Test Report

TEST ITEMS

12-weeks Muscle Implantation Study in Rabbits

TEST ARTICLE

Non-absorbable Surgical Suture (model: PTFE)
<Specification: Z21302-60 (USP2-0/EP2.5)>

IDENTIFICATION №

150643

MANUFACTURER

Foosin Medical Supplies Inc., Ltd.

SPONSOR

TÜV SÜD Certification and Testing (China) Co., Ltd.
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SUMMARY

The test article, Non-absorbable Surgical Suture (model: PTFE), was implanted in muscle tissue of the rabbit. The muscle tissue was evaluated for evidence of irritation based on the requirements of the International Organization for Standardization ISO 10993-6: 2007: Biological Evaluation of Medical Devices, Part 6: Tests for Local Effects after Implantation.

New Zealand White Rabbits with body weight ranging from 2.1kg to 2.6kg were utilized. Sterile four test samples were implanted by needle into the muscle along one side of spine. Four negative control samples were similarly implanted in the opposite muscle. The animals were euthanatized at 1w, 4w and 12w after implantation. Three animals with total of 24 implant sites were examined macroscopically at each time point and tissue reaction such as heamatoma, oedema, encapsulation was recorded. Subsequently, the muscle surrounding 10 test samples and 10 control samples were yielded. The microscopic evaluation was conducted to further define any tissue response including inflammation, neovascularisation, fibrosis and fatty infiltrate. Finally, according to the irritant ranking, degree of response was divided into 4 grades from non-irritant to severe irritant.

Under the conditions of this study, macroscopically, there was no visible abnormal reaction for test samples at any implant site of all observation periods. Microscopically, the average irritant scores were 0, 0 and 0 at 1 week, 4 week and 12 weeks respectively. According to the average irritant score at 12 weeks, the test article was considered a non-irritant as compared to the negative control.

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Apr 11, 2016
Date Completed

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INTRODUCTION

The purpose of this study was to evaluate the potential for a local irritant response to material [redacted] direct contact with muscle tissue. This test was conducted based on the requirements of [redacted] 2007: Biological Evaluation of Medical Devices, Part 6: Tests for Local Effects after Implantation. The test article was received on Jun. 16, 2015. The animals were implanted on Oct. 20, 2015 and the final observations were concluded on Mar. 17, 2016

This study, initiated by protocol signature on Aug. 20, 2015, was completed in the Lab of Shanghai Biomaterials Research & Test Center (SBRTC) and was conducted in accordance with the provisions of the ISO/IEC 17025-2005.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	Non-absorbable Surgical Suture (model: PTFE) <Specification: Z21302-60 (USP2-0/EP2.5)>
Identification No:	150643
Sterilization Status:	STERILE
Storage Conditions:	Room temperature
Test Article Preparation:	According to the requirement of sponsor, the samples with 0.303mm in wire diameter were supplied by the sponsor. The test samples were prepared into 10mm in length for testing.
Negative control:	According to the requirement of sponsor, High density polyethylene rod coming from Hatano Research Institute Food and Drug Safety Center was utilized as the negative control, and prepared into 1mm in width and 10mm in length for testing.

METHODS

Test System:

Species:	Rabbit
Strain:	New Zealand White
Source:	SHANGHAI SONGLIAN LAB ANIMAL-FEILD
Sex:	7 male, 7 female
Number of Animals:	14
Body Weight Range:	2.1 kg to 2.6 kg before implantation.

Animal №	1	2	3	4	5	6
Weight (kg)	2.1	2.2	2.6	2.3	2.2	2.4

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Animal №	7	8	9	10	11	12
Weight (kg)	2.1	2.6	2.4	2.3	2.2	2.2
Animal №	13	14	15	16	17	18
Weight (kg)	2.4	2.5	/	/	/	/

Age: Adult

Test Periods: 1w, 4w and 12w after implantation

Animal Management:

Husbandry: Conditions conformed to "Laboratory animal-Requirements of environment and housing facilities"; "ISO 10993-2:2006: Biological evaluation of medical devices Part 2: Animal welfare requirements".

Food: Diet was provided from Shanghai Pu Lu Teng Biological Technology Co. Ltd.

Housing: Healthy animals were acclimatized to the laboratory conditions for 7 days before the treatment, and then they were individually housed in stainless steel suspended cages identified by a card indicating the Identification № of the test article and first treatment date.

Environmental: The room temperature and humidity were monitored daily. The room temperature range was from 20℃ to 23℃. The room humidity range was from 51 % to 61 %.

Personnel: Associates involved were appropriately qualified and trained.

Selection: Healthy, previously unused animals were selected.

