12.1 STUDY INFORMATION

12.1.1 Protocol

Study No.: WLI-1196-PER

Clinical Investigation - Lima Peru

1. **Title:** Efficacy and Safety Investigation of SeraSeal

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3. **Monitors:** Felipe Plaza, MD
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4. **This protocol focuses on treatment**

5. **Location:** Edgardo Rebagliati Martins National Hospital, Lima, Peru
Guillermo Almenara I. EsSalud National Hospital, Lima, Peru
Jose Casimiro Ulloa Emergency Hospital
FAP- Peruvian Air Force Hospital, Lima, Peru
Military Hospital of Peru, Lima

6. **Research Plan:**

6.1 Purpose: The purpose of this study is to investigate the safety and effectiveness of SeraSeal on clotting time in surgical or trauma wounds. This study is designed to determine whether it is safe and effective in achieving hemostasis in different types of wounds.

6.2 Hypothesis or Research Questions or Objectives: The null hypothesis is that cauterization is more effective than SeraSeal in achieving hemostasis. The alternative hypothesis is SeraSeal will achieve hemostasis sooner than cauterization. A secondary endpoint is the measurement of blood loss.

6.3 Significance: SeraSeal shows promise of being an effective hemostatic agent applicable to wounds, surgical operations in which hemostasis is difficult (hepatic, renal and splenic trauma, debridement, burns, skin grafting, endoscopic procedures), control of epistaxis, esophageal variceal bleeding, bleeding peptic ulcers, and bleeding associated with proctitis and cystitis. For some of these conditions, there is no entirely satisfactory treatment.

6.4 Relevance: The availability of an easy to use, stable, safe, effective hemostatic agent, would be a tremendous advantage to the trauma patient (surgical or accidental), possibly reducing morbidity and mortality significantly, and conserving surgical and blood resources.

6.5 Background and Review of Literature:

Problem with Bleeding Wounds

Bleeding is still a major cause of morbidity and mortality in wounds. Thirteen percent of patients suffering acute trauma, die of bleeding[1]. Of the total deaths occurring on the battlefield, eight-five percent are due to blood loss[2]. Particularly vulnerable areas are the face, groin, pelvis and extremities, areas not protected by body armor. In head and neck trauma, uncontrolled bleeding can cause airway compromise and asphxiation[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 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. Outside of the hospital, the paramedic has only compresses to control bleeding. A quick, effective, and safe device to control bleeding, would be extremely beneficial.

Hemostatic agents in wounds

Commercially available topical thrombogenic agents include microfibillar collagen (Avitene®), collagen sponges (Gelfoam®, Instat®), and fibrin sealants (Tisseel®, AFTA®, ViGuard®). 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 inhibited, they are less effective.

Fibrin Sealants

These are prepared from homolgous, pooled human or bovine fibrinogen, Factor XII, and thrombin that must be combined to make the fibrin. Other components include calcium to provide activation of Factor XIII, fibronectin 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].

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 or trauma setting, adding to the operative time and delaying transportation to a stable operative setting. Previously, the FDA withheld approval from European products because they used pooled human plasma as a product source[6]. The fibrin sealants are somewhat difficult to use, as they must be mixed from four components, a process which takes approximately 15 minutes, and then must be used within four hours. If they are to be used endoscopically, they must be placed through a dual luman catheter and mixed in situ[7]. 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 repair[8]. The incorporation of fibrin sealants into a dry dressing has reduced some of these problems. The strength of the clot formation, when utilizing a dry dressing, can be sub-optimal. These shortcomings limit the utility of fibrin sealants for hemostasis in the battlefield, emergency response teams such as paramedics, and in the surgical arena.

Bovine topical thrombin in most cases, is a heterolgous plasma thrombin concentrate that has been used for hemostasis since the 1940's. Some commercial bovine thrombin preparations are highly immunogenic, and appear to be associated with an increased risk for adverse clinical outcomes during subsequent surgical procedures[9]. Antibody formation and anaphylaxis (rates 0.5 to 5.8%) have also been seen with Factor V and aprotinin, a polyvalent proteinase inhibitor isolated from bovine lungs, and used alone to enhance clot stability, or in combination with fibrin sealants[10, 11, 13]. These antibodies may interfere with the heparin anti-Xa assay, thereby complicating the monitoring of anticoagulant therapy[12]. The commercially available product, Tisseel VH fibrin sealant, contains bovine aprotinin, resulting in the risk of hypersensitivity.

