



TEST REPORT

STUDY REPORT NUMBER: MB-WH-281h-07

Study Completion Date: 20th June 2009

**Title: EFFICACY ASSESSMENT OF
'NATURAL SAGO HYDROGEL' FOR
HEALING OF SKIN ULCER OF
DIABETIC RATS**

Study Sponsor

Rumbia Bio-Tech Sdn Bhd
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Jalan Dengkil, Bangi,
43000 Kajang,
SELANGOR.

Testing Facility

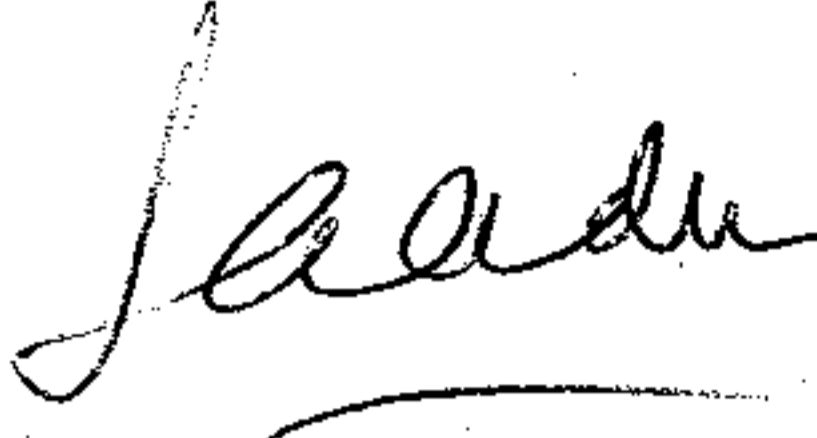
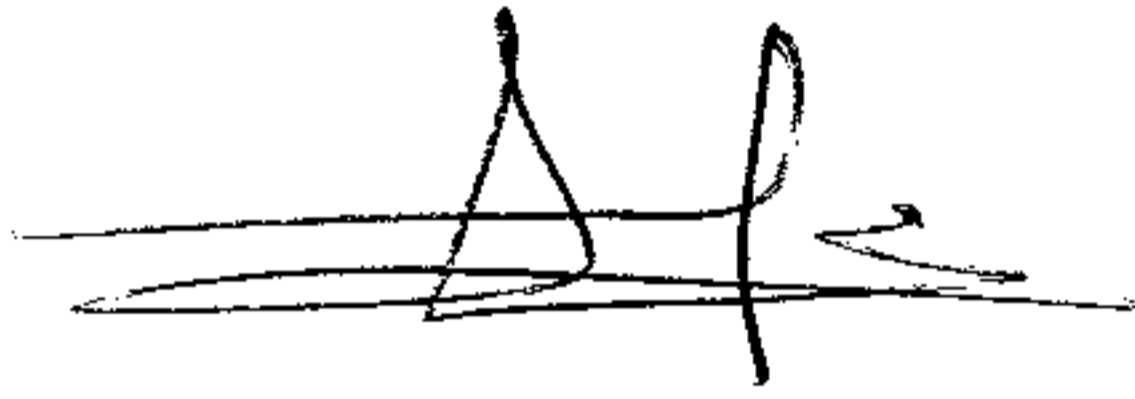

Animal Experimental Unit
Unit Makmal Haiwan,
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Study Director (2) : Assoc. Prof Dr. Md Mahmood Ameen
Abdulla

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- This test report concerns only the product being tested.

**EFFICACY ASSESSMENT OF 'NATURAL SAGO HYDROGEL' FOR
HEALING OF SKIN ULCER OF DIABETIC RATS**

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Study Director (2)	
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Sponsor	
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TEST REPORT - Efficacy Assessment of 'Natural Sago Hydrogel' for Healing of Skin Ulcer of Diabetic Rats

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SUMMARY

Efficacy Assessment of NSH for Healing of Skin Ulcer of Diabetic Rats

Protocol reference : Protocol AL - WH
Study completion date : 20/6/2009
Study reference number : WH-01-01-07
Study report number : MB-WH-281h-07
Job number : 281h-07-RBT
Test material : NSH
Conditions of use : Neat

1. **OBJECTIVE**

To evaluate the efficacy of a test material named as Natural Sago Hydrogel following topical administration on ulcer of diabetic rats.

