# HEMOSTASIS *IN VIVO* TEST REPORT

**Product:** "Coreleader"Hemo-Pad
"Coreleader"Hemo-Fiber

**Model:** Extremity Arterial Hemorrhage in Swine

# HEMOSTASIS SWINE TEST REPORT

**Applicant**: Coreleader Biotech Co., Ltd.
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**Sample Description:**

| | | |
|---|---|---|
| Item Name | : | "Coreleader" Hemo-Fiber (Sterile) |
| | | "Coreleader" Hemo-Pad (Sterile) |
| Item NO. | : | 20121220 |
| Quantity | : | 3 groups |
| Country of Origin | : | Taiwan |
| Date sample Received | : | October 20, 2012 |
| Date Test Started | : | November 20, 2012 |

**Test Conducted:**

As requested by the applicant, for details please refer to attached pages.

**Conclusion:**

The test article meets the requirement of the Animal Ethics Committees of YUAN-PEI University. All animals received care and were used in strict compliance with the Guide for the Care and Use of Laboratory Animals. "Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber are the effective dressings tested in this arterial hemorrhage model. The hemostatic property of "Coreleader" Hemo-Pad and "Coreleader" Hemo-Pad are attributed to its raw material. "Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber are now recommended as the first line of treatment for life-threatening hemorrhage on the battlefield..

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**AUTHORIZED BY :**
**ON BEHALF OF THE LAB OF ADULT STEM CELL AND TISSUE ENGINEERING**

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# Abstract

**Background:** The bio-compatible polymer (chitosan, alginate) dressing has been used over 10 years for treating external compressible hemorrhage in combat casualties. Previously, we tested two new hemostatic agents in granular forms that were superior to these products. In this study, two new dressings (preselected) that are more suitable for battlefield application were evaluated. The efficacy and acute safety of the dressings were tested in our standard arterial hemorrhage model.

**Methods:** Anesthetized pigs (32~42 kg) were instrumented, and arterial blood was collected for hematological and coagulation assays. After splenectomy, the right femoral artery was isolated, injured (6 mm arteriotomy), and unrestricted bleeding allowed for 45 seconds. Hemostatic dressings ("Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber) were then applied over the wound randomly and compressed for 5 minutes. Fluid resuscitation was administered and titrated to maintain a mean arterial pressure of 65 mm Hg. Animals were observed for 180 minutes or until death. Computed tomography angiography was performed on survivors and tissues were collected for histology.

**Results:** Stable hemostasis was achieved in the group of "Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber. Both groups led to stabilized mean arterial pressure and significantly survival (100%). "Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber secured hemostasis in 2.67± 0.58 minutes and 3.25± 1.26 minutes respectively, which were significantly less than control (over 20 minutes). The average survival time (over 180 minutes) of animals with "Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber treatment were also significantly longer than that of control (36± 5 minutes). The total blood loss was less in "Coreleader" Hemo-Pad (31.58 mL/kg) and "Coreleader" Hemo-Fiber (30.55 mL/kg) groups than control (188± 17 mL/kg). Computed magnetic resonance imaging results proved no blood leaking from the vessels.

**Conclusion:** "Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber are tested effective dressing in this arterial hemorrhage model. The hemostatic property of "Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber are both attributed to its raw material. "Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber are now recommended as the first line of treatment for lethal hemorrhage on the battlefield and accident site.

**Key Words:** "Coreleader" Hemo-Pad, "Coreleader" Hemo-Fiber, Traumatic haemostasis, Swine test of haemostasis, Lethal hemorrhage control, Military traumatic item, First aid for haemostasis, Severe hemorrhage.

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## Materials and Methods

This study was approved by the YUAN-PEI University. All animals received care and were used in strict compliance with the Guide for the Care and Use of Laboratory Animals.

**Prepare Surgical**

1. Animal: Yorkshire cross-bred swines (castrated males only): 34 kg ~ 42 kg
1-1. Fasted: 12~18 hrs
1-2. Water: free access

2. Name of test dressing :
2-1. Products " Coreleader" Hemo-Pad (Sterile)"
2-1-1. Hemo-Pad
2-1-2. Hemo-Fiber non-wovenc
2-1-3. Control dressing: commercially gauze patch
2-2. Storage condition: Room temperature

3. Physiological monitor:
3-1. mean arterial pressure (MAP)
3-2. Heart Rate (HR)
3-3. wound temperature

Before the surgery date:

