GLP Final Report

Report No.: M4662017-004
Date: 11/01/2017

Exclusively prepared for:

SPONSOR
Solaplus Biotech Co., Ltd.
No.75 FengFang Road, Ouhai Economic Development Zone, Wenzhou, China

STUDY TITLE
Hemostasis Efficacy Animal Study

TEST ARTICLE
Hemostatic xerogel sponge
Model: XLJ-I-4
Specification: 120×80×5mm

TESTING FACILITY
Mid-Link Technology Testing Co., Ltd.
B6-05, RongTong Building,
No. 80, Haiyun Street, TEDA
Tianjin, 300457, China

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SUMMARY

Purpose: This study was performed on an porcine model to evaluate the Hemostasis Efficacy of Hemostatic xerogel sponge for wound of skin surface and superficial vascular, by comparing with a legally marketed device, HemCon ® ChitoGauze® PRO.

Method: Yorkshire pig was used as the animal model; two cuts were created on each animal, one was the cut on the ear, which covered both superficial arterial and vein, the other is a "#" shape cut on the back, with injury to vascular under skin. Then dressing was applied and compressed, respectively, on each of the cut for 3 minutes. (for test group, Hemostatic xerogel sponge was used; for control group, HemCon ® ChitoGauze® PRO was used). After the 3 minutes, the dressing was removed and bleeding condition of the cut was observed. The dressing was weighed both pre- and post-treatment, and then absorbing amount was calculated.

Conclusion For cuts of superficial vascular, the successful rate of the test group was higher than that of the control group, for skin surface cuts, the successful rate of test group and control group were identical. The result demonstrated that Hemostatic xerogel sponge controlled the bleeding successfully.

Surgery performed by

Yuwei Wang and Ning Wang

Approved by 傅立 11/17/2017

Lee Fu, Study Director Date

GLP STATEMENT

This nonclinical laboratory study was conducted in accordance with the United States Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR Part 58.

There was no deviation to the protocol or provisions of GLP Regulation noted during the course of the study.

Approved by: 傅立

Lee Fu, Study Director

11/17/2017

Date

1. Generals

1.1 Purpose

Hemostatic xerogel sponge is intended for the external, temporary control of severely bleeding wounds. This study was performed on an porcine model to evaluate the Hemostasis Efficacy of Hemostatic xerogel sponge for wound of skin surface and superficial vascular, by comparing with a legally marketed device, HemCon ® ChitoGauze® PRO.

1.2 Compliance

This study was conducted based on the requirements of Code of Federal Regulations (CFR), Title 21, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

1.3 Dates

Test Article Received:	09/20/2017
Test Initiated:	09/20/2017
Concluded:	11/03/2017

2. Test Materials

Test Article	Hemostatic xerogel sponge
Model	XLJ-I-4
Manufacturer	Solaplus Biotech Co., Ltd.
Identification Number	201706001
Status	Sterile Finished Device Gamma Radiation Sterilization
Physical Description	White Gauze
Composition	
Stability	Stability testing is completed and on file with the sponsor Expiration Date: 06/17/2019
Strength	Not applicable, no active ingredient
Purity	Not applicable, no active ingredient
Storage Condition	Room Temperature
Control Article	HemCon ® ChitoGauze® PRO
Model	30-0049 (7.5cm x 3.7cm)
Manufacturer	HemCon Medical Technologies, Inc
Identification Number	300049
Status	Sterile Finished Device Gamma Radiation Sterilization
Physical Description	White Gauze
Composition	HemCon ChitoGauze PRO is a flexible hemostatic chitosan coated gauze dressing.
Stability	Marketed device, stability is characterized by labelling.
Strength	Not applicable, no active ingredient
Purity	Not applicable, no active ingredient
Storage Condition	Room Temperature