2. **EXPERIMENTAL PROCEDURE**

Animals: Fifteen Diabetic Sprague Dawley albino rats, (5 for negative control, 5 for positive control), all females

Weight: Test group : 160 - 184 g
Negative control group : 140 - 180 g
Positive control group : 160 - 180 g

Date of initiation : 15th August 2007

Date of completion : 1st March 2008

Treatment

A full-thickness circular skin graft with a surface area of 5.66 cm² was harvested from the dorsum of each rat using surgical scissors. The full-thickness skin defect was left to heal by secondary intention, and each rat was kept in a separate cage with the defect uncovered.

The rats were divided into 3 groups of 5 rats. The first group was the study group and was treated with NSH topically. The second group was the negative control and left untreated and the third group was the positive control and was treated with Intrasite gel as positive control. All topical application was done once daily until the ulcer completely healed.

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3. **OBSERVATIONS**

Clinical Examination

Animals were observed for improvement of ulcer daily. The day the ulcer is completely healed is taken as the end point.

Histopathological Examination

Upon healing, a full thickness biopsy of the healed ulcer is excised for histopathological examination.

4. **RESULTS**

The group treated with NSH took a significantly shorter duration of time for complete ulcer healing, i.e 15.6 days compared to 17.8 days for negative control, $p=0.000$ (i.e $p<0.05$). Although positive control had a shorter duration for complete ulcer healing compared to test group, i.e 15.2 days, the difference was not significant, $p=1.000$, (i.e $p>0.05$). Positive control took a significantly shorter duration for complete ulcer healing compared to negative control, $p=0.020$ (i.e $p<0.05$).

There was histopathological changes observed in all groups i.e group treated with NSH, untreated group (negative control) and group treated with Intrasite gel (positive control). NSH promotes ulcer healing activity in this excision ulcer model study, which is more or less equal to positive control (standard treatment- Intrasite gel). In all NSH treated ulcers, the histological appearance of skin showed less inflammation, increased collagen formation, fibroblast cells, new blood capillaries (angiogenesis) and re-epithelialization compared to the negative control. Granulation tissue of treated animals showed moderate deposition of collagen comparable to Intrasite gel treated animals. Scar area in ulcer closure was also noted to be smaller with NSH and Intrasite gel compared to negative control.

5. **CONCLUSION**

NSH is effective in promoting ulcer healing in diabetic rats, significantly shortens the duration of ulcer healing, and significantly promotes ulcer healing activity in the excised ulcer model. It is as effective as Intrasite gel in promoting ulcer healing in diabetic rat.

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TEST REPORT

1.0 SPONSOR OF TEST MATERIAL

- 1.1 Name : Rumbia Bio-Tech Sdn Bhd.
1.2 Address : Blok 43 ALURTON,
MOSTI Agensi Nuklear Malaysia,
Jalan Dengkil, Bangi,
43000 Kajang,
SELANGOR
1.3 Study report number : MB-WH-281h-07
1.4 Job number : 281h-07-RBT

2.0 DETAILS OF TEST MATERIAL

- 2.1 Name : Product named as Natural Sago Hydrogel subsequently referred to
as NSH in this report
2.2 Test material reference : GL-281h-RBT0507
2.3 Study reference number : WH-01-01-07
2.4 Pkg Lst Batch : 6147/54/40/51/52/53
2.5 Date sample received : 21 May 2007
2.6 Expiry date : Not applicable
2.7 Appearance : Solid
2.8 Colour : Transparent
2.9 Quantity : 300 pieces
2.10 Storage : Ambient
2.11 Condition of use : As supplied
2.12 Dates of study : 15th August 2007 to 1st March 2008
2.13 Date of completion : 1st March 2008

3.0 LABORATORY FACILITY

- 3.1 Collaboration with : Animal Experimental Unit,
Unit Makmal Haiwan,
Fakulti Perubatan Universiti Malaya,
60603 Lembah Pantai.
KUALA LUMPUR

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3.3 Project staff

3.3.1 Study Director (1)

Dr. Saadiah Sulaiman
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3.3.2 Study Personnel/Consultant

Assoc. Prof. Dr. Md. Mahmood Ameen Abdulla
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c/o

Fadhilah binti Manap

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3.5 Study Timetable

Date of initiation : 15th August 2007
Date of completion : 1st March 2008

3.6 Environment and Husbandry

3.6.1 Species and strain

Sprague Dawley albino rat.

3.6.2 Supplier

Animal Laboratory, Universiti Malaya and Institute Medical Research.

