Journal of Metals, Materials and Minerals. Vol. 14 No. 1 pp 11-19, 2004.

MECHANICAL TENSILE STRENGTH TESTS OF RAT SKIN WOUNDS DURING THE FIRST SEVEN DAYS OF THE HEALING PROCESS

Radovan HUDÁK[1], Peter GÁL[2], Tomáš TOPORCER[2], Róbert KILÍK[3], Ján SABO[4]

[1] Department of Instrumental and Biomedical Engineering, Faculty of Mechanical Engineering, Technical University of Košice, Slovakia

[2] Faculty of Medicine, P.J. Šafárik University, Košice, Slovakia

[3] Department of Surgery, Hospital in Revúca, Slovakia

[4]Department of Medical Biophysics, Faculty of Medicine, P.J. Šafárik University, Košice, Slovakia

ABSTRACT

The goal of our investigation was to evaluate the mechanical properties of the skin wounds during the first seven days of primary healing and to compare these results to simultaneous histomorphological studies.

Rats from the genus Spraque-Dawley (n=63) have been included into the experimental process. We performed two parallel symmetrical skin incisions (on the left and right side of the spine) under general anaesthesia. Rats were divided into 7 groups, in every group there were 9 rats. To six rats in every group the tensile strength of the wound was measured in 24, 48, 72, 96, 120, 144 and 168 hours after wounding. The other three rats in every group were assessed for histomorphological study.

After each day of measurement we obtained complex tensile strength diagrams of wound tensile strength-time dependence. We observed the highest absolute strength increase after 24 hours within 5 days after wounding.

In the period between the 2nd and 5th day of testing we observed stagnation of the strength, labelled as "plató phase". The next significant strength increase was observed only after 6 days, with a following increase of the tensile strength of the skin wound.

The tensile strength during wound healing was partially explained by simultaneous histomorphological study.

All the mentioned tensile strength tests were performed by a tensile strength tester, especially designed and constructed for our purpose.

Keywords: primary wound healing, mechanical properties of the skin wounds, rats from genus Sprague-Dawley, tensile strength tests

INTRODUCTION

Wound healing is defined as a replacing of dead tissue with living tissue, which does not just reproduce lonely(I suggest to use the word 'single' instead of lonely but I'm not sure) cells, but also recovers the damaged extracellular matrix (ECM) (Rubin and Faber, 1994). Every tissue is not able to repair ad integrum – accordingly to the initial state. Therefore, the healing of various tissues is possible to be divided into two basic groups, including regeneration and reparation (Rubin and Faber, 1994). The tissues unable to proliferate are healed by reparation as well as the tissues damaged by surgery or trauma. That kind of healing results in scar-creation. After the damaging of the tissue, the cells that did not lose the ability to proliferate, multiplied by mitosis, neosynthesis of the extracellular matrix and creation of the tissue identical or similar to the original tissue by a process called regeneration. Wound healing runs in three basic phases, inflammatory, proliferative and maturative (Whelan, *et al.* 2003). Individual phases of healing are characteristic by their typical processes. They are not strictly separated from each other, but their processes freely blend together.

With regard to the present stage of surgery, especially plastic surgery, a number of experimental studies deal with new approaches to improving the process of wound healing (Stadler, *et al.* 2001; and Whelan, *et al.* 2003). Experimental efforts are also focused on the reduction of visible skin scars. Early suture resection assures uniquely better cosmetic results (Burkitt, *et al.* 1990). Skin sutures can be removed as soon as the wound is fixed enough without mechanical support. Therefore, the tensile strength of the wound is the objective and preferred measure for wound healing evaluation and is adequate for experimental as well as for clinical results (Moelleken and Mathes, 1985).

The measurement of the tensile strength of the skin wound is possible to be performed by a biomechanical tensile strength test by means of a tensile strength tester adapted to that specific purpose. In rats the tensile strength of the wound achieves only 3% of healthy tensile strength after 7 days of healing (Andreassen, *et al.* 1987; and Stadler, *et al.* 2001). The low skin tensile strength of the wound during the first 7 days prompts us to the application of a specific high-sensitive tensile strength tester (Hudak, *et al.* 2003).