1. Venous blood samples were collected from pigs
1-1.complete blood count (CBC)
1-2.prothrombin time
1-3.activated partial thromboplastin time                within normal range
1-4.fibrinogen

On the day of surgery:

1. Anesthetized:
1-1.tiletaminezolazepam (Telazol, 4–6 mg/kg, [i.m.])
1-2. 5% isoflurane in oxygen via face mask

2. 100% oxygen in mechanically ventilated

## Surgical Procedures

Build up monitor system:

1. Right carotid artery was cannulated:
1-1. Recording of blood pressure (systolic, diastolic, and mean) and heart rate
1-2. blood draws
1-3.**9 mL** blood sample + 1 mL of Na citrate (3.2%)

2. Right jugular vein was also catheterized resuscitation fluid

3. Midline laparotomy:
3-1. splenectomy
3-2. cystostomy: drainage of urine
3-3. abdomen was then closed with suturing and skin stapled
3-4. arterial line collect blood sample:

Create a severe hemorrhage:

1. Groin area, ~ 5 cm of **femoral artery** dissected free from surrounding tissues
2. Measure wound temperature
3. Stabilization period to record baseline data:

Vascular punch:

1. Femoral artery was clamped proximally and distally
2. 6-mm diameter arteriotomy, on the anterior surface of the vessel
3. Pretreatment blood loss:

## Wound Treatment and Resuscitation

Wound dressing covered:

1. The surgeon was blinded to use of test dressing (applied over the wound randomly)

2. While bleeding continued:

2-1.the dressing was covered immediately with a folded laparotomy gauze

2-2. pressed the wound with sufficient pressure

2-3. occlude the artery and stop the bleeding

First check point: (compression after 30 seconds)

1. Fluid resuscitation to compensate pretreatment blood loss

Second check point: (Total compression 2 minutes)

then release compression and hemostasis observed 3 minutes

1. if re-bleeding happen:

1-1. laparotomy gauze was removed

1-2. replaced with fresh material

1-3. repeated   2-minute compression again

1-4. at most twice with each product regardless of hemostatic outcome

1-5. observed for the next 3 hours with laparotomy gauze left in place

1-6. shed blood was collected→ identified as post-treatment blood loss (Table 2)

Animals   were continued monitor by physiological monitor:

1. continued record 3 hours

2. or death: end tidal $P_{CO_2} < 15$ mm Hg and MAP $< 20$ mm Hg

**The Stability of the Hemostasis**

1. flexed and stretched their legs $\times$ 5 times (simulating walking condition)

2. removed dressing from the wound

3. check the status of injury and the patency of the vessel

**Results**

No differences were found in baseline blood measures, pretreatment blood loss or fluid infusion among groups. Stable hemostasis was achieved in the group of "Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber. Both groups led to stabilized mean arterial pressure and significantly survival (100%). "Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber secured hemostasis in 2.67± 0.58 minutes and 3.25± 1.26 minutes respectively, which were significantly less than control (over 20 minutes). The average survival time (over 180 minutes) of animals with "Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber treatment were also significantly longer than that of control (36± 5 minutes). The total blood loss was less in "Coreleader" Hemo-Pad (31.58 mL/kg) and "Coreleader" Hemo-Fiber (30.55 mL/kg) groups than control (188± 17 mL/kg). No significant rise noticed in wound temperature after treatment with dressings and computed magnetic resonance imaging results proved no blood leaking from the vessels.

