IMPLEMENTATION OF THE EXCISIONAL SKIN WOUND HEALING MODEL IN MINIPIG

Kučera J.[1], Daněk P.[2], Podhorná I.[1], Seifert J.[2], Velebný V.[1], Klein P.[1]*

[1] *Contipro Group, Dolní Dobrouč 401, 561 02*
[2] *Výzkumný ústav živočišné výroby, v.v.i., Oddělení chovu prasat, Kostelec n. Orlicí*

Abstract

For the physiological and structural similarities between pig and human skin, the pigs represent irreplaceable *in vivo* models of wound healing. Nevertheless, success of the wound healing experiment is also subject to several technical details such as anaesthesia, surgical technique, appropriate housing and animal care during experiments, or sufficient fixation of wound dressing which could minimize its damage or even its undesirable removal followed by wound contusion and contamination. Present contribution describes the porcine model of full-thickness excisional wounds which allows testing of wound dressings under standardized conditions.

Porcine and human skin share several similarities, such as epidermal and dermal-epidermal thickness ratios, mosaic hair growth or hair and blood vessel distribution (Nanney et al., 2008). Both man and pig show well-developed rete-ridges and dermal papillary bodies, as well as abundant subdermal adipose tissues. Moreover, porcine dermal collagen is biochemically similar to human dermal collagen. Functionally, pig and man are similar in terms of epidermal turnover time, type of keratinous proteins, and lipid composition of the *stratum corneum*. Also wounds in pig and human heal through physiologically similar processes. Whereas most small animals have a *panniculus carnosus* and rely on wound contraction for wound closure, man and pig close partial-thickness wounds largely though reepithelisation. These similarities between both species make pig an excellent model for human wound healing (Sullivan et al, 2001).

Unfortunately, many wound healing studies in pigs or minipigs give any or very little information describing technical details of the experimental protocol, e.g. how to fix wound dressings, how to safely house the animals or how to manipulate with them without damaging experimental wounds and the wound dressings. The aim of this paper is therefore to describe in detail some proven procedures necessary for wound healing experiment in porcine model.

Material and Methods

Animals, housing, diet

The experiments were carried out on 10 castrated male minipigs of the minnesotta type breed. Age and weight of the animals varied between 6 and 13 months and from 22 to 64 kg, respectively.

During experiments animals were penned individualy, in the mobile pens made of polypropylene with washable walls (Figure 1). The floor of the pens was covered with 5 mm thick rubber sheeting. The way the pens were constructed reduced the risk of the animals damaging their wound dressings, this was mainly due to an absence of any sharp protuberant parts.

To avoid undesirable obesity, animals were fed with complete pelleted food for minipigs with reduced content of metabolisable energy and enhanced content of crude fibre (Daněk, 2009). Food was administered twice a day, water was administered *ad libitum*. For administration of both food and water stoneware feeders were used.

Anaesthesia

Induction of the experimental wounds is a very painful intervention and anaesthesia is therefore necessary. There are several options how to induce anaesthesia in pigs, nevertheless some techniques require access to vein or usage of ventilation apparatus for the inhalation anaesthesia. These procedures are time-consuming and therefore restrict the amount of animals that can be involved in the study.

In our conditions, we therefore optimized the surgical intervention which is performed completely within 45 to 60 minutes and allowed us to use only ketamine after previous application of a tranquilizer. This short-term anaesthesia also allowed us to create groups of 5 animals which were in the same phase of the experiment.

Two combinations of drugs were tested:

1) Azaperone i.m. and after 10 minutes Ketamine HCl i.m.

2) Acepromazine p.o. and after 15-20 minutes Ketamine HCl i.m.

Because wound induction and sampling is such a painful process, field blocking anaesthesia of each wound was induced with subcutaneous application of 1 ml of 2% lidocaine (Egis Pharmaceutical, Hungary).