The goal of our study was to evaluate the tensile strength of the skin wounds during healing and find the correlation to morphology. We focused on the first 7 days as a time period of the most dynamic changes of the complete healing process.

MATERIALS AND METHODS

Forty-two female Spraque-Dawley rats of a similar age of 4-5 months were used for experiments. Animals were divided into groups of 9 animals per group. Atropin (0,05 mg/kg body weight, Hoechst-Biotika, Slovakia) was applied to animals 20 minutes before anaesthesia with ketamine-xylazine mixture (10mg/kg-30 mg/kg body weight, ketamine Calypsol, Richter Gedeon, Hungaria, xylazine Rometar a.u.v., Spofa, Czech Republic) and analgetic tramadol (5 mg/kg body weight, Tramadol-K, Krka, Slovenia).

The shaved surface of the dorsum of each subject was first cleaned with an alcohol pad, followed by the application of Betadine for skin disinfection. Two longitudinal incisions of identical length (3,5cm) were made on the dorsal skin. The incisions were then closed in a conventional manner by four simple sutures (Chiraflon 3/0, Chirmax Czech Republic). The animals were caged individually after undergoing wounding to avoid damaging the wound.

Equal numbers of animals from each group were euthanized by inhalation of ether 24, 48, 72, 96, 120, and 144 hours after injury.

The wounds were carefully excised immediately after euthanasia to prevent post mortem transformation of skin wounds. The

strip from the wounded area 50 x 50 mm was prepared without any undesirable mechanical damage of the sample. The sample was dampened by physiological solution during the measurement procedure to prevent drying up and the following changes of mechanical properties. The sutures were removed and the skin strip was adjusted to an optimal 2 cm width.

The skin strip was placed between the two clamps of the tensiometer and the clamps were secured to avoid any slippage of the sample. The Subcutaneous tissue was scarified near both c lamps. T hat approach w as a pplied as the large tensile strength of subcutaneous tissue did not allow it to be defined exactly what the breaking point of the sample was (Figure1a, b).

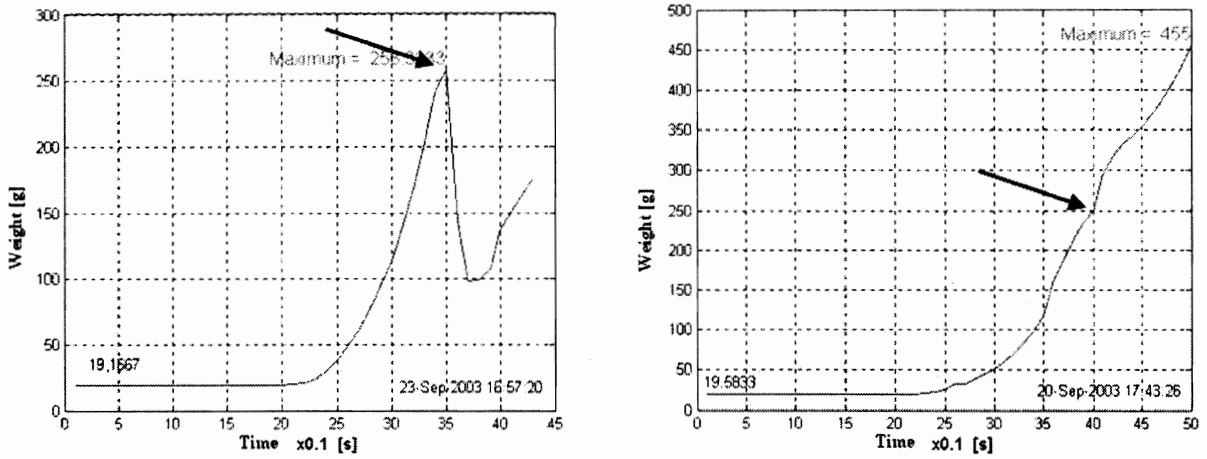

Figure 1 Tensile strength after 144 hours of wound healing. A-measurement with sliced subcutaneous tissue B-measurement without sliced subcutaneous tissue.

Pulling was performed vertically and perpendicularly to the original direction of the incision. Maximal breaking strengths were registered for each sample.