Experimental Procedure:

The fur was clipped from the area over paravertebral muscles with appropriate antiseptic and then injected with 3.0% pentobarbital sodium (1.0ml/kg) to achieve whole anesthesia by an ear intravenous injection. After the anesthetic had taken effect, the back was wiped with 0.1% benzal konium bromide tincture and draped.

An incision large enough to accommodate the needle was made through the skin over the dorsolumbar region of the vertebral column. A stylet was placed in the needle and the needle withdrawn, leaving the sample in the paravertebral muscle. 2.5-5 cm from the midline and parallel to spinal column, four test samples were implanted and each implanted site was approximately 2.5 cm apart. In the opposite muscle, four negative controls were similarly implanted. Rabbits were returned to the respective cages following the procedure. All the animals were observed daily for general health.

At 1w, 4w and 12w, the rabbits were euthanasia and the representative tissue implant sites (test and control) from each animal were excised, the area of the tissue surrounding the center portion of each implant strip was examined macroscopically.

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Evaluation and Statistics:

A sufficient area around the implant site was allowed for proper histological preparation and accounted for capsule formation or other signs of irritation under microscope. The scores were recorded according to table 1 and table 2.

Table 1 Histological Scoring System

Cell Type/ Response	Score				
	0	1	2	3	4
Inflammation:					
Polymorphonuclear cells	0	Rare,1-5/phfa[a]	5～10/phf	Heavy infiltrate	Packed
Lymphocytes	0	Rare,1-5/phf	5～10/phf	Heavy infiltrate	Packed
Plasma cells	0	Rare,1-5/phf	5～10/phf	Heavy infiltrate	Packed
Macrophages	0	Rare,1-5/phf	5～10/phf	Heavy infiltrate	Packed
Giant cells	0	Rare,1-2/phf	3～5/phf	Heavy infiltrate	Sheets
Necrosis	0	Minimal	Mild	Moderate	Severe
[a] phf = per high powered (400x) field					

Table 2 Histological Scoring System

Response	Score				
	0	1	2	3	4
Neovascularisation	0	Minimal capillary proliferation, focal, 1-3 buds	Groups of 4-7 capillaries with supporting fibroblastic structures	Broad band of capillaries with supporting structures	Extensive band of capillaries with supporting fibroblastic structures
Fibrosis	0	Narrow band	Moderately thick band	Thick band	Extensive band
Fatty infiltrate	0	Minimal amount of fat associated with fibrosis	Several layers of fat and fibrosis	Elongated and Broad accumulation of fat cells about the implant site	Extensive fat completely surrounding the implant
[a] phf = per high powered (400x) field					

The histological scoring systems as described in Table 1 and Table 2 are converted to an implant evaluation system by calculating the average irritant score shown as below.

$$\text{Average score} = \frac{\text{total score of Table 1} \times 2 \ + \ \text{total score of Table 2}}{\text{animal number in each group}}$$

Average irritant score = average score of test sample — average score of control sample
(A negative difference is recorded as zero)

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Average irritant score is used to determine irritant ranking shown below as the conclusion.
Irritant ranking:

__ Non Irritant (0.0 to 2.9)
__ Slight Irritant (3.0 to 8.9)
__ Moderate Irritant (9.0 to 15.0)
__ Severe Irritant (>15.0)

DEVIATIONS

There were no deviations from the ISO 10993-6:2007 and SBRTC-PROTOCOL-15-13.

RESULTS

Clinical Observations:
All animals tolerated the surgical procedures well and appeared clinically normal throughout the duration of the study. During the experimental periods there was no evidence of infection or disturbed wound healing.

Macroscopic Observations:
There was no visible abnormal reaction at any implanted site of all observation periods. No signs of implant rejection, necrosis or infection were found.

Microscopic Observations:
Negative control group:
1 w: Rare polymorphonuclear cell, lymphocyte and giant cell infiltration was found. The fibrotic cyst wall was moderately thick. Groups of 4-7 of capillaries with supporting structures were observed.
4 w: Rare lymphocyte infiltration was found. The fibrotic cyst wall was narrow, and minimal capillary proliferation was found.
12 w: Nearly no inflammatory reaction was observed. The fibrotic cyst wall was narrow, and minimal capillary proliferation was found.

Test group:
1 w: Rare lymphocyte infiltration was found. Other histological expression was similar to the negative control group.
4 w: No inflammatory reaction was observed. Other histological expression was similar to the negative control group.
12 w: The histological expression was similar to the negative control group.

The average irritant scores in 1 week, 4 weeks and 12 weeks were 0, 0 and 0, respectively.