3.6.3 Number of animal and sex

Fifteen Sprague Dawley albino rats; 5 for test group (females), 5 for negative control and 5 for positive control (females).

3.6.4 Body weight at initiation of treatment

Weight: Test group	: 160 - 184 g
Negative control group	: 140 - 180 g
Positive control group	: 160 - 180 g

3.6.5 Housing

Animals were housed in a plastic polypropylene caging with perforated base.

3.6.5.1 Size of cage: Plastic cage internal dimension: 22cm x 15cm x 37cm. Each rat was housed in a separate cage and assigned specific numbers from 1 to 5.

3.6.5.2 Photoperiod

12 hour light/dark cycle.

3.6.6 Diet

Mouse pellet: Gold Coin Brand Animal Feed (Cahaya Suria Sdn Bhd) and Australia pellet

3.6.7 Water

Filtered tap water *ad libitum* continuously supplied through water dispensing bottles.

3.6.8 Contaminant

There were no known contaminants reasonably expected to be found in the food or water at levels which would interfere with the results of the study.

3.7 Pre-Treatment Procedures

3.7.1 Check for ill health

On arrival, daily and just before commencement of treatment to ensure only healthy animals were used in the study.

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3.7.2 Body weight

All animals were weighed on the day just before treatment, once weekly and at termination of study.

3.7.3 Selection and allocation of animal

Rats were selected randomly at the start of the acclimatization period.

3.7.4 Identification

Cages : Labeled with animal number, sex of animal and study reference number.

Animal : Each cage of rat was marked with a color code (permanent marker pen) and given a sequential animal number assigned to study reference number, which constitutes a unique identification system.

4.0 TEST METHOD

4.1 Name of test: Efficacy Assessment of NSH for Healing of Skin Ulcer of Diabetic Rats

4.2 Objective

To evaluate the efficacy of a test material named as Natural Sago Hydrogel following topical administration on ulcer of diabetic rats.

4.3 Material

4.3.1 Test Material: Topical product named as Natural Sago Hydrogel (NSH)

Positive Control: Intrasite Gel, which contains 2.3% of a modified carboxymethylcellulose (CMC) polymer together with propylene glycol (20%) as a humectants and preservative.

4.3.2 Test system

Fifteen Sprague Dawley albino rats that were successfully induced with diabetes; five for test group (females), 5 for negative control (females) and 5 for positive control (females).

4.3.2.1 Preparation of animal model 'diabetic rats'

Procedure: Induction of Diabetes

Fasting blood glucose of each rat was measured by using glucometer (Advantage II Strips) following overnight fast. A day later, all rats were induced intraperitoneally with Streptozotocin 70 mg/kg body weight. Twenty-four hours later, fasting blood glucose was again measured. Rats with fasting blood sugar of more than 11 mg/dl were considered as diabetic and were enrolled in to the study.

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4.4 Preparation of test material

4.4.1 Route of test material administration

The route of test material administration was by topical application. Test material was applied as delivered.

4.4.2 Method of test material administration

The test material (2x2cm) was administered directly on to the designated treatment site.

4.5 Procedure

4.5.1 Preparation and selection of animals

On the day before initiation, the fur of rats was removed by clipping on the dorsal neck by electrical clipper. The rats were anesthetized with local anesthesia, i.e subcutaneous injections of lignocaine 2%. A 1.8-cm diameter circle was drawn with a marker pen on the test area of the dorsum of the neck. A full-thickness circular skin graft with a surface area of 5.66 cm² was harvested from the dorsum of each rat using surgical scissors. The full-thickness skin defect was left to heal by secondary intention, and each rat was kept in a separate cage with the defect uncovered.

4.5.2 The rats were divided into 3 groups of 5 rats. The first group was the study group and was treated with NSH topically. The second group was the negative control and left untreated and the third group was the positive control and was treated with Intrasilite gel as positive control. All topical application was done once daily until the ulcer completely healed.

4.5.3 Treatment and observation period

Each rat was observed for improvement of ulcer daily. The day the ulcer is completely healed is taken as the end point.

Histopathology

Upon healing, a full thickness biopsy of the healed ulcer is excised for histopathological examination.

4.6 Method of assessment

1. Clinical assessment

Mean duration of time taken for complete healing of ulcer i.e clearance of raw surface.

2. Histological assessment

Full thickness biopsy of each ulcer area was excised, stored in formalin and subsequently processed into tissue block. Sections were then made and stained with Hematoxylin and Eosin (H&E) staining for histological assessment.