3. Reagent, Instrument and Disposables

Anaesthetic	Pentobarbital sodium
Manufacturer	Shanghai Rongbai Biological Technology Co., Ltd
Lot No.:	57-33-0
Stability	Marketed device, stability is characterized by labelling.
Storage Condition	Room Temperature
Anaesthetic	Xylazine hydrochloride
Manufacturer	Jilin Province Huamu Animal Husbandry Animal Health Products Co., Ltd
Lot No.:	151207
Stability	Marketed device, stability is characterized by labelling.
Storage Condition	Cool and Dry
Disinfectant	Medical Alcohol
Manufacturer	Shandong Lierkang Medical Technology Co., Ltd.
Lot Number	20170417/1
Stability	Marketed device, stability is characterized by labelling.
Storage Condition	Light prevention, cool
Instrument	Patient Monitor
Manufacturer	Contec Medical Systems Co., Ltd
Model	CMS8000VET
Serial No.:	17080300001
Instrument	Stop Watch
Manufacturer	Shanghai Diamond Stop Watch Co., Ltd
Model	DM1-001
Serial No.:	EQ093
Instrument	Coagulation Analyzer
Manufacturer	Perlong Medical Co., Ltd
Model	PUN-2048A
Serial No.:	N20415120022
Disposable	Blood Collection Tube
Manufacturer	Guangzhou Improve Medical Instrument Co., Ltd
Model	Sodium citrate 9:1
Lot Number:	160218
Disposable	Blood Collection Needle
Manufacturer	Guangzhou Improve Medical Instrument Co., Ltd
Lot Number:	150814

Disposable	Introducer Sheath
Manufacturer	Beijing Target Medical Technologies Inc.
Model	6F
Lot Number:	201705011

4. Test System, Group and Justification

Species	Swine
Breed	Yorkshire
Source	Tianjin Bainong Laboratory Animal Breeding Technology Co., Ltd
Sex	Male and female, females were nulliparous and non-pregnant
Age	Young adults
Acclimation Period	Minimum 7 Days
Number of Animals	28
Weight	73.3±3.3 (kg)
Group	Test Group 14 animals and Control Group 14 animals.

Justification: The principle for selection of animal model was to select an animal that could represent the bleeding and coagulation condition of human. The swine has been widely used in the studies of bleeding control dressing[1,2]. For the cut of the skin surface, the swine has a skin with structure closer to human beings compared with other animals, specifically, the thickness of its dermis layer is similar to (or more than) that of human and contains all dermal matrix. As for the cuts of the superficial vascular, the swine has vascular with large diameter, which is closer to human than other animals. In addition, the swine has a big body size which can facilitate the operation.

Sample size was calculated according to the following formula:
n=ln(1-q)/ln(p), where n was the sample size, q was the confidence level which was 95% and p was the reliability which was 80%.
The calculated result was 13.42, therefore, 14 animals for each group were used.

5. Animal Management

5.1 Husbandry, Housing and Environment

Conditions conform to MID-LINK Standard Operating Procedures that are based on the "Guide for the Care and Use of Laboratory Animals." Animals will be individually housed in cages identified by a card indicating the animal number, test identification.

5.2 Food, Water and Contaminants

A commercially available porcine feed was provided daily. Potable water was provided ad libitum through species appropriate water containers or delivered through an automatic watering system. No contaminant present in the feed and water was expected to impact the results of this study.

5.3 Personnel

Associates involved in this study were appropriately qualified and trained.

5.4 Veterinary Care

All anesthetics, analgesics, and other medications are given or altered at the discretion of the attending veterinarian in accordance with standard veterinary practice and the study objectives. This applies to specific medication, dose, and dosing intervals. In the unlikely event that an animal should become injured, ill, or moribund, care was

conducted in accordance with current veterinary medical practice. If warranted for humane reasons, euthanasia will be conducted in accordance with the current report of the American Veterinary Medical Association's Guidelines on Euthanasia. The objective of the study is given due consideration in any decision and the study sponsor will be advised.

6. Method

6.1 Fasting and anesthesia

Food was withheld at least 8 hours prior to the surgery, each animal was weighed, and general anesthesia was induced with an intramuscular injection of xylazine hydrochloride, then with an intravascular injection of 3% Pentobarbital sodium. When the sufficient level of anesthesia was reached the animal was maintained on deep anesthesia status, with monitoring of vital signs, including respiration, heart rate and temperature.