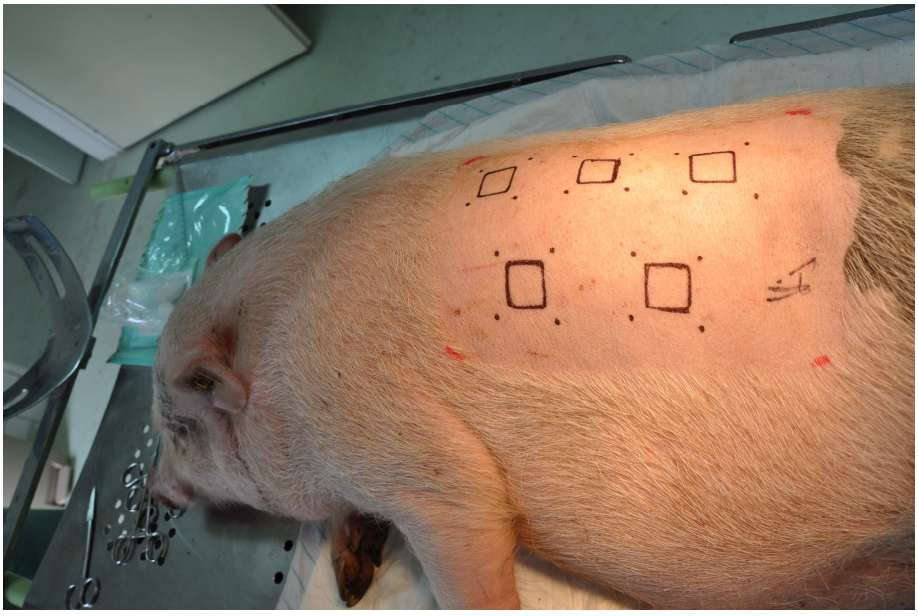
Figure 1. Mobile pen

Wounding and wound dressing

Before the creation of wounds, the animals were anaesthesised, cleaned down and their flanks were clipped and and the area to be surgically operated on was shaved. The surgical area was spread with a 10% iodopovidone solution (Alfadine, Bioveta, Czech Republic) or alternatively with a 1% aqueous octenidine solution.

Five full thickness wounds 25 x 25 mm excised to muscle fascia were placed on each flank of the animal(s). The lay-out of the wounds was the same as previously described (Van Dorp et al., 1998), i.e. 3 wounds were placed in the upper line and 2 in the lower line (Figure 2). Tissue was removed using a standard surgical lancet (size 22). Depth of the wounds and exposure of the fascia was checked visually. The distance between each wound was approximately 40 mm to minimize mutual effects of contraction and inflammation.

Figure 2. Location of the wounds



The experimental wound dressings (Contipro C, Czech Republic), were changed twice a week until healing up, they had the tea-bag structure; it consisted of a contact layer (polyamide, 40 g/m^2), two absorption layers (mixture of polyester and viscose, 140 g/m^2 each) and a covering layer (polyester, 30 g/m^2). The core of the bag was made up of absorption layers. The tested healing substance was spread in the form of thin film at the contact layer. The size of each bandage was 100 x 200 mm. Control wounds were covered with the same dressing with exclusion of the healing substance. Under our experimental conditions, each animal was treated with both control and experimental dressing, e.g. each wound had its own control at the same position, but located on the opposite side.

In order to completely overlay the wound area, 3 bandages were put side by side (Figure 3) and attached using polypropylene water-proof duct tape (Patex Power Tape, Henkel, Germany). The tape was then fixed with several sutures to the skin (Figures 4 and 5). Alternatively, the wound dressings were fixed only using the duct tape. To protect the wounds and wound dressings properly against secondary contamination, whole wounded area was finally covered using unwoven textile material with attached nanoparticles of silver (Inotex, Czech Republic). Before transport to the cage, trunk of the animals was clamped with elastic tube bandage and fixed with the duct tape (Figure 6).