5.0 DATES OF TEST PROCEDURE

Date of initiation : 15th August 2007

Date of completion : 1st March 2008

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6.0 SUMMARY OF RESULTS

- 6.1 A total of 60 rats were induced intraperitoneally with Streptozotocin at 70 mg/kg body weight. Forty rats died following induction, twenty rats of which died during procedure due to complication of diabetes. A total of 15 diabetic rats completed the study, five for test group treated with NSH, five for negative control (left untreated) and five for positive control (treated with Intrasite gel).
- 6.2 The duration the ulcer take to heal is as tabulated in Table 1. The group treated with NSH took a significantly shorter duration of time for complete ulcer healing, i.e 15.6 days compared to 17.8 days for negative control, $p=0.000$ (i.e $p<0.05$). Although positive control had a shorter duration for complete ulcer healing compared to test group, i.e 15.2 days, the difference was not significant, $p=1.000$, (i.e $p>0.05$). Positive control took a significantly shorter duration for complete ulcer healing compared to negative control, $p=0.020$ (i.e $p<0.05$).

Table 1: Duration of the time taken for complete ulcer healing by Group Treated with NSH, compared to Negative Control (untreated) and group Treated with Intrasite gel (Positive Control).

Group	Animal number	Test animal sequences	n	Day of complete healing	No of days ulcer take to hea	Mean
Test Group (NSH)	rwt01	02-1	5	Day 17	17	15.6
	rwt02	02-2		Day 16	16	
	rwt03	02-3		Day 14	14	
	rwt04	02-4		Day 15	15	
	rwt05	02-5		Day 16	16	
Negative control	rwn01	nc-1	5	Day 17	17	17.8
	rwn02	nc-2		Day 17	17	
	rwn03	nc-3		Day 17	17	
	rwn04	nc-4		Day 19	19	
	rwn05	nc-5		Day 19	19	
Positive control	rwp01	pc-1	5	Day 15	15	15.2
	rwp02	pc-2		Day 15	15	
	rwp03	pc-3		Day 16	16	
	rwp04	pc-4		Day 17	17	
	rwp05	pc-5		Day 13	13	

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Table 2: Comparison of p value for each group in diabetic rats

Comparison	Actual p value	p value	
NHS vs PC	1.000	p>0.05	Not significant
NHS vs NC	0.050	P<0.05	Significant
PC vs NC	0.020	P<0.05	Significant

6.2.1 PICTURES

1. Picture 1

Demonstrates the difference in ulcer healing between rats treated with NSH compared to untreated group (negative control).

Pictures 1a and 1b:

At Day 7, group treated with NSH had smaller diameter, 1.3 cm x 1.3 cm compared to those left untreated (negative control) 1.8 cm x 1.5 cm.

Pictures 1c and 1d:

Complete ulcer healing is faster in group treated with NSH - Day 15 compared to the untreated group (negative control) - Day 18 (See Appendix I)

2. Picture 2

Histopathological examination of ulcer treated with NSH on diabetic rats (See Appendix II)

3. Picture 3

Histopathological examination of ulcer left untreated (negative control) on diabetic rats (See Appendix III)

6.3 Histopathology

There was histopathological changes observed in all groups i.e group treated with NSH, untreated group (negative control) and group treated with Intracel gel (positive control). NSH promotes ulcer healing activity in this excision ulcer model study, which is more or less equal to positive control (standard treatment- Intracel gel). In all NSH treated ulcers, the histological appearance of skin showed less inflammation, increased collagen formation, fibroblast cells, new blood capillaries (angiogenesis) and re-epithelialization compared to the negative control. Granulation tissue of treated animals showed moderate deposition of collagen comparable to Intracel gel treated animals. Scar area in ulcer closure was also noted to be smaller with NSH and Intracel gel compared to negative control.

7.0 CONCLUSION

NSH is effective in promoting ulcer healing in diabetic rats, significantly shortens the duration of ulcer healing, and significantly promotes ulcer healing activity in the excised ulcer model. It is as effective as Intracel gel in promoting ulcer healing in diabetic rat.