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Table 3 Scoring System at 1 week after implantation

	Test Sample			Control Sample		
Animal Number:	1	2	3	1	2	3
Inflammation:						
Polymorphonuclear	0	0	0	1	0	2
Lymphocytes	1	1	1	1	1	1
Plasma Cells	0	0	0	0	0	0
Macrophages	1	0	0	0	0	0
Giant Cells	0	0	0	1	1	1
Necrosis	0	0	0	0	0	0
SUB TOTAL (X2)	4	2	2	6	4	8
Neovascularisation	2	2	1	2	2	3
Fibrosis	2	2	1	2	2	3
Fatty Infiltrate	0	0	0	0	0	0
SUB-TOTAL	4	4	2	4	4	6
TOTAL	8	6	4	10	8	14
GROUP TOTAL	18			32		
AVERAGE IRRITANT SCORE	TEST - CONTROL = 18/3 - 32/3 = -4.7 (regarded as 0)					
Traumatic Necrosis	/	/	/	/	/	/	/	/	/	/
Foreign Debris	/	/	/	/	/	/	/	/	/	/

Table 4 Scoring System at 4 week after implantation

	Test Sample			Control Sample		
Animal Number:	4	5	6	4	5	6
Inflammation:						
Polymorphonuclear	0	0	0	0	0	0
Lymphocytes	0	0	0	1	1	0
Plasma Cells	0	0	0	0	0	0
Macrophages	0	0	0	0	1	0
Giant Cells	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0
SUB TOTAL (X2)	0	0	0	2	4	0
Neovascularisation	1	1	1	1	1	1
Fibrosis	1	1	1	1	1	1
Fatty Infiltrate	0	0	0	0	0	0
SUB-TOTAL	2	2	2	2	2	2
TOTAL	2	2	2	4	6	2
GROUP TOTAL	6			12		
AVERAGE IRRITANT SCORE	TEST - CONTROL = 6/3 - 12/3 = -2.0 (regarded as 0)					
Traumatic Necrosis	/	/	/	/	/	/	/	/	/	/
Foreign Debris	/	/	/	/	/	/	/	/	/	/

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Table 5 Scoring System at 12 week after implantation

	Test Sample			Control Sample		
Animal Number:	7	8	9	7	8	9
Inflammation:						
Polymorphonuclear	0	0	0	0	0	0
Lymphocytes	0	0	0	0	0	0
Plasma Cells	0	0	0	0	0	0
Macrophages	1	0	0	1	0	0
Giant Cells	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0
SUB TOTAL (X2)	2	0	0	2	0	0
Neovascularisation	1	1	1	1	1	1
Fibrosis	1	1	1	1	1	1
Fatty Infiltrate	0	0	0	0	1	0
SUB-TOTAL	2	2	2	2	3	2
TOTAL	4	2	2	4	3	2
GROUP TOTAL	8			9		
AVERAGE IRRITANT SCORE	TEST - CONTROL = 8/3 - 9/3 = -0.3 (regarded as 0)					
Traumatic Necrosis	/	/	/	/	/	/	/	/	/	/
Foreign Debris	/	/	/	/	/	/	/	/	/	/

According to the irritant ranking, 12 weeks muscle implantation test of the test sample was regarded as non-irritant.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by Shanghai Biomaterials Research & Test Center.

CONCLUSION

Under the conditions of this study, macroscopically, there was no visible abnormal reaction for test samples at any implant site of all observation periods. Microscopically, the average irritant scores were 0, 0 and 0 at 1 week, 4 week and 12 weeks respectively. According to the average irritant score at 12 weeks, the test article was considered a non-irritant as compared to the negative control.

RECORD STORAGE

All raw data, paraffin blocks and tissue slides pertaining to this study and a copy of the final report are to be retained in designated SBRTC archive files.

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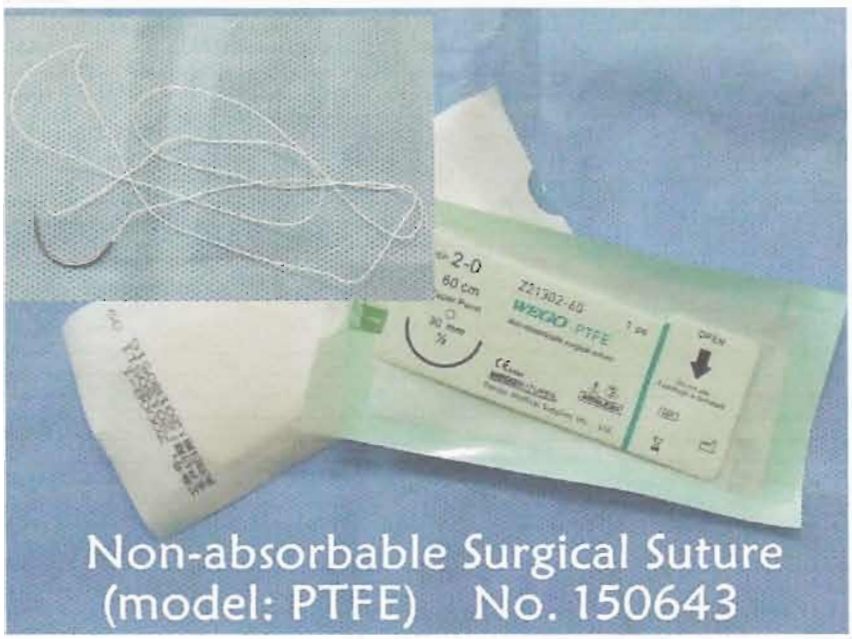
PHOTOGRAPH OF THE TEST ARTICLE



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APPENDIX

Light microscopic evaluation of each implant site was shown in Table 6~8.