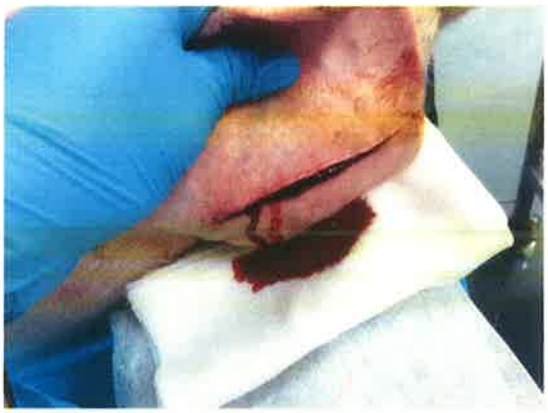
6.2 Preparation

Animal was fixed on the operation table, hair on the surgery site was shaved with electric clippers. Medical alcohol was used to disinfect all surgical site for three times. Neck skin was cut, left jugular artery was exposed, 5ml blood was taken for coagulation analysis. Then an introducer sheath was inserted into the artery and connected with invasive blood pressure (IBP) sensor for continuous monitoring. Both the test article and control article were weighed before the surgery as initial weight w_0.

6.3 Ear Cut Study

A cut was made by scalpel on one ear to create an injury cover both superficial artery and vein (see Fig 1). Wait for five (5) seconds, then the test article (for test group) or control article (for control group) was applied on the cut. During the application, one hand of the surgeon was placed under the ear for supporting, and the dressing was compressed by the other hand (palm was used to ensure sufficient compressing area). The compression lasted for three (3) minutes, and then withdraw the application slowly, while the dressing was still sit on the ear without compression and wait for ten (10) minutes. During this period, observe whether there was secondary hemorrhage. And then the dressing was removed and weighed as final weight w_1.

Fig 1 Illustration of Ear Cut

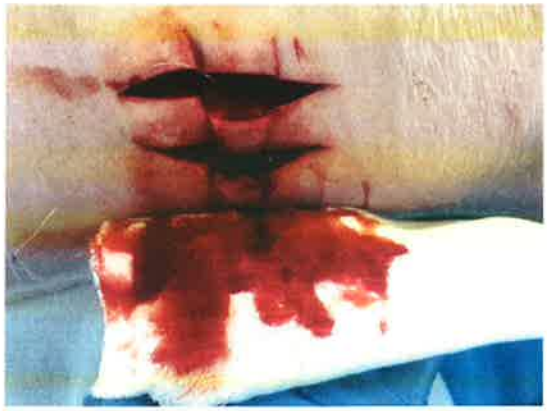


6.4 Back "#" Shape Cut Study

Four cuts were made by scalpel to create a "#" shape injury on the side of the body (see Fig 2). The depth of the cut was approximate 3-4cm with length of 8 cm. The vascular under the skin was cut to create an injury with severe bleeding. Wait for five (5) seconds, then the test article (for test group) or control article (for control group) was applied on the cut. During the application, the dressing was compressed by the hand (palm was used to ensure sufficient compressing area). The compression lasted for three (3) minutes, and then withdraw the application slowly, while the dressing was still sit on the ear without compression and wait for ten (10) minutes. During this period, observe whether there was secondary hemorrhage. And then the dressing was removed and weighed as

final weight w_1.

Fig 2 Illustration of "#" Shape Cuts



6.5 Terminal Procedures

At completion of the surgical procedures, each animal was euthanized.

7. Evaluation and Statistical Method

7.1 Coagulation Condition

Before the surgery, the coagulation condition of the animals was evaluated, including the prothrombin time (PT), Activated partial thromboplastin time (APTT) and Fibrinogen (FIB). The results were expressed as Means ± Standard Deviation. Student-T Test was performed for test group and control group to determine whether there is a statistical significance ($P<0.05$)

7.2 Hemostatic Efficiency

1) Observe whether the bleeding was controlled after application and whether there is secondary hemorrhage;
2) Document the Mean Blood Pressure (MAP) before the cut and at the end of compression; The results were expressed as Means ± Standard Deviation.
3) Absorbing amount Calculation:

Absorbing Amount (ml)= $(w_1-w_0)(g) \div 1.05$. The density of blood of Yorkshire white is about $1.05\times10^3 kg/m^3$.The results were expressed as Means ± Standard Deviation.