What this study will determine

This study is designed to determine whether SeraSeal is safe, effective, and will improve the quality of care, in achieving hemostasis in surgical wounds or other emergent hemorrhagic episodes. The effectiveness will be determined by achieving hemostasis after application of the product. The safety will be determined by monitoring outcomes from application: recurrence of bleeding, infection, lack of healing, and presence of hypersensitivity reactions, e.g. pruritus, fever, rash, anaphylaxis. An improved quality of care will be achieved by reducing or eliminating the hospital length of stay.

Characteristics of SeraSeal

SeraSeal is a bovine protein-derived accelerator of hemostasis interacting with the intrinsic and extrinsic pathways. It is obtained from pathogen and prion free cows. SeraSeal can be provided as a solution, spray, foam, incorporated into an absorbable dressing or a non-absorbable removable dressing. Its active ingredients are the bovine proteins Factors II, VII, IX, and X.

Toxicity of SeraSeal

A total dosage level of SeraSeal over 13,000 IU/Kg (2.4 mg protein) in leporids and canines had no adverse effects[14]. The total expected treatment levels to be used in this study is less than 1,000 IU/Kg, a 130 fold lower level than the no toxic effect. Cell culture studies reveal that SeraSeal is not directly toxic for any cells tested. Thus, drug related toxicity is not expected to be seen in this clinical study.

6.6 Research Design and Methods: This clinical trail will be a difference study where SeraSeal will be used as a liquid, administered by a syringe (300 IU/0.1ml), and compared to standard surgical technique as the control. Patients receiving treatment at Edgardo Rebagliati Martins Hospital, the National Guillermo Hospital, Peruvian Air Force Hospital, and the Military Hospital of Peru will serve as study volunteers. Prior to enrollment in the study, an initial screening test and subsequent studies will take place, including: informed consent, collection and recording of subject demographic information, medical history, current medications, assessment of inclusion/exclusion criteria, physical examination, with blood draws for hematological assessment and coagulation studies for bleeding and time to hemostasis. The location of the study wounds will be invasive. The study wound sites will involve bone and soft tissue.

This is a single-blinded, parallel, randomized clinical trial, where the primary endpoint is hemostasis. Secondary endpoints will include reduction of blood loss, fewer blood transfusions. There are 18 designated surgical faculty members from Head & Neck, Vascular, Thoracic Cardiovascular, Neurosurgery, Orthopedics, and General Surgery participating in this study, with a minimum of 10 surgical procedures being studied. All of the participating surgeons are highly skilled and have extensive expertise in their surgical specialty.

Randomization: The patients will be randomized within each surgical department by drawing a SeraSeal or Cauterization slip. Which ever slip is drawn the next participating patient having the same surgical procedure will

be given the opposite method to control bleeding at same specific bleeding wound sites.

In this trial, the dosage level of SeraSeal is 3,000 IU/ml, with a maximum expected dosage of 10,000 IU, 50 times less than the pre-clinical toxicity studies[14]. After hemostasis is achieved the excess SeraSeal will either be removed through irrigation and suction or absorption onto a surgical sponge. Blood loss will be measured and recorded by reading the volume in the suction canister, or by weighing the blood soaked surgical sponges (1 ml whole blood = 1.04 g). A time to hemostasis is measured from the time when the product or the control is first applied to the wound, until no bleeding or oozying is observed for the entire wound. A nurse will use a stopwatch to record the time to hemostasis. SeraSeal is expected to achieve hemostasis 25% faster than the control. Stopping criteria for treatment in the investigational group will be when hemostasis fails to be achieved within 10 minutes for a particular wound, or at the discretion of the surgeon. If this should happen, the patient will be switched over to standard surgical practices. There are no stopping criteria for the control, since the control employ standard surgical procedures.