Table 6 Scoring System at 1week after implantation

	Test Sample			Control Sample		
Animal Number:	1	2	3	1	2	3
Inflammation:						
Polymorphonuclear	0,0,0	0,0,0	0,0,0,0	1,1,1	1,0,0	2,2,2,2
Lymphocytes	1,1,1	1,1,1	1,1,1,1	1,1,1	2,1,1	1,1,1,1
Plasma Cells	0,0,0	0,0,0	0,0,0,0	0,0,0	0,0,0	0,0,0,0
Macrophages	0,1,1	0,0,0	1,0,0,0	1,0,0	0,0,0	0,0,0,0
Giant Cells	0,0,0	0,0,0	1,0,0,0	1,1,1	1,1,1	1,1,1,1
Necrosis	0,0,0	0,0,0	0,0,0,0	0,0,0	0,0,0	0,0,0,0
Neovascularisation	1,2,2	2,2,2	2,1,1,1	2,2,2	2,2,2	3,3,3,3
Fibrosis	1,2,2	2,2,2	2,1,1,1	2,2,2	2,2,2	3,3,3,3
Fatty Infiltrate	0,0,0	0,0,0	0,0,0,0	0,0,0	0,0,0	0,0,0,0
Traumatic Necrosis	///	///	////	///	///	////
Foreign Debris	///	///	////	///	///	////

Table 7 Scoring System at 4week after implantation

	Test Sample			Control Sample		
Animal Number:	4	5	6	4	5	6
Inflammation:						
Polymorphonuclear	0,0,0	0,0,0	0,0,0,0	0,0,0	0,0,0	0,0,0,0
Lymphocytes	0,0,0	0,0,0	0,0,0,0	1,1,1	0,1,1	0,1,0,0
Plasma Cells	0,0,0	0,0,0	0,0,0,0	0,0,0	0,0,0	0,0,0,0
Macrophages	0,0,0	0,1,0	0,0,0,0	0,1,0	0,1,1	0,0,0,0
Giant Cells	0,0,0	0,0,0	0,0,0,0	0,0,0	0,0,0	0,1,0,0
Necrosis	0,0,0	0,0,0	0,0,0,0	0,0,0	0,0,0	0,0,0,0
Neovascularisation	1,1,1	1,3,1	1,1,2,1	1,1,1	1,1,1	1,1,1,1
Fibrosis	1,1,1	1,3,1	1,1,2,1	1,1,1	1,1,1	1,1,1,1
Fatty Infiltrate	0,0,0	0,0,0,0	0,0,0,0	0,0,0	0,0,0,0	0,0,0,0
Traumatic Necrosis	///	///	////	///	///	////
Foreign Debris	///	///	////	///	///	////

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Table 8 Scoring System at 12week after implantation

	Test Sample			Control Sample		
Animal Number:	7	8	9	7	8	9
Inflammation:						
Polymorphonuclear	0,0,0	0,0,0	0,0,0,0	0,0,0	0,0,0	0,0,0,0
Lymphocytes	0,0,0	0,0,0	0,0,0,0	0,0,0	0,0,0	0,0,0,0
Plasma Cells	0,0,0	0,0,0	0,0,0,0	0,0,0	0,0,0	0,0,0,0
Macrophages	1,1,1	0,1,0	0,0,0,0	0,1,1	0,0,0	0,1,0,0
Giant Cells	0,0,0	0,0,0	0,0,0,0	0,0,0	0,0,0	0,0,0,0
Necrosis	0,0,0	0,0,0	0,0,0,0	0,0,0	0,0,0	0,0,0,0
Neovascularisation	1,1,1	1,1,1	1,1,1,1	1,1,1	1,1,1	1,1,1,1
Fibrosis	1,1,1	1,1,1	1,1,1,1	1,1,1	1,1,1	1,1,1,1
Fatty Infiltrate	0,0,0	0,0,0	0,0,0,0	0,0,0	1,1,0	1,0,0,0
Traumatic Necrosis	///	///	////	///	///	////
Foreign Debris	///	///	////	///	///	////

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