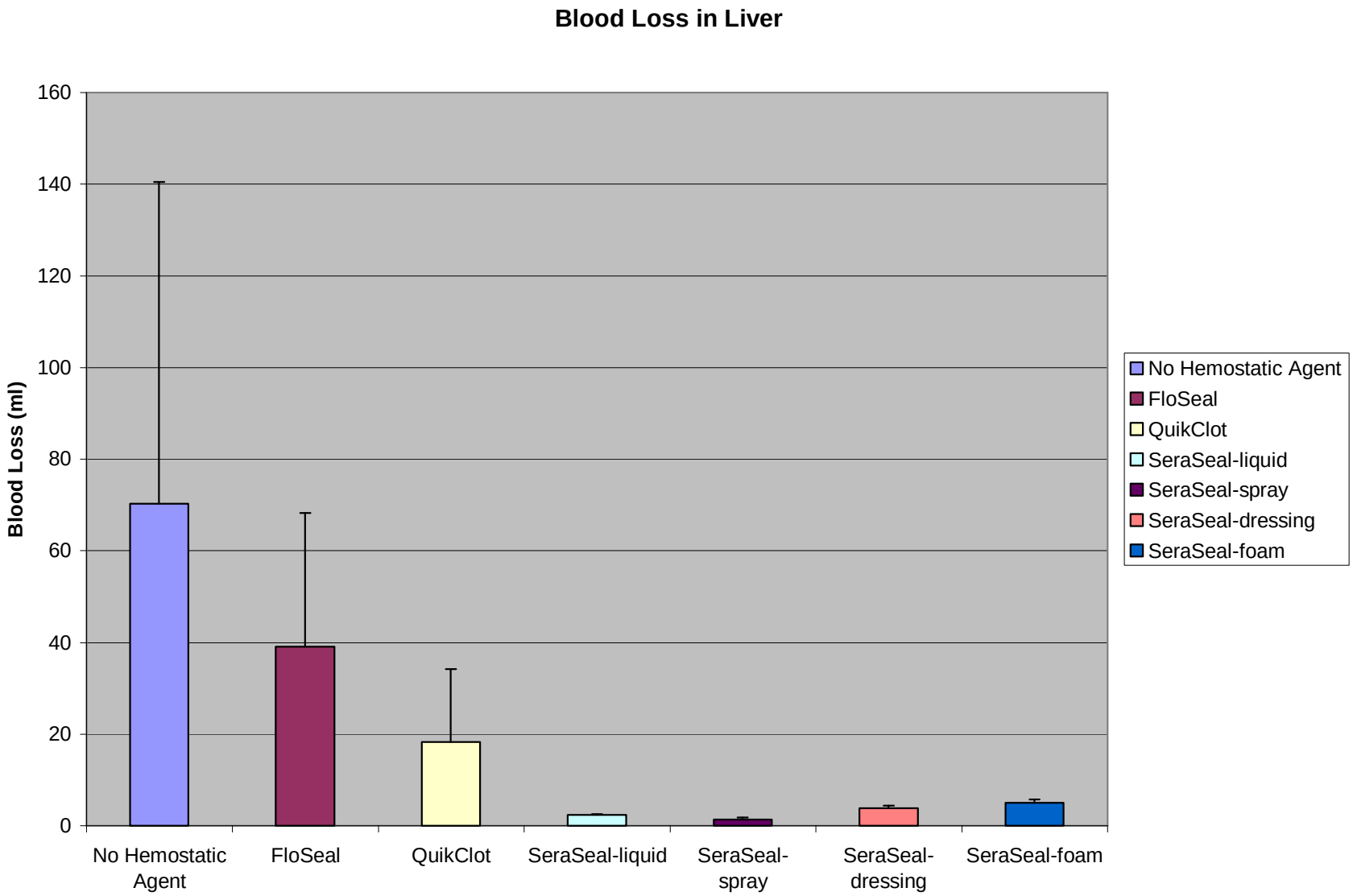
Graph 7:


Blood Loss in Skin
Blood Loss (ml)
20
18
16
14
12
10
8
6
4
2
0
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam