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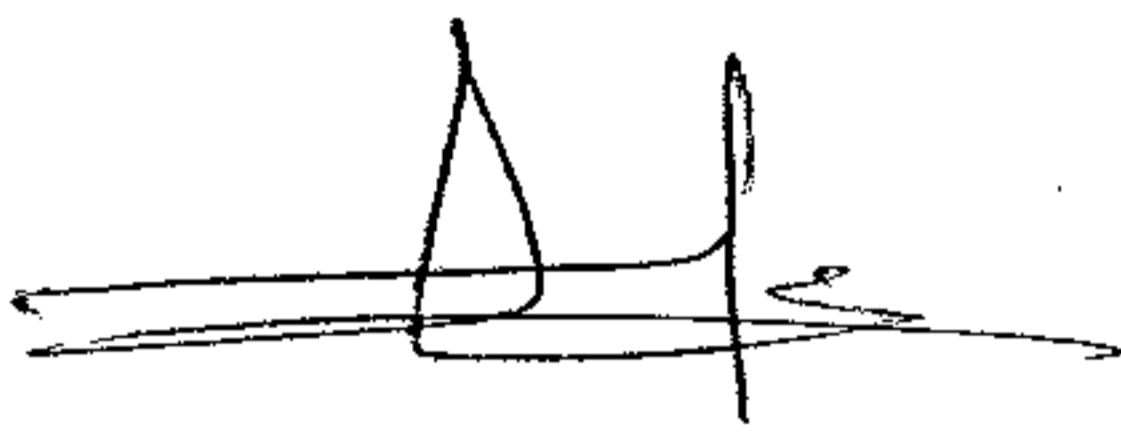
8.0 VERIFICATION

We the undersigned declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.



.....
Dr. Saadiyah Sulaiman
Study Director 1

20/6/09
.....
Date



.....
Assoc. Prof. Dr. Md. Mahmood Ameen Abdulla
Study Director 2

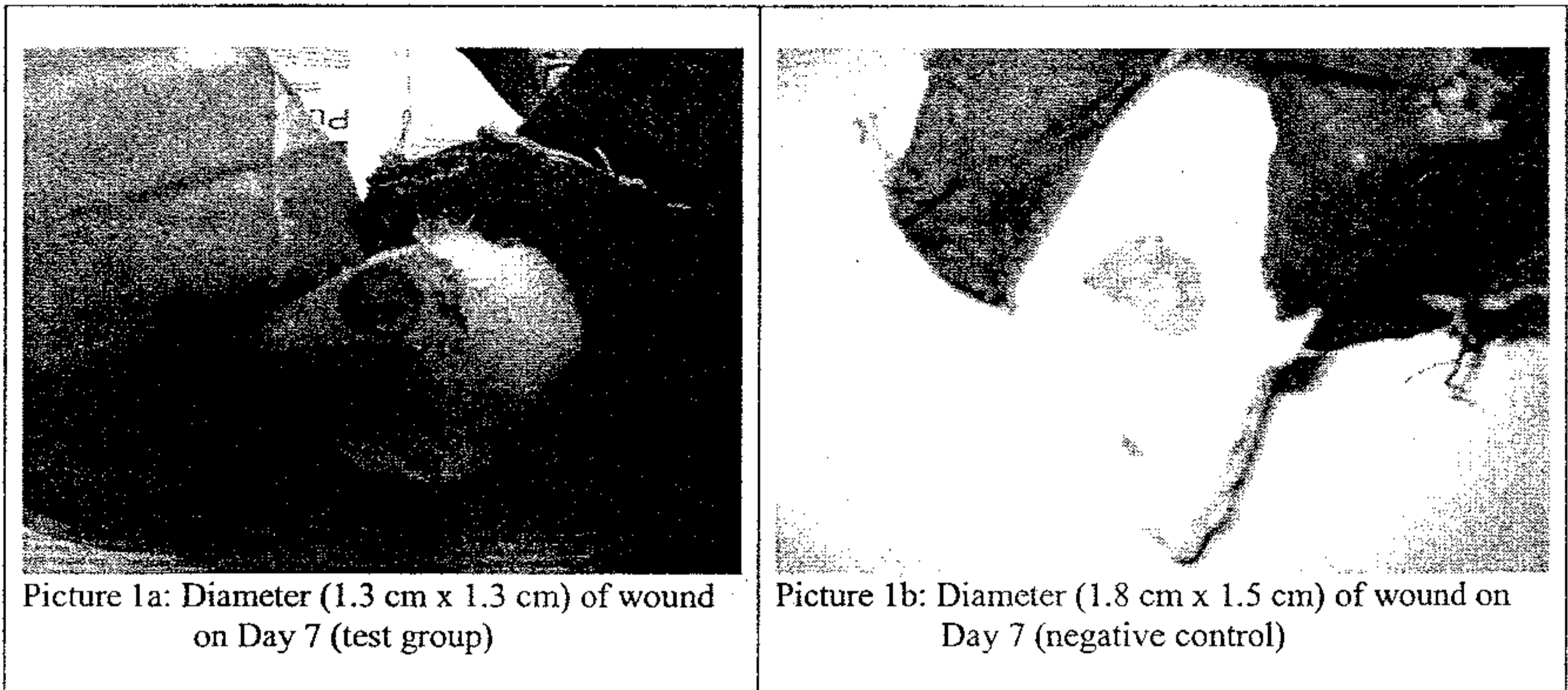
20/6/09
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Date