8. Results

8.1 Coagulation Conditions

Table 1 Coagulation Conditions

Item	Test Group (n=14)	Control Group (n=14)	P Value
PT	20.98±2.86	23.04±4.13	0.15(P≥0.05)
APTT	30.40±8.77	30.53±9.73	0.97(P≥0.05)
FIB	140.73±58.27	183.43±89.66	0.16(P≥0.05)

Note: There was no statistical significance of coagulation condition between test group and control group before the surgery.

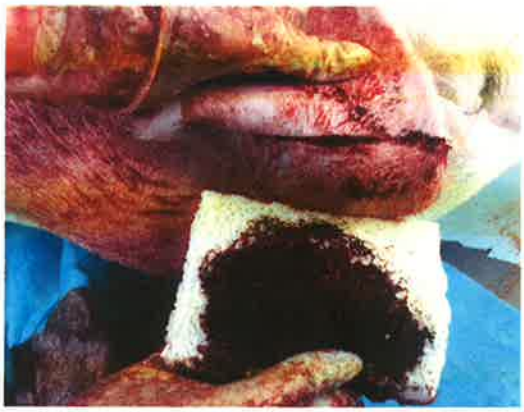
8.2 Hemostatic Efficiency (Ear Cut)

As shown in Table 2, the test group had a higher successful rate than the control group, with less absorbing amount. the MAP was not significantly affected by the cut of superficial vascular.

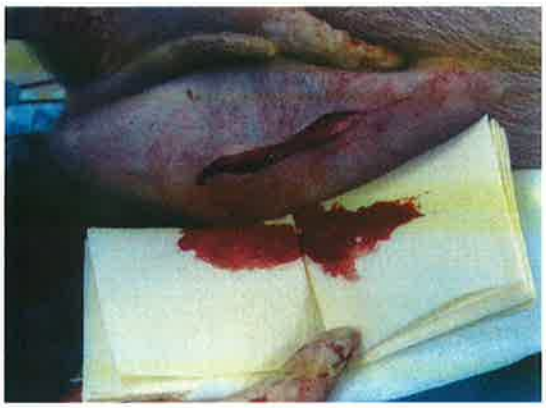
Table 2 Hemostatic Efficiency (Ear Cut)

Item	Test Group (n=14)	Control Group (n=14)
Number of Animal with Successful Bleeding Control	14	12
Compression Time (minutes)	3	3
Pre-Cut MAP (mmHg)	124±7	125±6
Post Compression MAP (mmHg)	130±7	127±5
Absorbing amount (ml)	12.90±6.04	16.84±9.06

Fig 3 Typical Illustration of Ear Cut



(1)



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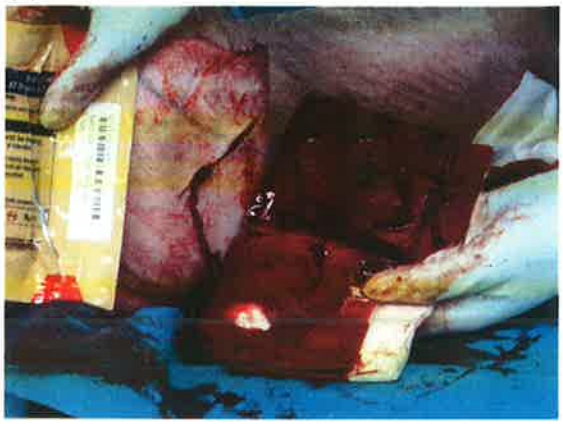


(3)

Note

(1) Successful bleeding control of test group;
(2) Successful bleeding control of control group
(3) Unscucccessful bleeding control of control goup