On days 1 to 7 after the operation, 3 animals from every group were killed and skin wounds were removed from the body and histopathologically evaluated. The specimens were processed classically for light microscopy (fixation, dehydration, embedding, cutting, staining with hematoxylin-eosin, van Gieson and Mallory´s phosphotungstic hematoxylin).

APPARATUS

The main construction unit of the tensile strength tester is the stand with the moving arm, which transfers force from the sample to connected Honeywell's piezoelectric sensor FSG15N1A, working with a frequency of 10 Hz.

We used as the sensor-computer interface an intelligent module ADAM 4011, developed by Advantech Company.

The fixation mechanism fulfils the following parameters:

- simplicity of manipulation.
- elimination of shear and slide forces in the place of fixation.
- minimal mass.

To a chieve a vertical tensile force, we used a servomechanism with a power supply ±3V and t he range of o utput f orce from 0 to 30N, compatible with the range of the piezoelectric sensor and covering continual tensile force increased to the breaking point of the sample (Figure 2).

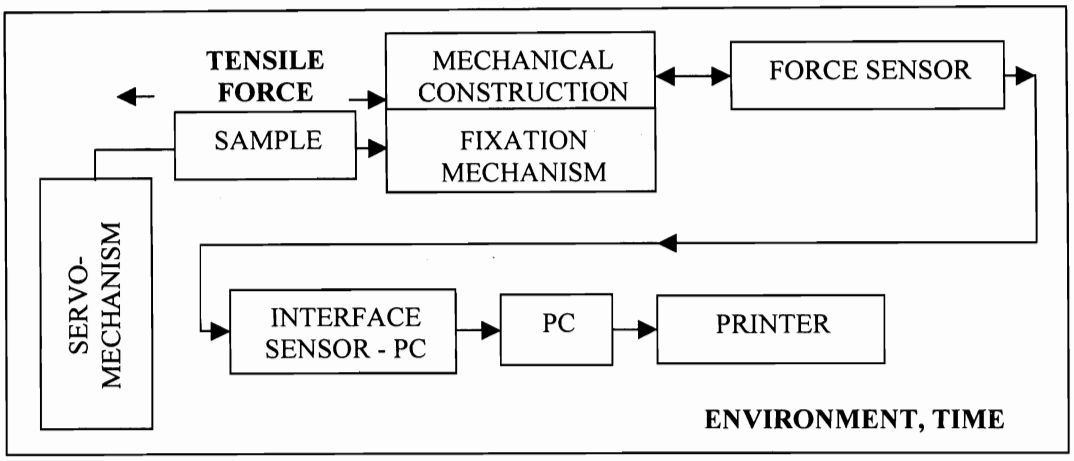

Figure 2 Measuring network of the tensile strength tester system.

DATA PROCESSING AND ANALYSING

Data transmission to the computer was realized through the serial port. Software solution of data processing, recording and analysing has been realized by means of Matlab software.

The data were recorded and processed into a beforehand-defined file with the possibility of graphical visualisation.

STATISTICAL EVALUATION

Data were statistically evaluated by program Statistica Cz 6.1 (StatSoft USA, Inc.) to determine the significant differences at the $p<0.001$.

The data were divided into three groups (0-24 hours, 48-120 hours and 144-168 hours) and compared to each other by the Kruskal-Wallis test.