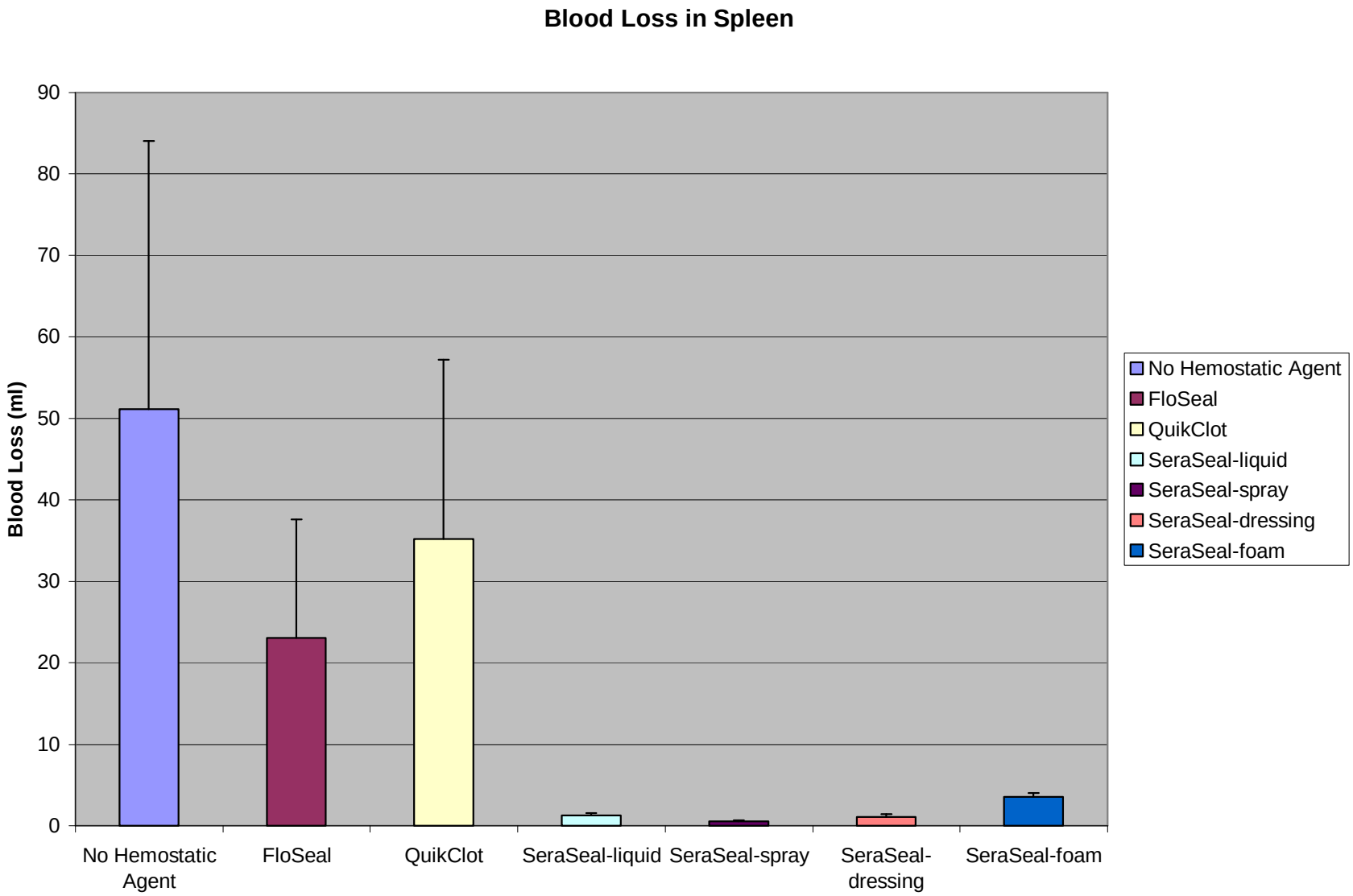
Graph 8:


Blood Loss in Muscle
Blood Loss (ml)
16
14
12
10
8
6
4
2
0
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam

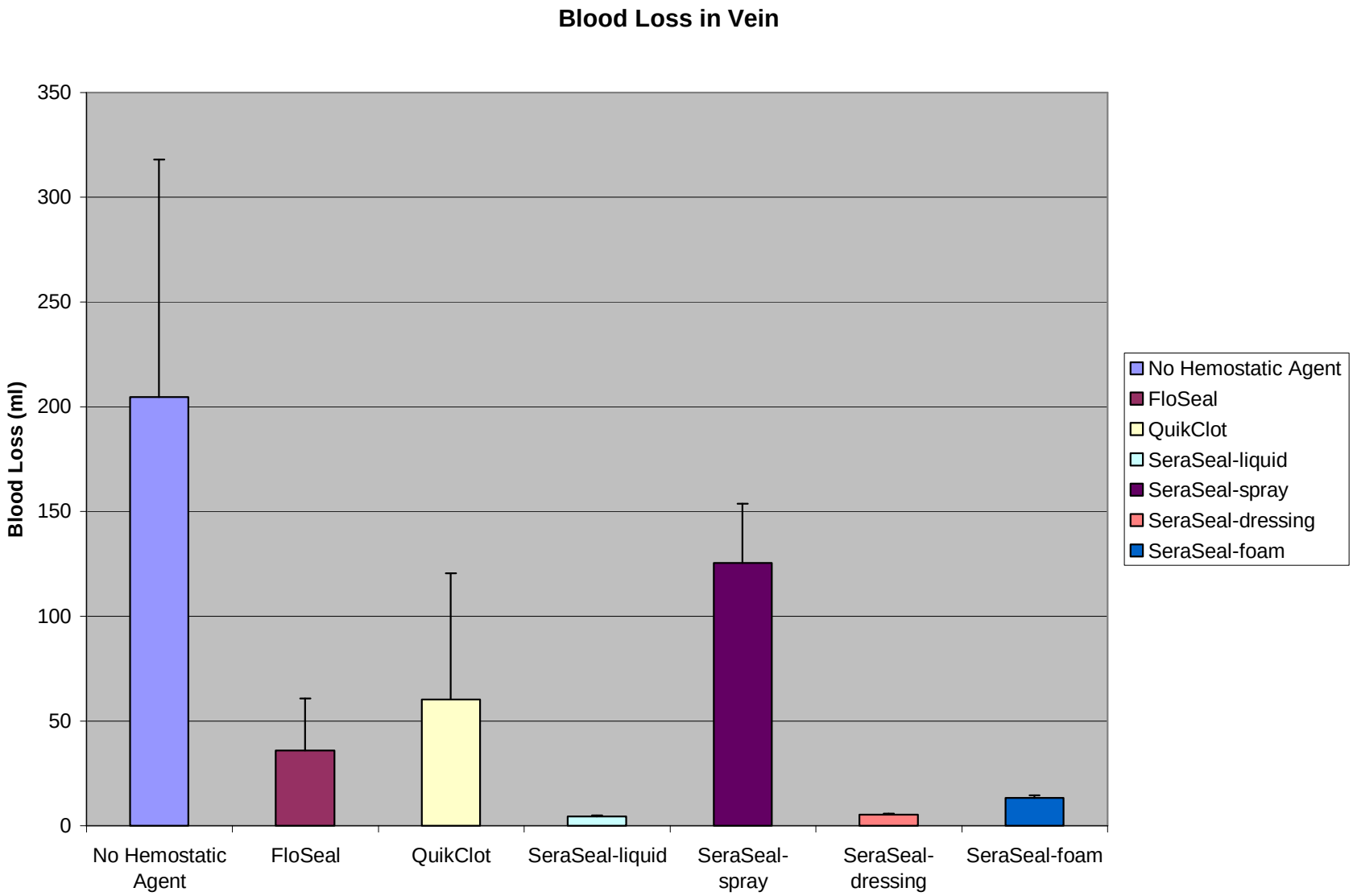
Graph 9:


Blood Loss in Liver
Blood Loss (ml)
160
140
120
100
80
60
40
20
0
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam

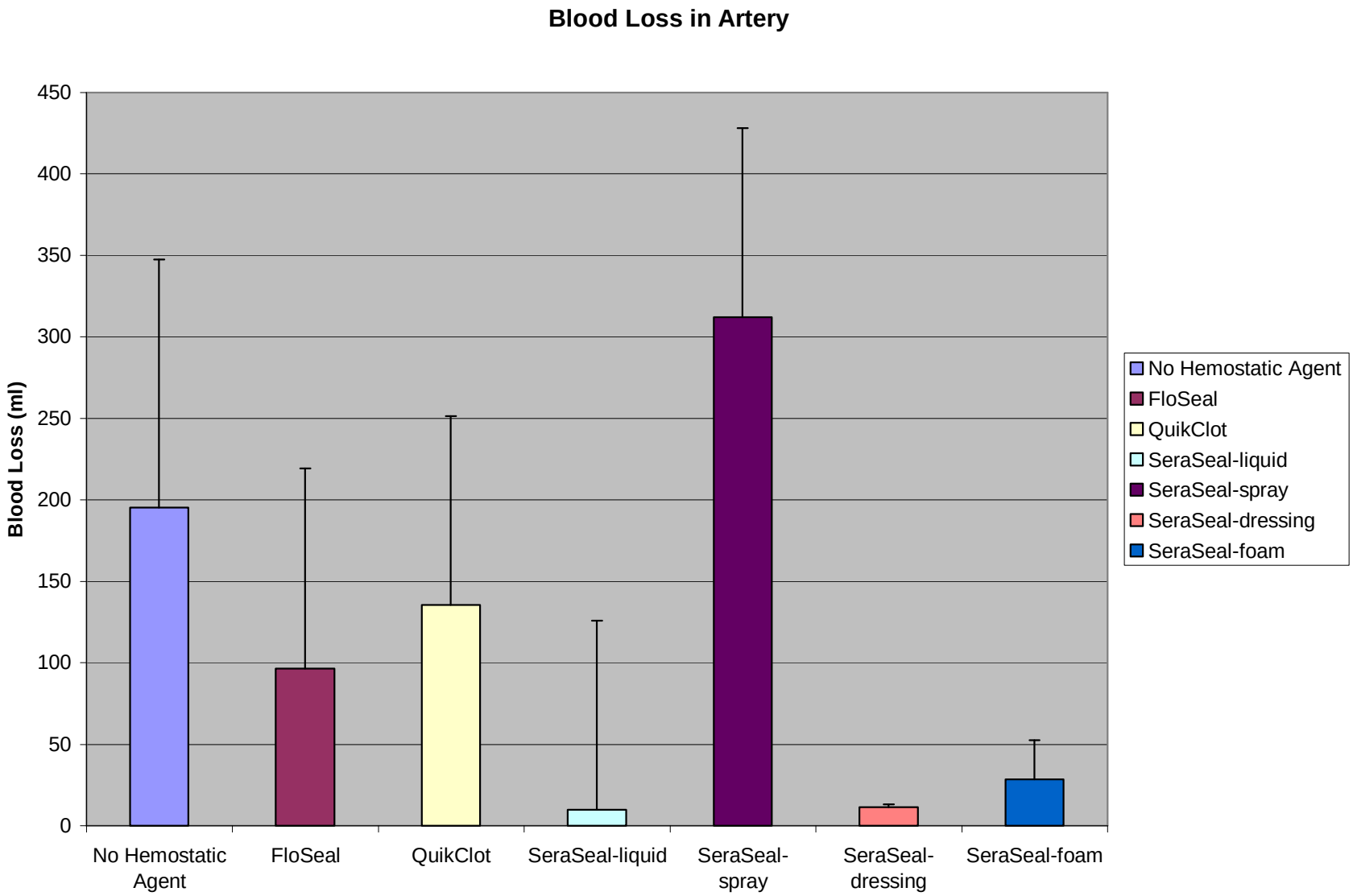
Graph 10:


Blood Loss in Spleen
Blood Loss (ml)
90
80
70
60
50
40
30
20
10
0
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam

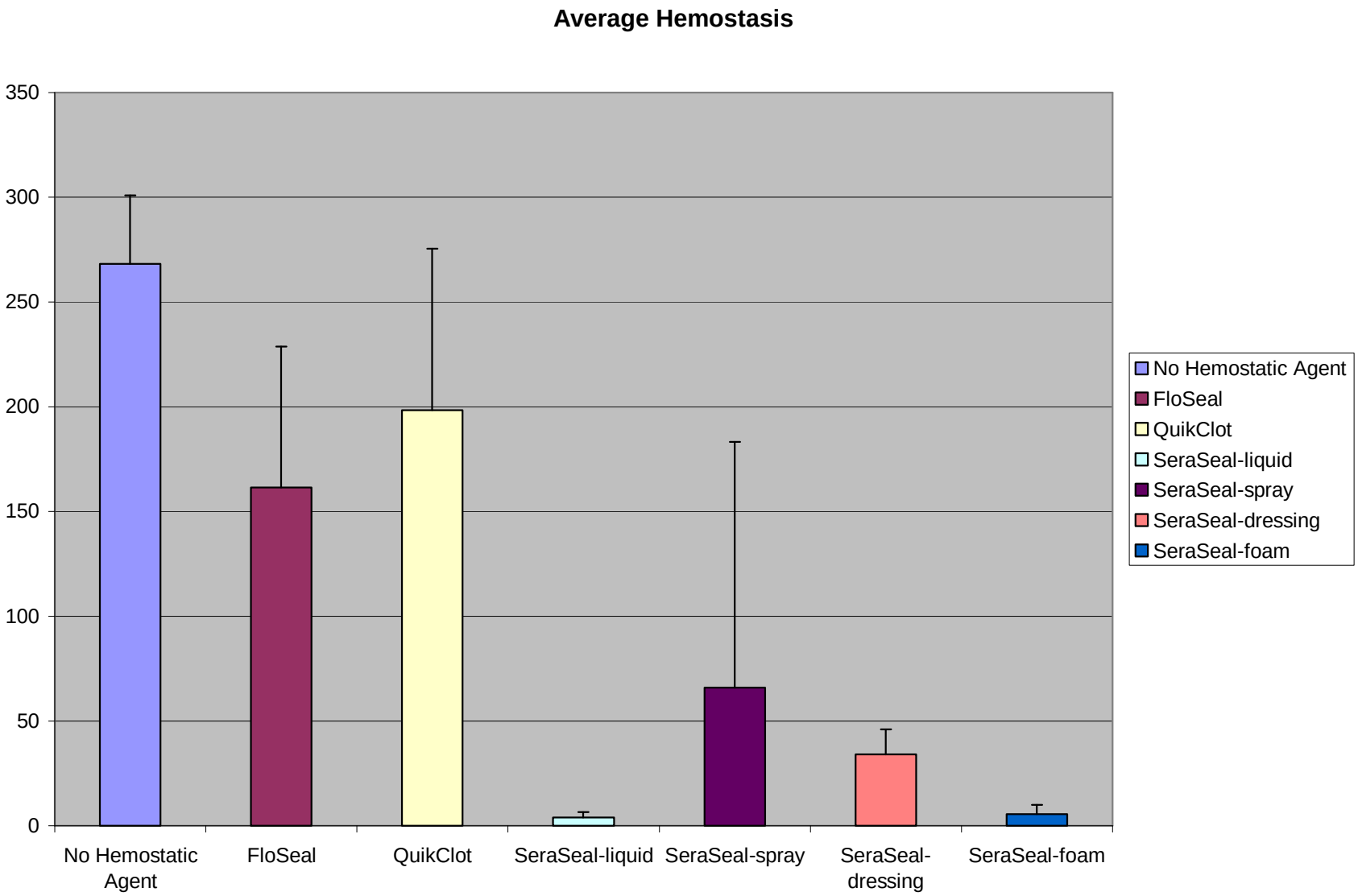
Graph 11:


Blood Loss in Vein
Blood Loss (ml)
350
300
250
200
150
100
50
0
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam

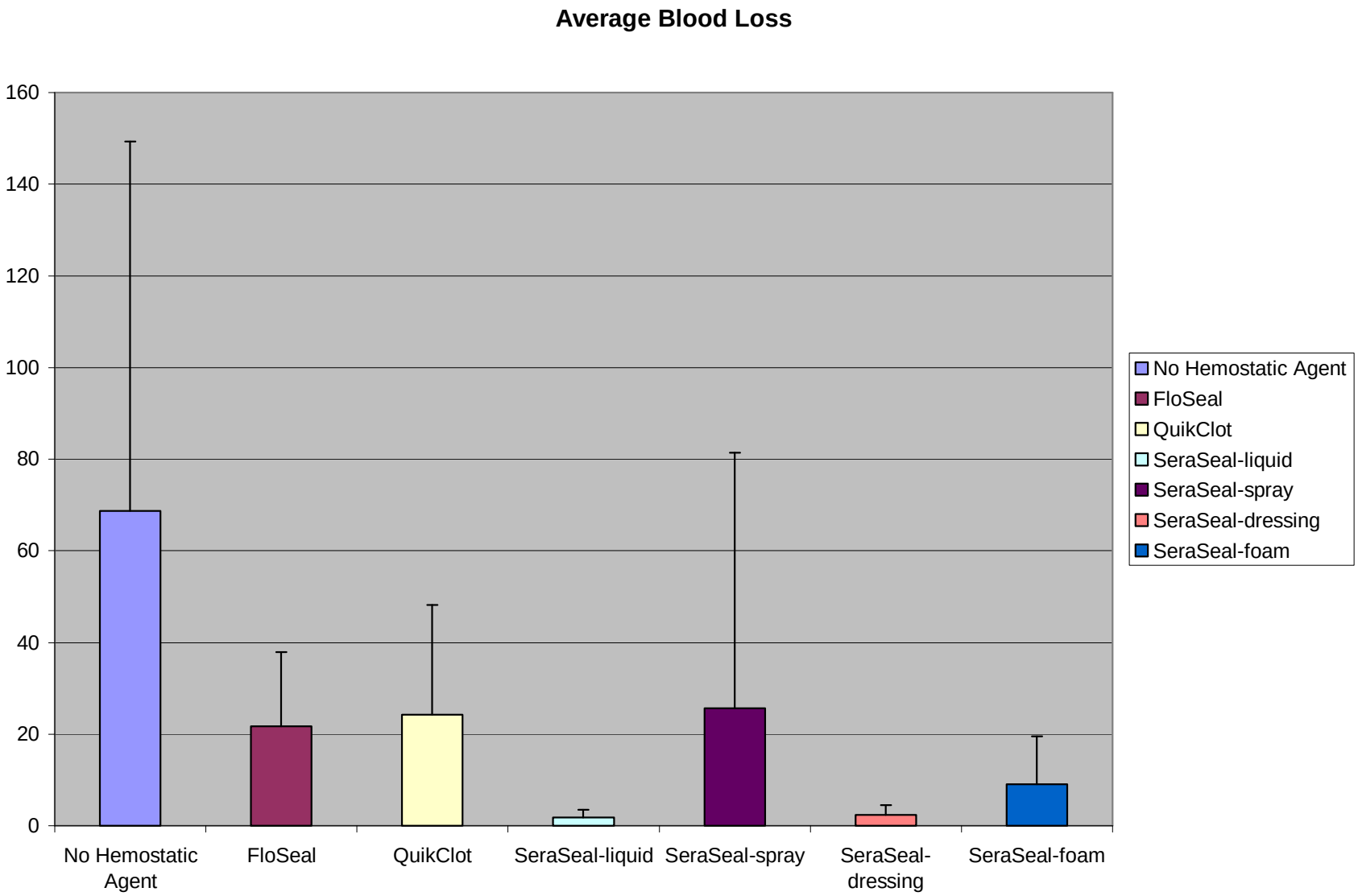
Graph 12:


Blood Loss in Artery
Blood Loss (ml)
450
400
350
300
250
200
150
100
50
0
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam

Graph 13:


Average Hemostasis
350
300
250
200
150
100
50
0
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam

Graph 14:


Average Blood Loss
160
140
120
100
80
60
40
20
0
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam
No Hemostatic Agent
FloSeal
QuikClot
SeraSeal-liquid
SeraSeal-spray
SeraSeal-dressing
SeraSeal-foam

Clinical Outcome

FloSeal

No adverse healing reactions attributable to the use of FloSeal were noted.

Skin An occasional eschar was observed, however this was not beyond the extent expected during normal healing.

Muscle Minimal scarring was observed.

Liver/Spleen Some adherence of the omentum occurred, however this was not beyond the extent expected.

Femoral Wound Occasional seroma and scar tissue were noted, most likely due to the surgical procedure, and not a direct result of the hemostatic agents used.

QuikClot

Skin This granular hemostatic agent (GHA) caused superficial burns at the margin of the partial thickness skin wound, as well as in the deep dermis and fat. At seven and thirty days postoperatively the GHA treated wounds had failed to heal completely. Injury to the skin at the site of the groin wound was extensive from GHA. In several cases there were areas of full thickness burns at the wound margin and where GHA had spilled during application. The extent of skin injury from GHA caused us to euthanize one animal at seven days (rather than at the planned thirty day point) and contributed to groin abscesses in all three surviving animals. Cultures from the groin wounds grew fecal organisms.

Muscle The muscle wounds appeared edematous and slightly ecchymotic immediately after application of the GHA. At seven and thirty days there was abscess formation in some wounds and scar tissue in other wounds treated with GHA.