Figure 3.Bandage application

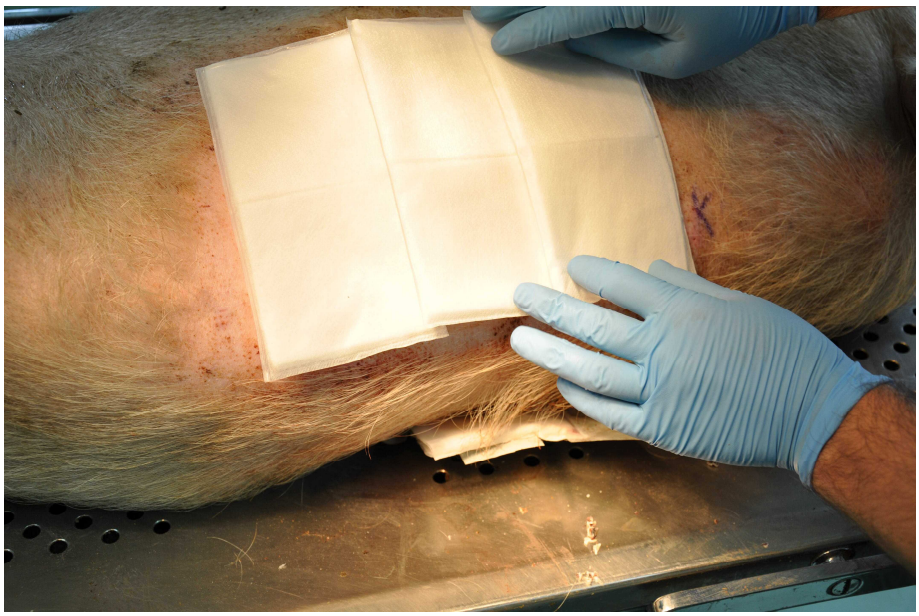


Figure 4. Bandage taping

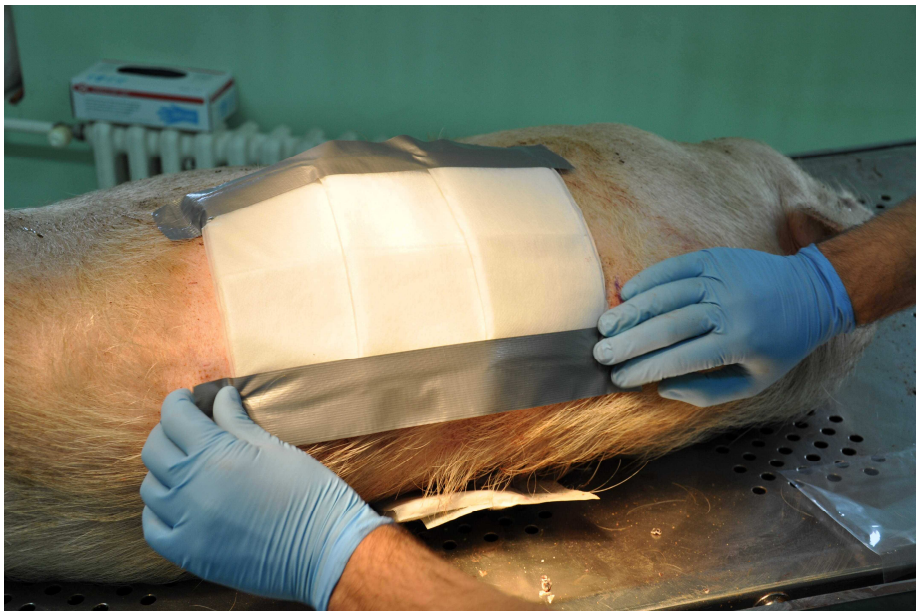


Figure 5. Bandage fixation

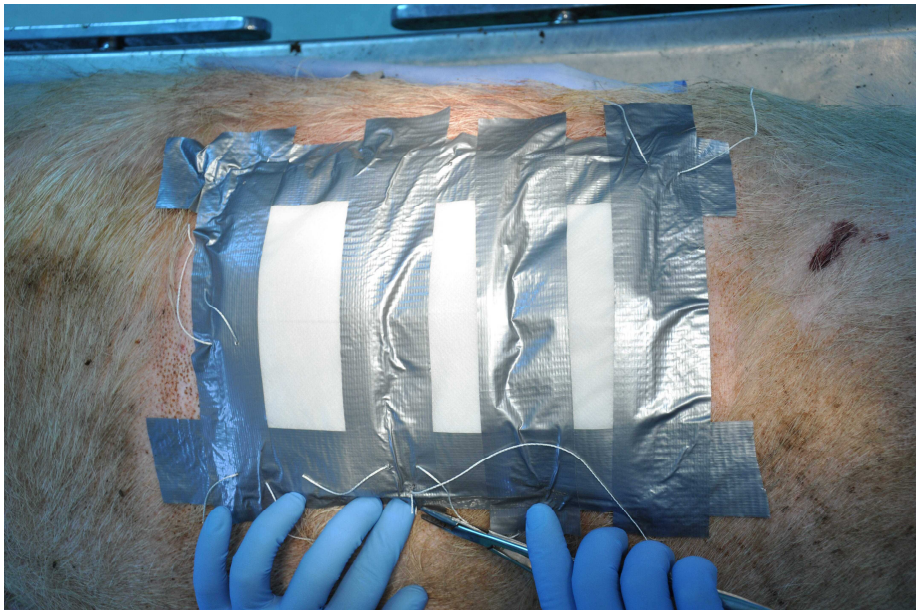
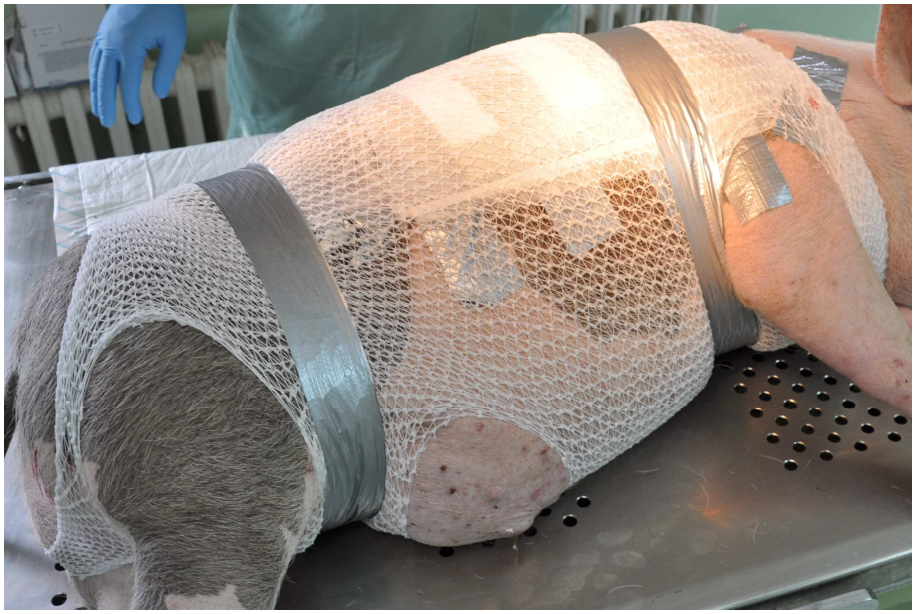


Figure 6. Final body covering



Monitoring the healing process

Wound documentation

Wounds were documented at time of induction and then at every re-dressing using a D90 digital camera (Nikon, Japan) equipped with an accessory SB-900 external flash (Nikon, Japan). Standardised capturing has been warranted using semi-manual mode with aperture priority set to lowest ISO (200) for lowest signal-noise ratio and highest obtainable *F*-number (in our case f/29) to achieve full depth of focus. Exposure time ie. shutter speed for these settings was 1/60 s. In order to achieve sharp images even when captured "wildly" (without using the viewfinder), autofocus function was enabled. White balance was set by the system automatically ("flash" function). Exposure compensation was used in range of ±1,7 EV in steps of 0,3 when needed to obtain accurately exposed pictures. A standard gauge was placed in each area captured to enable calibration of the images for subsequent measurements and analysis.

The wound area on each image was determined manually by a trained person blinded to treatment process using image analysis software NIS-Elements AR 3.1 (Laboratory imaging, Czech republic) and expressed as a per cent ratio of original wound size with standard deviation.

Wound sampling

On days 3, 7, 14, 21 and 35, samples of wound tissue for histological evaluation and qRT-PCR analysis has been recovered using a standard surgical lancet. For histology, middle-part strip of approximately 5 mm width including 5 mm of surrounding unwounded tissue in dorso-lateral direction was recovered and after a quick wash off in PBS to remove blood, immediately fixed in 4% paraformaldehyde-PBS. Care was taken to sample the whole healing granulation tissue down and exceeding the depth of the healing wound by 2-3 mm.

For qRT-PCR wound tissue of approximately 3x3x3 mm was sampled in the middle part adjacent to histology sample and immediately placed into RNAlater Solution (Albion, USA).

The sample location is shown in figure 7. Samples of the wounds were taken from lower hind position to the top front position during the run of the experiment (Figure 8). Due to the sampling-caused disruption of wound healing process, the sampled wound was after withdrawal excluded from further observation.

In order to evaluate wound microbiological colonisation, skims from 2 randomly chosen wounds on treated resp. control flank were made using sterile single use sample system ESwab (Copan, Italy) for subsequent cultivation. Swabbing was performed until the healing process was complete. Control skim samples were taken from the surrounding dressed unwounded skin.