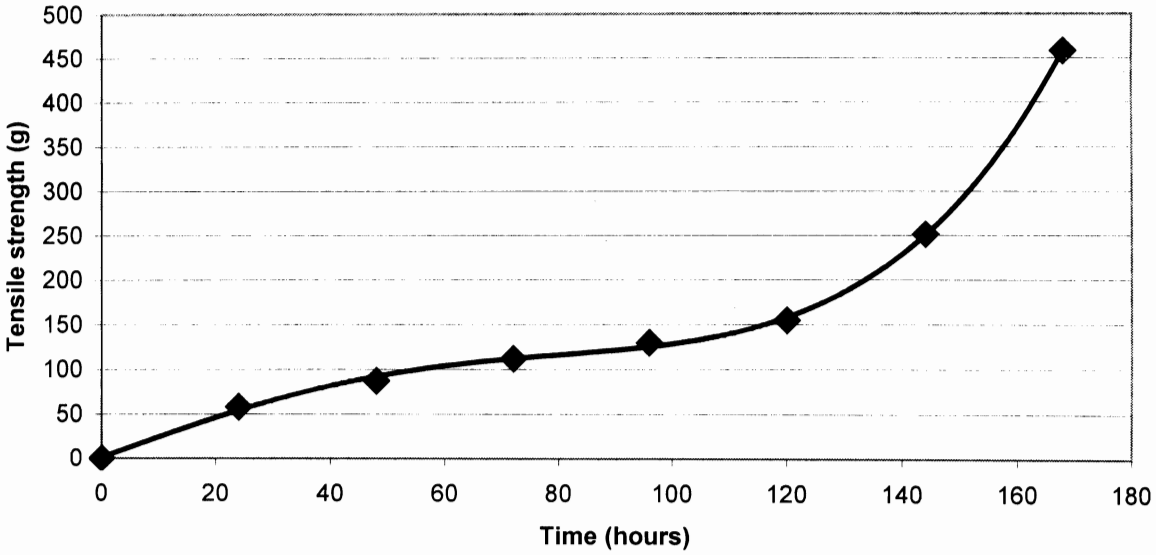

Figure 3 Tensile strength of wound versus time of healing. The values of tensile strength were calculated as the average value of all samples on the day.

Then values deviations were evaluated after 48-120 hours (increased between the 2nd and 5th day) and after 120-144 hours (increase during the 6th day) by application of the unpaired Student t-test.

RESULTS

We evaluated the increase of the tensile strength of skin wounds in rats during seven days after wounding (Figure 3).

During the inflammatory and the beginning of the proliferative phase (days 1 to 5) we observed the largest increase of the tensile strength of wound within 24 hours after wounding (0 to 58. 27g, p<0, 0001) (Figure 6). Histomorphological study has explained this increase in the creation of a fibrin network (Figure 4).

In the time period between 48 and 120 hours, only minimal increase was visible on the ascendant tensile strength curve. Among any of the evaluated groups (between 48 and 72; 72 and 96; 96 and 120 hours) no statistically significant difference was found (p=0, 2843; p=0, 1856; p=0, 2146). Therefore, we denoted the mentioned period as "plató phase". Without immunohistochemical techniques it is not possible to clearly explain histomorphological changes of wound healing (remodelling of ECM and granulation tissue).

The tensile strength increase during the 2nd – 5th day was compared to the tensile strength increase during the 6th day. Using the unpaired student t-test, a statistically significant difference was found (67.85g – from 2nd to 5th, 96.78 – during 6th day, p<0, 0001), which signified the end of a "plató phase" (Table 1).

Table 1 Tensile strength of skin wound.

Time	Tensile Strength (g)	Minimal Tensile Strength (g)	Maximal Tensile Strength (g)	Standard Deviation	Standard Error
24 hours	58,27	45,30	68,96	8,25	2,38
48 hours	87,15	72,92	99,79	10,87	3,14
72 hours	112,03	98,13	119,37	7,05	2,03
96 hours	129,86	120,63	137,71	5,15	1,49
120 hours	154,73	143,54	170,83	7,57	2,18
144 hours	251,51	218,75	263,96	13,70	3,96
168 hours	458,15	392,29	511,46	35,35	10,21

The first morphological manifestations of the maturation phase of wound healing in rats were observable after 144 hours of wound healing by cross linking the incisions with collagen I fibers (Figure 5).

Therefore the ending of the "plató phase" and the increase of tensile strength between 120 and 168 hours (from 154.73g at 120 hours to 251.51g at 144 hours, p<0, 0001, from 251.51 at 144 hours to 458.15g at 168 hours, p<0, 0001) were related to the processes of the maturation phase (Table 1).

According to obtained data the skin wound healing in rats from the point of view of tensile strength can be divided into three basic intervals: I. interval (0-24 hours) – initial rapid increase of the tensile strength – wound sticking by fibrin, II. interval (48-120 hours) "plató phase", ECM re modelation, III. interval (144-168 hours) – next rapid increase of tensile strength- maturative healing phase formation (Table 2).