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Appendix I

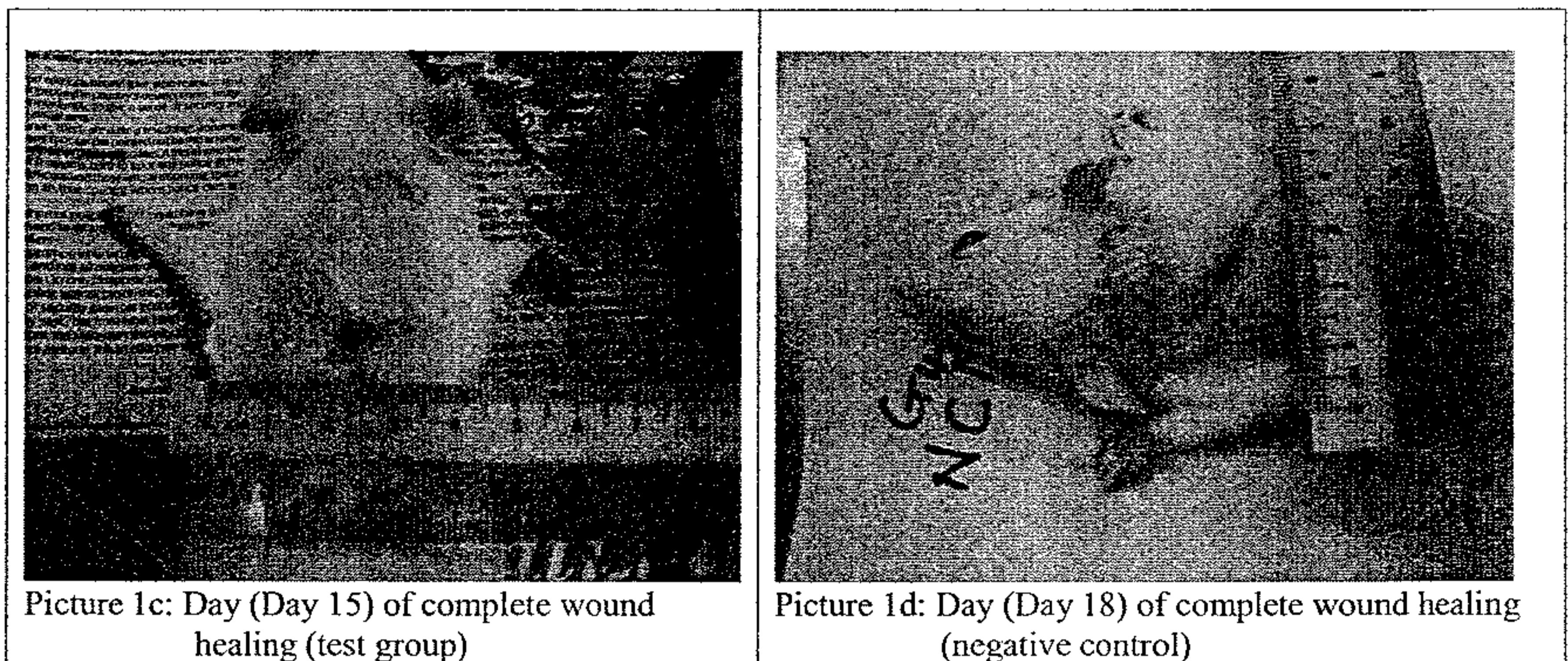
Pictures 1:

Demonstrates the difference in wound healing between rats treated with NSH, compared to rats from untreated group (negative control).



Pictures 1a and 1b at Day 7:

Demonstrate that a rat from the group treated with NSH had smaller diameter, 1.3 cm x 1.3 cm compared to a rat from untreated group (negative control), 1.8 cm x 1.5 cm.