Liver The liver wounds were difficult to evaluate after application of GHA as much of the compound adhered to the cut surface of the wounds. Attempts at removal caused bleeding. Therefore what could not be irrigated from the wound was left in place. Some burning of the surface of the liver appeared to take place, presumably from application of GHA. At seven and thirty days the omentum was firmly adherent to the site of wounding.

Spleen The splenic wounds were difficult to evaluate immediately after application of GHA for the same reason as the hepatic wounds. - adherence of the GHA to the cut surface. At seven and thirty days the omentum was firmly adherent to the site of wounding.

Artery Arterial wounds were encased in a firm mixture of blood and GHA after application and appeared to have suffered thermal injury. In every case it was impossible to repair the vein as

the vessel had become friable. At seven and thirty days the vessels were thickly encased in scar and abscess.

Vein The venous wounds were encased in a firm mixture of blood and GHA after application and appeared to have suffered thermal injury. In every case it was impossible to repair the vein as the vessel had become friable. At seven and thirty days the vessels were thickly encased in scar and abscess.

SeraSeal

No adverse healing reactions attributable to the use of SeraSeal have been noted.

Liquid, Spray & Foam These delivery systems of the product was effective in achieving hemostasis in all of the studied wounds, and did not appear to harm tissue.

Dressing This product was effective in achieving hemostasis and did not appear to harm tissue. Removing the gauze from the bleeding site also provoked further bleeding. Irrigation of the dressing before removing it from the wound prevented further rebleeding.

Histopathology

FloSeal FloSeal was not interpreted histopathologically as being harmful to tissue. Some gelatin granules were present at seven days post injury. As with SeraSeal, changes noted histologically were interpreted as being consistent with the normal course of healing.

QuikClot

Skin Histologic examination of sections of skin taken from the area of the groin lesion within hours after treatment with GHA revealed full-thickness necrosis consistent with a third-degree thermal injury. In some areas, the necrosis extended into underlying subcutaneous adipose tissue. Sections of skin taken from the groin lesion seven days post-GHA application displayed focally extensive dermal necrosis and contraction. By thirty days post-GHA application, skin from the lesion in the groin area was characterized by hyperplastic (regenerative) epithelium overlying a dermis expanded by fibroplasia with neovascularization (granulation tissue), which extended deep into the underlying subcutis. These changes were characterized by coagulative necrosis of surface epithelium, and dermal connective tissues, blood vessels, and adnexa. All dermal epithelial elements were included within the necrosis. Frequently, the necrotic changes affected underlying adipose tissue.

Muscle Histomorphologically, the skeletal muscle collected from the area of the groin lesion within hours after treatment with GHA exhibited degeneration and necrosis of myofibers. By

seven days post-GHA, multifocal areas of granulation tissue border degenerate and necrotic muscle within inflammation and mineralization. Gram-positive bacteria were observed in the muscle interstitium in some sections examined. By 30 days post-GHA application, the sections taken from muscle tissue in the groin lesion contain abundant granulation tissue with multifocal abscess formation. The sections examined frequently contained embedded foreign material compatible with GHA granules.

Liver Some sections submitted from the area of the surgically created liver laceration contained a superficial layer of subcapsular hepatocytes (approximately 2mm deep from the cut surface) exhibiting mild degenerative changes characterized by hypereosinophilia of cytoplasm without clear evidence of necrosis. By seven days post-GHA application, the abdominal omentum was adhered to the surgically cut surface of the liver. The omental tissue contained multifocal areas of increased fibrous connective tissue, mineralization, and granulomatous inflammation surrounding foreign material compatible with GHA granules. The subjacent hepatocytes were essentially normal.

Spleen No significant histologic lesions were observed in the parenchyma of the spleen taken immediately after application of GHA. There were degenerative changes in the collagen of the overlying splenic capsule. At seven and 30 days post-GHA application, abdominal omentum adhered to the surgically cut surface of the spleen contained changes similar to that previously described from the omentum adhered to the liver. The subjacent splenic parenchyma was essentially normal.

Artery There were no significant histologic lesions observed in the walls of arteries taken immediately after application of GHA. However, degenerative changes were apparent in the collagen of periarterial connective tissue associated with the tunica adventitia. At seven days post-GHA application, a section of artery within abundant granulation tissue from the area of the groin was thrombosed and degenerative changes within the arterial wall were characterized by hypereosinophilia, hemorrhage, cellular debris, a few neutrophils.