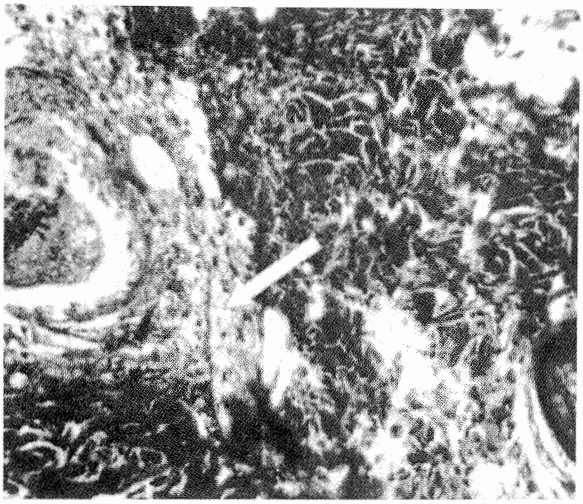

Figure 4 Wound after 24 hours - fibrins deposits. (Stained with Mallory´s phosphotungstic hematoxylin)

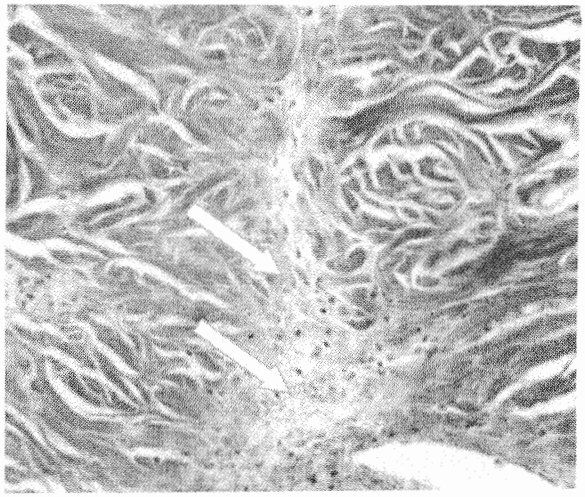

Figure 5 Wound after 6 days - collagen fibres. (Stained with van Gieson)

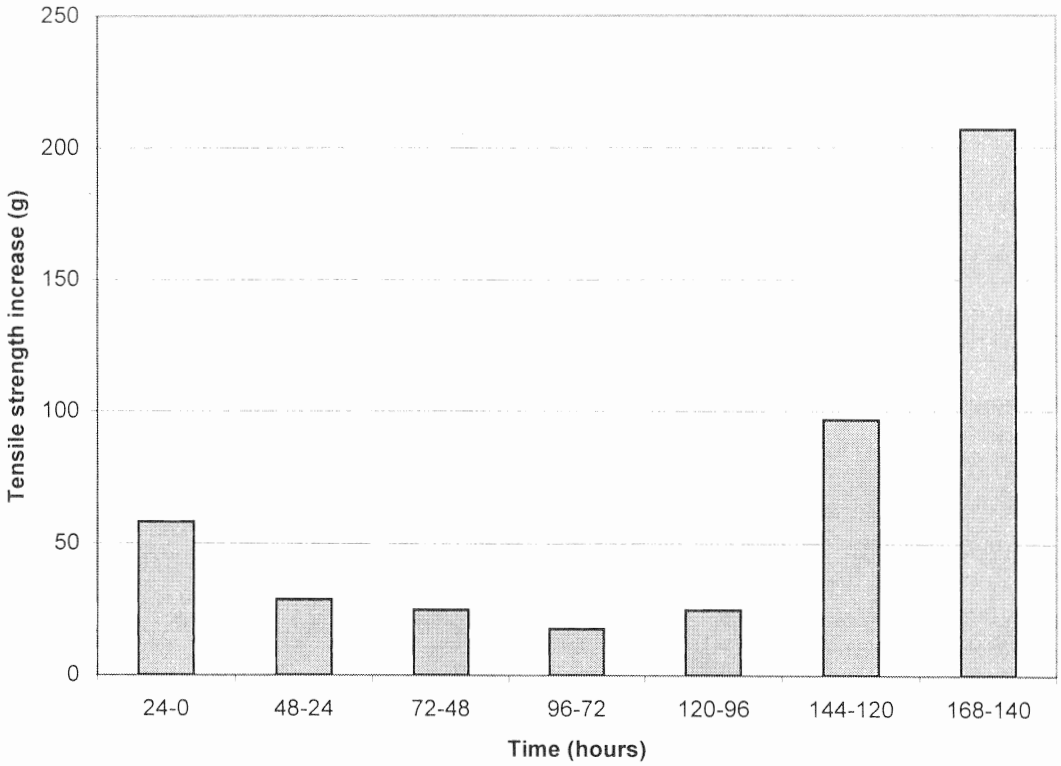

Figure 6 Change in tensile strength on particular days. Values were calculated as a difference of average values of the tensile strength on two consecutive days.