The patients will be monitored for 30 days, and will be measured by the amount of post-operative bleeding and overall wound healing for those wounds that can be observed. A base line hemoglobin (HGB) and hematocrit (HCT) blood count, prothrombin time (PT), partial thromboplastin time (PTT), will be drawn and measured 24 hours, and also at 48 hours after surgery. The HGB, HCT, PT and PTT will be drawn 30 days after surgery (Table 1). The patients will be observed and assessed for a minimum of 60 minutes after application of SeraSeal for signs of hypersensitivity to the investigational product, and throughout the 30 days. They will be assessed for, but not limited to, skin rash; wheezing; anaphylaxis; pruritus; prolong coagulation tests; delayed healing or infection through quantitative cultures. In the event there is progression of edema leading to respiratory distress, anaphylaxis, or shock due to blood loss, a crash cart and/or any additional standard and customary treatment(s), will be available. This study will not limit the standard of care performed at both investigational hospitals. The following components of wound care are "standard practice" at each of the hospitals: daily dressing changes; quantitative cultures when infection may be present; routine blood tests. All enrolled study subjects may request to be withdrawn from the study at any time, and will be terminated from the study and provided standard of care. All adverse events will be recorded according to whether the event was unrelated, unlikely, possibly or probably related to the study treatment.

Table 1: Schedule of Assessments

Test	Pre-Op	Surgery	24 Hrs Post Op	48 Hrs Post Op	30 Days
Medical History	X				
Physical Examination	X				
Informed Consent	X				
Designated Delivery System		X			
HGB	X		X*	X+	X
HCT	X		X*	X+	X
PT	X		X*	X+	X
PTT	X		X*	X+	X
ECG	X				
X-Ray Work-UP	X				
Wound care			X	X+	
Microbiological work-up			X*	X+	
Adverse Events			X	X	
Primary Variable		X	X	X	
Secondary Variable		X	X	X	
Safety					
Adverse Events		X	X	X+	X

* measured as often as needed

\+ monitored beyond 48 hours as needed

6.7 Inclusion/Exclusion Criteria

Inclusion criteria:

1. All ages and both genders
2. Wounds of a similar type, size, location and bleeding tendency listed in Table 1.
3. Currently on anticoagulation therapy with no dosage limitations.
4. Diagnosed with a coagulation disorder.
5. Participants must be able to participate for the 30 day duration of the study.

Exclusion criteria:

1. Any clinically infected wound, draining pus, surrounding erythema or edema, or patients with systemic signs of infections.
2. Subjects on antibiotic therapy prior to enrollment.
3. Subjects with known allergy to bovine proteins, atopic reactions, history of anaphylaxis.
4. HIV virus infection
5. Sensitivity to idodine
6. Inability to give informed consent.
7. Inability to return for a 30 day follow-up visit.

6.8 Source of Research Material

6.9 Number of Subjects: A total of 200 subjects, are to participate in this study.

7.0 Recruitment: Subjects will be randomly selected from within each participating surgical department at Edgardo Rebagliati Martins National Hospital, Guillermo Almenara I. EsSalud National Hospital, Peruvian Air Force Hospital, and the Military Hospital of Peru. The faculty from all of the hospitals will obtain an informed consent, prior to their participation in the study. No promotional fliers are planned. The consent form will be provided by the hospitals in Spanish. Subjects will not be paid for participation in this study.

7.1 Benefits: The possible benefit of the patient's participation in this study is a faster time to clotting, less blood loss, faster wound healing, and less loss of tissue. However, the participant should understand there is no guarantee or promise that they will receive any benefit from this study, other than knowing that the information may help future patients.