Vein Histologically, sections of vein taken from the area of the groin within hours after application of GHA exhibited degeneration characterized by hypertrophy and loss of endothelial cells and hypereosinophilia of subendothelial connective tissue. These changes only affected the tunica intima. **(Table 4)**

SeraSeal products SeraSeal did not appear to produce specific histopathology in the wounds. Changes noted were interpreted as consistent with the normal wound healing process.

Table 4: Pathology Scores

Wound						
	Skin	Muscle	Liver	Spleen	Vein	Artery
FloSeal	1	1	2	2	2	2
QuikClot	4	4	4	4	4	4
SeraSeal liquid	0	0	1	1	1	1
SeraSeal spray	0	0	1	1	0	0
SeraSeal dressing	0	0	1	1	1	0
SeraSeal foam	0	1	1	0	0	0

Score	Definition
0	No changes, normal tissue
1	Minimal changes consistent with normal healing
2	Moderate changes consistent with moderate inflammation, acceptable healing
3	Inflammation or other changes, not acceptable healing
4	Marked change, clear interference with wound healing

Advantages/Disadvantages

FloSeal Advantages of FloSeal were 1) It is FDA approved for hemostasis; 2) It was moderately effective overall. Disadvantages of FloSeal were: 1) It was time consuming and cumbersome to mix and therefore not applicable to a combat setting; 2) The hemostatic effect was only moderate and not strong enough to warrant deployment in a combat.

QuikClot QuikClot has an advantage of achieving hemostasis. However, QuikClot also created exothermic reaction with the potential of tissue damage and full thickness burns.

SeraSeal-Liquid SeraSeal-Liquid demonstrated to be effective to control bleeding in all wounds and meet the desirable standard of hemostasis within 60 seconds of application to warrant deployment in a combat.

SerSeal-Spray One advantage to the spray was the ease of use. Disadvantages of the SeraSeal-Spray were: 1) Application needs to be direct, therefore leaving wounds deep inside the body untreatable; 2) the agent did not work well on rapidly bleeding wounds.

SeraSeal-Dressing The dressing worked well when applied to the wound with pressure. An advantage of the SeraSeal-Dressing is it does not require refrigeration. However, a surgical dressing cannot be used on deep wounds because manual pressure needs to be applied to keep the surgical dressing in place for effective hemostasis.

SeraSeal-Foam SeraSeal-Foam was very effective to control all bleeding wounds without pressure and meet the desirable standard of hemostasis within 60 seconds of application to

warrant deployment in a combat. An advantage to using the foam is that it covered all parts of the wounds and could reach the deep wounds inside the body. Disadvantage of the foam application to arterial hemorrhages on flat surfaces pushed the product away from the site of bleeding, rendering it less effective. The foam is most effective if the product can be contained within the site of bleeding.

Discussion

Through application of each agent, we found that: SeraSeal liquid, dressing, and foam performed in all wounds (4%, 6%, and 14%, respectively of control blood loss); SeraSeal spray performed best in dermal, muscle, liver, and spleen (2%, 8%, 2%, and 1% of control); QuikClot performed best in liver and vein (26% and 29% of control); FloSeal performed best in the spleen and vein (29% and 18% of control). **(Table 3)**

We believe FloSeal would be of greater use if there were a one step application procedure. For use in combat, FloSeal would need to be pre-mixed decreasing the amount of time needed for application. QuikClot was found to cause third degree burns and necrosis when applied to the wound and the area around the wound. QuikClot was efficient in some of the wounds, however the thermal injury that resulted decreased its value for field use. SeraSeal liquid and spray although effective to control bleeding wounds, both delivery systems have refrigeration storage requirements limiting their usefulness for field use. SeraSeal dressing when held with direct contact with the bleeding source it was consistently effective. The dressing does not require refrigeration making it suitable for combat wounds. SeraSeal foam was able to reach and cover all wounds and effectively controlled the bleeding. The challenge facing the foam for field use is its refrigeration storage requirement.

All of the agents were able to reduce the amount of blood lost and the time to hemostasis, but FloSeal, QuikClot and SeraSeal spray were not consistently effective for all wounds. The SeraSeal foam is quite suitable for combat use, particularly to treat bleeding wounds in a closed cavity. However, the storage requirement will have to be resolved for field use.