Table 2 Data of selected time periods. Values were obtained by the summary of all measured data.

Interval	Time period	Average of Tensile Strength (g)	Standard Deviation	Standard Error
1	0 -24 hod.	58,27	8,25	2,382
2	48 120 hod.	120,94	26,12	3,771
3	144 – 168 hod.	354,82	108,75	22,198

DISCUSSION

This study presents a unique model of tensile strength properties of the skin wounds. Most of the studies published on that topic deal with mechanical properties of skin wounds only after 4, 7 and more days from wounding. The time dependence of tensile strength in those studies was constructed simply by linking zero with the values of tensile strength measured on days 4, 7, 8 and was therefore linear within the first days after wounding (Jorgensen, *et al.* 1995; Lu, *et al.* 2000; Oxlund, *et al.* 1996; and Quirinia, *et al.* 1992). Later on the dependence had a parabolic pattern (Achauer, *et al.* 2000; and Larrabee, 1995). Besides it should be mentioned for that kind of measurement it was not necessary to construct a high sensitive tensile strength tester.

According to our results the dependence of tensile strength on time is not linear within the first days after wounding. The tensile strength of skin wound increases particularly during 24 hours. We interpreted this increase aetiologically mainly by fibrinous sticking of the wound borders, also confirmed by morphological studies (Connally, *et al.* 1997; and Vidinsky, *et al.* 2004).

Concerning the mechanical properties, healing has a specific course between the first and fifth day, denoted as the "plató phase", with a minimal increase of the tensile strength. The mechanical properties of the wound correlate with the results of the morphological studies.

The crucial effect on the tensile strength of the wound had the dynamic, self-remodelling, macromolecular complex of the extracellular matrix synthesized especially by fibroblasts (Kadler, 1995; Kumar, *et al.* 2003; and Menetrey, *et al.* 2000). The presence of fibroblasts in healing tissue was proved morphologically after 24 hours (Vidinsky, *et al.* 2004). Therefore we suppose the initial increase of the tensile strength of the wound is related to the process of the inflammatory phase running during the first and second day and to the function of fibroblasts.

Continuance of the "plató phase" correlated with the proliferative phase of wound healing that started on the second day and persisted all seven days.

The presence of collagen in wound tissue was immunohistochemically confirmed after 24 hours (Oxlund, *et al.* 1996). However, according to the published data, the synthesis of collagen prevails its degradation just after day five, in the period when the tensile strength of the wound has an increasing pattern (Achauer, *et al.* 2000; and Larrabee, 1995). According to StadlerStadler, *et al.* (2001), the increase in the tensile strength of the healing wounds is only 3% of non-wounded skin at 1 week after wounding.

After seven days our results were consistent with other published studies, where the trend curve of wound tensile strength is similar (Burkitt, *et al.* 1990; Greenwald, *et al.* 1993; and Savunen, *et al.* 1992). Comparable results prove the quality of our testing apparatus and credibility and accuracy of our measurements.

CONCLUSION

We evaluated the mechanical properties of skin wounds primary healing in rats. We obtained graphical time dependence of tensile strength within seven days after wounding, in 24 hour intervals. We confirmed the correlation between tensile strength of the wound and histopathological changes.

We are planning to use the designed and examined measurement technology of the tensile strength of the wound for further experimental studies of the other physical factors effects (magnetic fields, low level laser radiation) on the wound healing.

REFERENCES

Achauer, B. M., Eriksson E., Guguron, B., Vander Kolk, C.,Coleman, Hohn J., and Russell, R.C. 2000. *Plastic Surgery : indications, operations and outcomes.* St. Louis, A Harcourt Health Sciences.