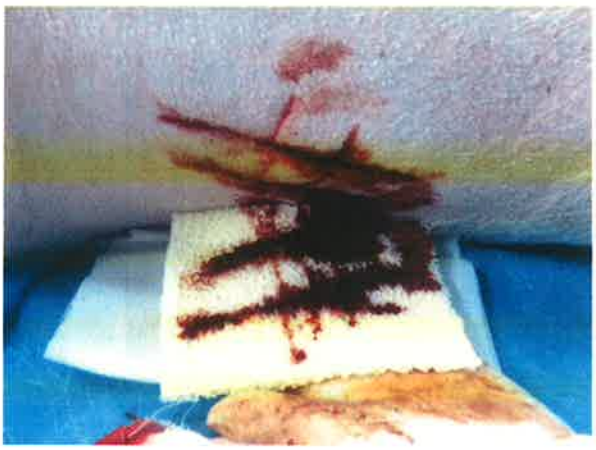
8.3 Hemostatic Efficiency (Back "#" Shape Cut)

As shown in Table 3, there was no difference of bleed control between test group and control group. The blood formed thrombus in the injury and prevent from the further bleeding. The MAP was slight increased during the procedure, this was because of the response. The absorbing amount of the test group and control group was similar.

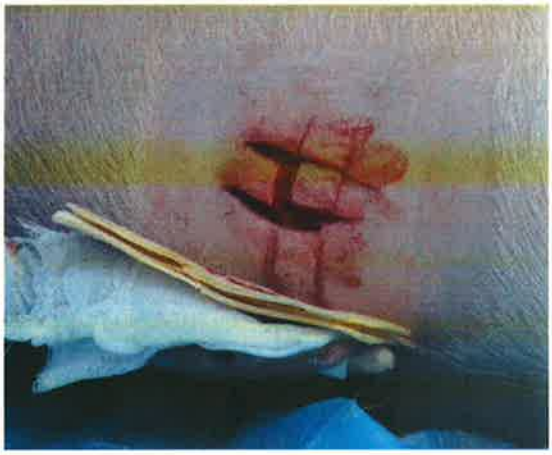
Table 3 Hemostatic Efficiency (Back "#" Cut)

Item	Test Group (n=14)	Control Group (n=14)
Number of Animal with Successful Bleeding Control	14	14
Compression Time (minutes)	3	3
Pre-Cut MAP (mmHg)	129±8	127±4
Post Compression MAP (mmHg)	131±8	128±4.
Absorbing amount (ml)	11.67±4.40	11.73±8.49

Fig 4 Typical Illustration of Back Cut



Test Group



Control Group

9. Conclusion

For cuts of superficial vascular, the successful rate of the test group was higher than that of the control group, for skin surface cuts, the successful rate of test group and control group were identical. The result demonstrated that Hemostatic xerogel sponge controlled the bleeding successfully.

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility.

10. Records

All raw data pertaining to this study and a copy of final report are retained in designated Mid-Link's archive files in accordance with Mid-Link SOP.

11. Reference

1) Code of Federal Regulations (CFR), Title 21, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies;
2) Eric M. Acheson, Bijian S. Kheirabadi, et al, Comparison of Hemorrhage Control Agents Applied to Lethal Extremity Arterial Hemorrhages in Swine, The Journal of Trauma Injury, Infection and Critical Care, Volume 59, Number 4.;
3) J. M. Davidson, Animal models for wound repair, Arch Dermatol Res (1998) 290 (Suppl) :S1–S11;

STATEMENT OF QUALITY ASSURANCE ACTIVITIES

Phase Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Study (Test Article)	09/11/2017	09/12/2017	09/13/2017
Study (Control Article)	10/09/2017	10/10/2017	10/10/2017
Study Data Review	09/27/2017	09/28/2017	09/29/2017
Final Report Review	09/29/2017	10/11/2017	10/16/2017

Based on a review of this study, it has been concluded that this report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study. This study has been reviewed in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, part 58).

QA Representative 梁芸源 Liyuan Liang, Senior QA Specialist

11/17/2017 Date

Appendix: Illustration of Test Article and Control Article