7.2 Risks: The risks of application of SeraSeal are theoretical. These risks may be bleeding, infection, and allergic reaction[14]. Anaphylaxis is a possible risk, but the incidence is not clearly known. In clinical case studies, SeraSeal was used in Greece on 39 dialysis patients[15]. These patients received SeraSeal 3-4 times each over a period of two weeks, and no reactions or adverse sequeal were noted[16]. Aprotinin, a bovine protein derived from the bovine lung, is used in cardiac surgery and is the component of some fibrin sealants. It has an anaphylaxis rate of 2.5% on second exposure, with a mean interval of 1,654 days (range, 16-7,136 days)[16,17]. SeraSeal contains no aprotinin. Bovine gelatin is a component of some vaccines and anaphylaxis has been reported with this protein[18]. Other bovine proteins for which anaphylaxis and hypersensitivity reactions have been reported, are bovine serum albumin[19], thrombin[20,21], recombinant Factor VIII[22], insulin[23], and tissue sealants[24]. The risk of anaphylaxis appears to be lower for topical devices than injected ones, and repeated administration with a break, as proposed in this study, also appears to have a lower incidence of anaphylaxis[25]. We surmise that anaphylaxis may be a risk of application of SeraSeal. Measures to reduce and treat anaphylaxis are to exclude those patients who are sensitive to bovine proteins from the study, and to provide a crash cart, and/or any additional standard and customary treatment(s). One of the purposes of this study is to determine that risk. Reports of an autoimmune reaction to fibrin sealants containing bovine Factor V mimicking hemophilia have been reported. SeraSeal does not contain Factor V.

7.3 Safeguards for Protecting Subjects: The risks of SeraSeal treatment are minimized by the patient selection

process. Should an allergic reaction occur, the participant will be withdrawn from the study and standard of care will be administered. Physicians, nurses, and technicians, will be on hand for all procedures. Patients will be monitored through routine post-operative care. Should any adverse event occur during treatment, immediate intervention will be taken. A crash cart is kept in the treatment room and on the hospital floor. Patient records will be kept confidential.

7.4 Alternatives: Alternatives to SeraSeal are the application of no hemostatic agents, electric cauterization, ligature, pressure dressings, collagen dressings, and the use of fibrin sealants.

8.0 Data Analysis:

8.1 Data Collection: There will be one study form for each patient. The surgery form will have all of the outcome data, including blood loss and complications. Once a patient is placed on the surgery schedule, the patient will be asked to participate in the clinical trial by the participating investigator surgeon. The only patients that will require special action are the SeraSeal patients. For those patients the need for SeraSeal will be made known only to the attending surgeon and/or fellows, and made available to the OR room. At the time of the pre-op exam, the inclusion/exclusion of participation in the study will be filled out. The research nurse will collect all data forms and will be responsible for evaluating them for completeness and accuracy, except for time to hemostasis supplied by the surgeon.

8.2 Statistical Analysis: We will compare continuous outcomes such as time to hemostasis (primary outcome), pre and post operative blood loss, surgery duration, (log) bacterial titer, and length of hospital stay in SeraSeal versus conventional treatment, using factorial analysis of variance (ANOVA) methods.

For continuous lab outcomes measured on four occasions (base, 24 hours, 48 hours, 30 days), we will use repeated measure analysis of variance methods to compare mean lab values for conventional versus SeraSeal overall.

For binary outcomes, (including adverse event outcomes) that are not quantified or that may be dichotomized for clinical convenience and interpretability such as fever (yes or no) or pruritus (yes or no) we will use chi-square methods to compare conventional versus SeraSeal treatment proportions overall. Since the total follow up times may differ between groups, we will also compare rates per person-time of binary outcomes under conventional versus SeraSeal treatment overall.

Sample size: The sample size is based on the primary outcome, time to hemostasis. We consider at least a 25% mean reduction in time to hemostasis to be the smallest clinically important average improvement. Historically, the mean time to hemostasis for liver surgery is 30 minutes. A 25% reduction from 30 minutes corresponds to a mean time to hemostasis of 30 x 0.75 = 22.5 minutes under SeraSeal. Based on this calculation, a sample size of 20 persons per group provides 80% power for confirming this mean difference using the $p<0.05$ two sided significance criterion (alpha =0.05). Assuming that all of the surgical procedures can be collapsed within their respective departments, and assuming that the 25% mean difference or greater difference occurs in all 5 departments, the required total sample size is 20 x 2 x 5 = 200.

9. Duration of Study: Subject participation is 30 days post study procedure. Expected enrollment time is 12 months.

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