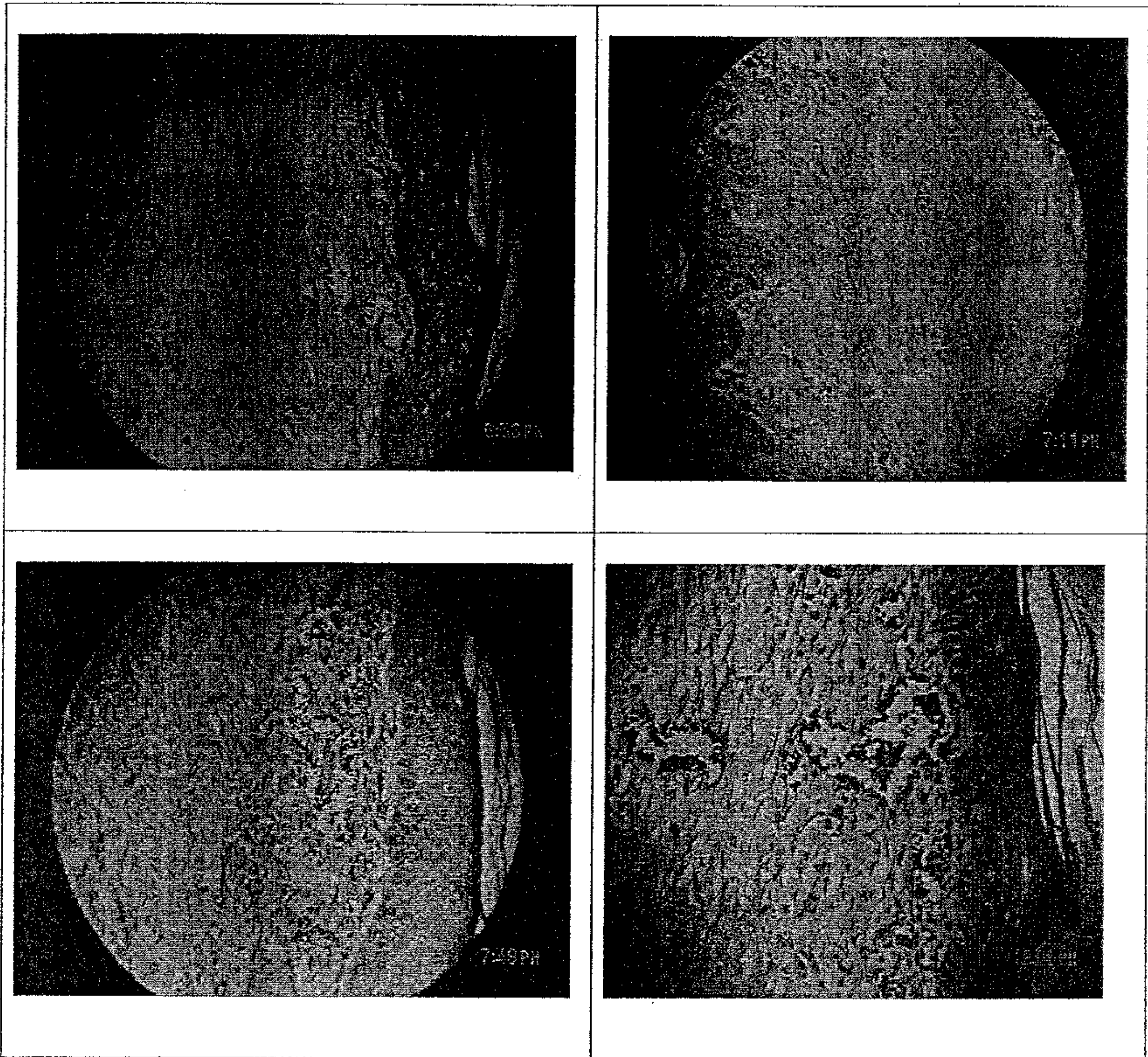
Pictures 1c and 1d:

Complete wound healing is faster in a rat from the group treated with NSH - Day 15 compared to the rat from untreated group (negative control) - Day 18

Appendix II

Picture 2:

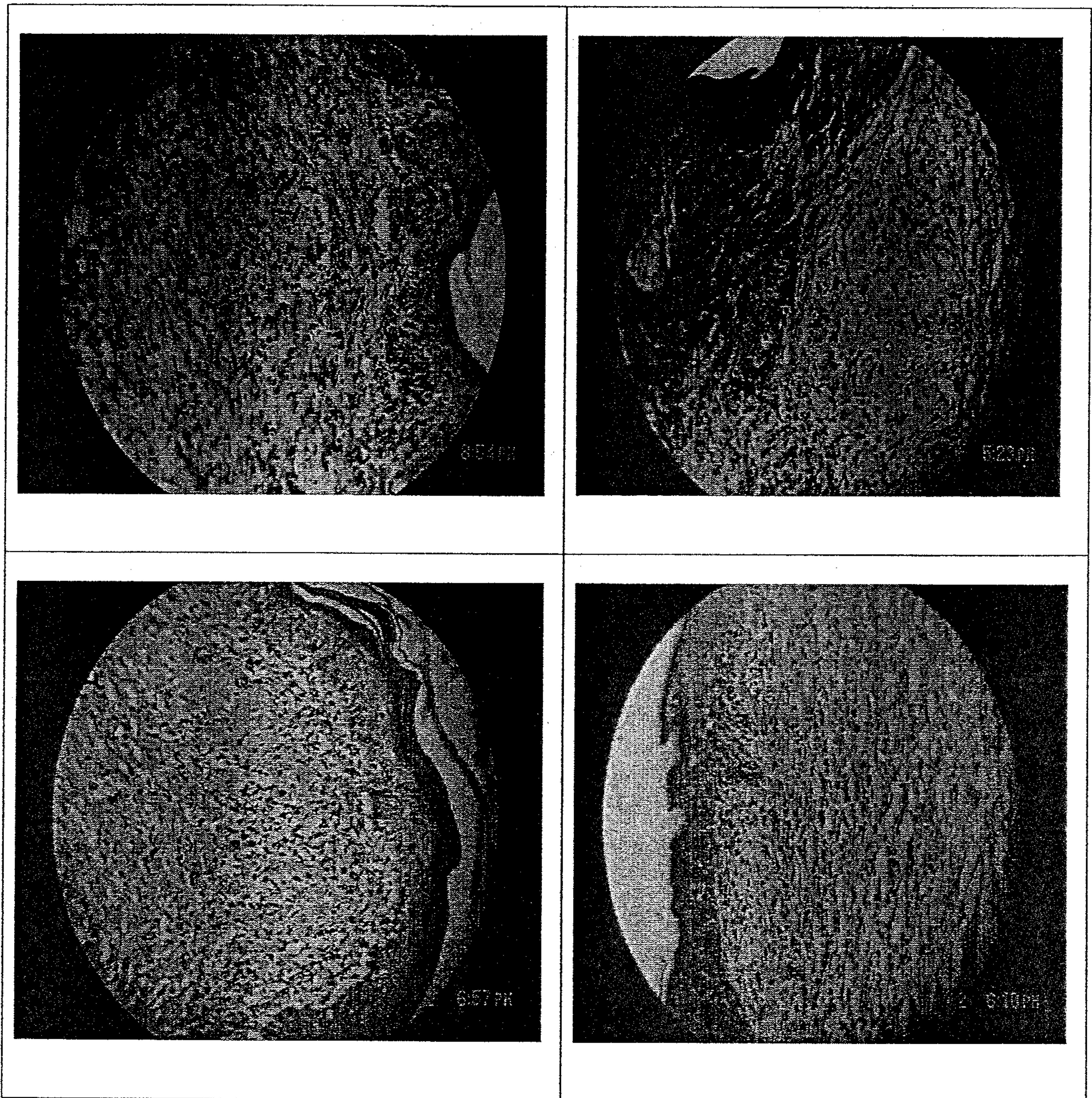
Histopathological Examination of Wound Treated with NSH on Diabetic Rats



Moderate granulation tissue, collagen, angiogenesis and re-epithelization, and moderate fibroblast and myofibroblasts and less inflammatory cells compared to untreated diabetic wounds. Wound enclosure and scar are moderate and regular.

Appendix III

Picture 3:
Histopathological Examination of Wound Left Untreated (Negative Control) on Diabetic Rats



Poor wound healing in untreated diabetic rats contained reduced granulation tissue, decreased collagen deposition, decreased re-epithelialization and angiogenesis, and few fibroblast and myofibroblasts and more inflammatory cells compared to diabetic wound treated with NHS. There was a delay in wound filling by granulation tissue. The enclosure of wound are slow, more scar and irregular.