**Table 1**

Baseline Physiological and Hematological Measurements in the Swines

| Parameters \ Dressing | Hemo-Fiber (N=4) | | | | | Hemo-Pad (N=3) | | | | Commercially Gauze (N=3) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **HGB (g/dL)** | $n_1$ | $n_2$ | $n_3$ | $n_4$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. |
| | 17.10 | 15.90 | 16.40 | 16.20 | 16.40±0.51 | 17.30 | 15.90 | 15.70 | 16.30±0.87 | 17.00 | 16.70 | 15.30 | 16.33±0.91 |
| **HCT (%)** | $n_1$ | $n_2$ | $n_3$ | $n_4$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. |
| | 61.50 | 50.80 | 60.10 | 60.30 | 58.18±4.96 | 61.30 | 55.50 | 49.20 | 55.33±6.05 | 61.10 | 57.00 | 48.40 | 55.50±6.48 |
| **PLT (1,000/μL)** | $n_1$ | $n_2$ | $n_3$ | $n_4$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. |
| | 734 | 597 | 724 | 633 | 672.00±67.56 | 761 | 809 | 491 | 687.00±171.43 | 801 | 784 | 820 | 801.67±18.01 |
| **The Power of Hydrogen (pH)** | $n_1$ | $n_2$ | $n_3$ | $n_4$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. |
| | 7.263 | 7.382 | 7.384 | 7.507 | 7.384±0.100 | 7.326 | 7.394 | 7.439 | 7.386±0.057 | 7.167 | 7.381 | 7.313 | 7.287±0.109 |
| **$HCO_3$ (mM)** | $n_1$ | $n_2$ | $n_3$ | $n_4$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. |
| | 19.00 | 22.70 | 21.00 | 24.00 | 21.68±2.17 | 22.30 | 24.90 | 20.90 | 22.70±2.03 | 6.40 | 21.80 | 21.40 | 16.53±8.78 |
| **$PCO_2$ (mM)** | $n_1$ | $n_2$ | $n_3$ | $n_4$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. |
| | 41.70 | 37.80 | 34.90 | 30.00 | 36.10±4.93 | 42.30 | 40.30 | 30.60 | 37.73±6.26 | 17.50 | 36.40 | 41.80 | 31.90±12.76 |

| PO$_2$ (mM) | n$_1$ | n$_2$ | n$_3$ | n$_4$ | Mean±Std. | n$_1$ | n$_2$ | n$_3$ | Mean±Std. | n$_1$ | n$_2$ | n$_3$ | Mean±Std. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 24.20 | 77.40 | 67.10 | 75.50 | 61.05±24.97 | 35.70 | 55.00 | 72.10 | 54.27±18.21 | 134.80 | 360.30 | 22.60 | 172.57±171.99 |
| HR (time/min) | n$_1$ | n$_2$ | n$_3$ | n$_4$ | Mean±Std. | n$_1$ | n$_2$ | n$_3$ | Mean±Std. | n$_1$ | n$_2$ | n$_3$ | Mean±Std. |
| | 53.00 | 52.00 | 56.00 | 50.00 | 52.75 ± 2.50 | 55.00 | 60.00 | 53.00 | 56.00 ± 3.61 | 54.00 | 55.00 | 57.00 | 55.33 ± 1.53 |
| RR (time/min) | n$_1$ | n$_2$ | n$_3$ | n$_4$ | Mean±Std. | n$_1$ | n$_2$ | n$_3$ | Mean±Std. | n$_1$ | n$_2$ | n$_3$ | Mean±Std. |
| | 18.00 | 16.00 | 20.00 | 19.00 | 18.25 ± 1.17 | 17.00 | 19.00 | 16.00 | 17.33 ± 1.53 | 18.00 | 15.00 | 17.00 | 16.67 ± 1.53 |

**Table 2**

Outcomes of Treating a Groin Arterial Hemorrhage with Different Hemostatic Dressings in Swine †Initial hemostasis was considered to occur when bleeding was stopped for at least 3 minutes after compression.

| Dressing \ Parameters | Hemo-Fiber (N=4) | | | | | Hemo-Pad (N=3) | | | | Commercially Gauze (N=3) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total Time Bleeding Stopped (min)** | $n_1$ | $n_2$ | $n_3$ | $n_4$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. |
| | 3.00 | 5.00 | 3.00 | 2.00 | 3.25 ± 1.26 | 2.00 | 3.00 | 3.00 | 2.67 ± 0.58 | >20 | >20 | >20 | >20 |
| **Total Resuscitation Fluid (mL/kg)** | $n_1$ | $n_2$ | $n_3$ | $n_4$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. |
| | 31.10 | 30.00 | 30.45 | 30.65 | 30.55 ±0.46 | 31.88 | 30.86 | 32.00 | 31.58 ± 0.63 | 188.63 | 188.00 | 188.33 | 188.32 ±0.32 |
| **Survival Time (min)** | $n_1$ | $n_2$ | $n_3$ | $n_4$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. | $n_1$ | $n_2$ | $n_3$ | Mean±Std. |
| | >180 | >180 | >180 | >180 | >180 | >180 | >180 | >180 | >180 | 61.20 | 59.97 | 61.58 | 60.92 ± 0.69 |
| **Survival Rate (%)** | 100% | | | | | 100% | | | | 0% | | | |

## Conclusion

"Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber are tested effective dressing in this arterial hemorrhage model. The hemostatic property of "Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber are both attributed to its raw material. "Coreleader" Hemo-Pad and "Coreleader" Hemo-Fiber are now recommended as the first line of treatment for lethal hemorrhage on the battlefield and accident site.

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