Andreassen, T. T. and Oxlund, H. 1 987. The influence of experimental diabetes and insuline treatments on the biochemical properties of rat skin incisional wounds. *Acta Chirurgica Scandinavia.* **408** : 405-413.

Burkitt, H. G., Quick, G. R. G. and Gatt, D. 1990. *Essential Surger : problems, diagnosis and management.* Edinbourg; New York, Churchill Livingstone.

Connolly, A. J., Suh, D., Hunt, T. H., et al 1997. Mice lacking the thrombin recoptor, PAR1, have normal skin wound healing. *Am. J. Path.* **1199** :1199-1208.

Greenwald, D. P., Shumway, S., Zachary, L. S., et al. 1993. Endogenous versus toxin-induced diabetes in rats – a mechanical comparison of 2 skin wound-healing models. *Plastic & Reconstructiva Surgery.* **1090 :** 1087-1093.

Hudák, R., Tóth T., Živčák, J., et al. 2003. Tester Constructions for examination of the biomechanical properties of skin sutures. *Měřící a řídicí technika v biomedicíně.* **189** : 188-192.

Jorgensen, P. H., Bang, C., Andreassen, T. T., et al. 1995. Dose-response study of the effect of growth-hormone on mechanical-properties of skin-graft wounds. *J.Surg.Res.* **295** : 295-301.

Kadler, K. 1995. Extracellular matrix .1. fibril-forming collagens. *Protein profile.* **496** : 491- 619.

Kumar, V., Cotran, R. Z. and Robbins, S. L. 2003. *Basic Pathology.* 7th ed. Philadelphia, Saunders.

Larrabee, W. F. 1995. *Principles of facial reconstruction.* Philadelphia.

Lu, W. W., Ip, W. Y., Jing, W. M., et al. 2000. Biomechanical properties of thin skin flap after basic fibroblast growth factor (bFGF) administration. *Brit. J. . Plast. Surg..* **225** : 225-229.

Menetrey, J., Kasemkijwattana, C., Day, C. S., et al. 2000. Growth factors improve muscle healing in vivo. *J. Bone Joint Surg.* **131** : 131-145.

Moelleken, B. R. W. and Mathes, S. J. 1985. *Plastik & Reconstructive Surgery.* Beverly Hills, Yale University School of Medicine.

Oxlund, H., Christensen, H., SeyrHansen, M., et al. 1996. Collagen deposition and mechanical strength of colon anastomoses and skin incisional wounds of rats. *J .Surg. Res.* **25 :** 25-30.

Quirinia, A., Viidick, A.1992. Ischemia in wound-healing .2. design of a flap model biomechanical properties. Scandinavian. *J. Plast . Reconstructive Surg. Hand Surg.* **133** :133-139.

Rasik, A. M., Shukla, A., Patnaik, G. K., et al. 1996. Wound healing activity of latex of euphorbia neriifolia linn. *Indian J. Pharm.* **107** : 107-109.

Rubin, E. and Faber, J. L. 1994. *Pathology.* London, J. B. Lippincot Co.

Savunen, T. and Viljanto, J. 1992. Prediction of wound tensile-strength – an experimental – study. *Br. J. Surg.* **402** : 401-403.

Stadler, I., Lanzafame, R. J., Evans, R., et al. 2001. 830-nm Irradiation Increases the Wound Tensile Strength in a Diabetic Murine Model. *Laser Surg. Med.* **220** : 220-226.

Syk, I., Agren, M. S., Adawi, D., et al. 2001. Inhibition of matrix metalloproteinases enhances breaking strength of colonic anastomoses in an experimental model. *Br. J. .Surg.* **228** : 228-234.

Vidinský, B., Gál, P., Lakyová, L., et al. 2004. Histomorphological Study of the Rat Skin Wound Healing, Lojda Symposium on Progress in Basic. *Applied Diagnost. Histochenistry,* Košice, Slovakia.

Whelan, H. T., Buchmann, E. V., Dhokalia, A., et al. 2003. Effect of NASA Light-Emitting Diode Irradiation on Molecular Changes for Wound Healing in Diabetic Mice. *J.Clinic. Laser Med. Surg.* **67** : 67-74.

(Revised version accepted September 11, 2004)