Figure 7. Sample's location

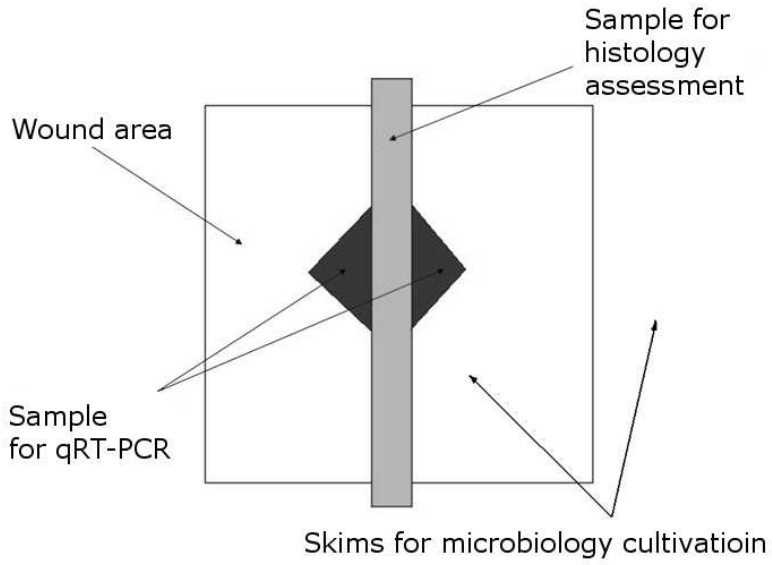
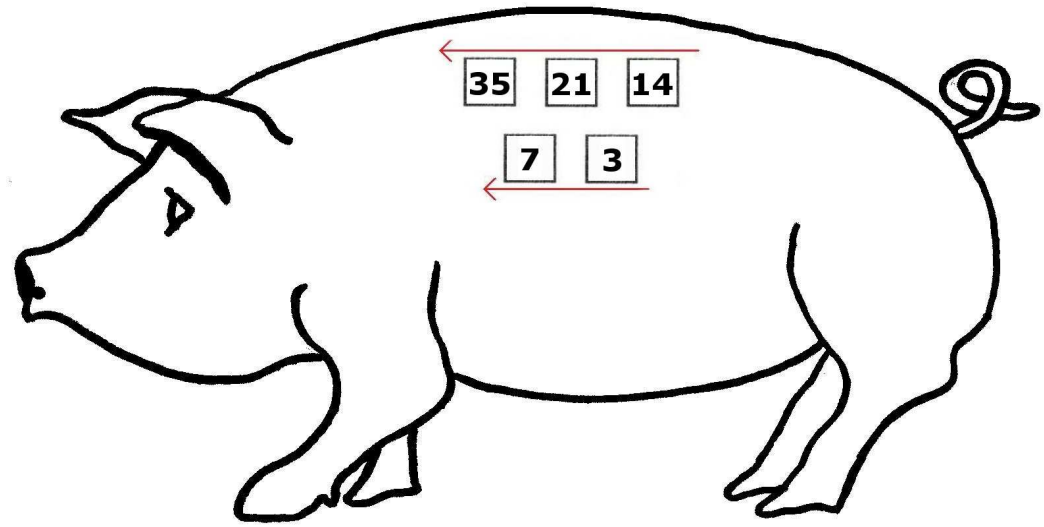


Figure 8. Sequence of wound withdrawal (the number means the day of sampling)



Results and Discussion

Anaesthesia

The documentation of wounds, taking samples from them and re-dressings required the animals to be anasthesised twice a week for the duration of the experiment. The total number of anaesthesias was therefore 11. The anaesthesia process had to be optimised with respect to this need.

The first tested combination of anaesthetics led to excess salivation in some animals and additional application of atropine was necessary. The dose of ketamine (Narkamon, Bioveta, Czech Republic or Narketan, Vétoquinol, France) necessary for induction of anaesthesia was about 23 $mg.kg^{-1}$. In contrast, the consumption of ketamine in the second combination was lower (15 $mg.kg^{-1}$) and patterns, as well as reversion from anaesthesia, appeared less complicated. Complications associated with refusing the orally administered acepromazine by animals were successfuly minimized using the gelous form (Sedalin gel, Vétoquinol, France). Although Linkenhoker et al. (2010) found this combination less suitable for increased heart rate and risk of vomiting, in our conditions the combination acepromazine / ketamine was found to be satisfactory and no apparent adverse effects of this anaesthesia were observed during the experiment.

Nevertheless, great differences in doses necessary for induction of anaesthesia have been observed among the animals, especially at the beginning of the experiment. In this case, doses of ketamine were increased gradually following the manufacturer's instructions. Also reversion from the first anaesthesia was longer in all the animals. To avoid complications caused by dehydration, hypoglycaemia and hypothermia, animals were supplied with 7-9 $ml.kg^{-1}$ of physiological solution with 3% glucose. The solution was applied subcutaneously as described in human geriatric practice (Šťastná et al., 2009). Moreover, up to the reversion, animals were warmed up by ultra-red dark radiators installed in their pens.

Wound dressing

During the wound healing study, the wound dressings have to be appropriately fixed to the body. Nevertheless, satisfying this obvious condition is not easy because the animals naturally tend to remove it.

Fixation using sutures, although very resilient, showed to be time-consuming and painful for the animals. As the duct tape exerts good adhesivity to dry and unshawed skin, we decided to fix the bandages only by taping. The shaved area has been reduced only in the vicinity of experimental wounds and the elastic tube bandages were doubled and additionally fixed with passing limbs through holes and using the duct tape (Figure 6). This way of dressing the wound area has been shown to be optimal.

The only disadvantage of this fixation technique is that after longer periods of time, strangulations on the legs may occur. In this case, it is necessary to protect these areas using roller or tube bandage made from soft material.

Wound documentation

The unhealed wounds represent a wet surface producing many reflections when illuminated in same axis as camera during documentation i.e. when the flash is attached directly at the top of camera. Such reflections appear as over-lightened areas in the image which make recognition of the underlying surface difficult. If the additional light is needed for lighting the wound area enough, it must be diffused as much as possible and placed out of axis regarding documentation device. Also, use of polarised light or LED-light would probably improve the results (Bae et al., 2010).

Further analysis that can be performed with wound healing tissue samples are tissue micro-array analysis in order to specify genes resp. mRNAs that are useful to trace and quantify with qRT-PCR. Another analysis depicting the healing process is a hydroxyproline assay which enables biochemical quality evaluation of newly formed collagen.

For further assessment of inflammatory phase, cytokines can be evaluated in saline lavage of a wound. In our pilot experiments we have used 1 ml of saline for irrigation. Care must be taken when collecting the lavage, in order not to damage the healing tissue. Alternatively to the lavage, a mild scratch off of the wound surface can be used.

Conclusion

Considering that every invasive intervention of a model organism is associated with an increase in stress levels our aim was to gather as much information as possible in order to have workable analytical material and data in order to produce the most relevant results.

During our research we reduced time costings and also the distress caused by the model induction. The following maintenance was also kept to a minimum as a result. We also successfully reduced the costs of the minipig anaesthesia, which represents a considerable entry in such *in vivo* experimentation.

References

Bae EJ, Seo SH, Kye YCh, Ahn HH: A quantitative assessment of the human skin surface using polarized light digital photography and its dermatologic signifikance. Skin Res Tech. 2010;16: 270–274

Daněk, P. Krmná směs pro miniaturní prasnice, 2009, Užitný vzor, U1 20133, Úřad průmyslového vlastnictví České Republiky

Linkenhoker JR, Burkholder TH, Linton CG, Walden A, Abusakran-Monday KA, Rosero AP, Foltz CJ: Effective and safe anesthesia for Yorkshire and Yucatan swine with and without cardiovascular injury and intervention. J Am Assoc Lab Anim Sci. 2010;49(3):344-51.

Nanney LB, Woodrell CD, Greives MR, Cardwell NL, Pollins AC, Bancroft TA, Chesser A, Michalak M, Rahman M, Siebert JW, Gold LI: Calreticulin enhances porcine wound repair by diverse biological effects. Am J Pathol. 2008;173(3):610-30.

Sullivan TP, Eaglstein WH, Davis SC, Mertz P: The pig as a model for human wound healing. Wound Repair Regen. 2001;9(2):66-76.

Šťastná V, Soukupová K, Kučerová H: Naše zkušenosti se subkutánní rehydratací. Čes.Ger.Rev. 2009;7(1):46-8.

Van Dorp AG, Verhoeven MC, Koerten HK, Van Der Nat-Van Der Meij TH, Van Blitterswijk CA, Ponec M: Dermal regeneration in full-thickness wounds in Yucatan miniature pigs using a biodegradable copolymer.Wound Repair Regen. 1998;6(6):556-68.

This work was supported by a grant from EU funds and a budget of the Ministry of Industry and Trade of the Czech Republic.

* corresponding author tel.: +420-465-519-542; klein@contipro.cz