*Int. J. Morphol.,*
*41(4)*:1053-1057, 2023.

# Thermal Injury Protocol to Study Healing, With and Without Infection, in Guinea Pig (*Cavia porcellus*) Model

## Protocolo de Lesión Térmica, para Estudio de Cicatrización, en Modelo Animal Cobayo (*Cavia porcellus*)

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**SALVO, J.; SCHENCKE, C.; PÁVEZ, M.; VEUTHEY, C. & DEL SOL, M.** Thermal injury protocol to study healing, with and without infection, in guinea pig (*Cavia porcellus*) model. *Int. J. Morphol., 41(4)*:1053-1057, 2023.

**SUMMARY:** Experimental healing studies in humans are complex and difficult to replicate *in vitro*. Hence, animal models are needed to study the different stages involved. The guinea pig (*Cavia porcellus*) is a model close to human physiology, including the lack of vitamin C synthesis, a precursor of collagen fibers for healing. The thermal injury in this animal makes it possible to study all the stages of healing, taking few days to show tissue repair in the processes with and without localized infection. The aim of this work was to systematize an experimental guinea pig (*Cavia porcellus*) animal model protocol for studies on healing with and without localized infection.

**KEY WORDS:** Animal model; *Cavia porcellus;* Wound healing.

## INTRODUCTION

Studies on the healing of skin wounds are relevant when facing the biopsychosocial sequelae that cause the major congenital, pathological, or traumatic defects that wounds in humans manifest. The healing process, widely described in the literature, begins early after an injury. Its different stages overlap in its evolution: hemostasis, inflammation, granulation, and remodeling, which involve a series of molecular, biochemical, and cellular regulatory processes, including cytokines, extracellular matrix, metalloproteinases, and growth factors, among others (Monavarian *et al*., 2019; Zommer & Trentin, 2018).

Experimental or quasi-experimental healing studies in humans are complex and difficult to replicate *in vitro* because many related factors affect the variation of the response, for example, comorbidities such as diabetes mellitus or immunological diseases, aging, and nutrition, among others, which can bias the results or require a large sample to be able to standardize an objective result (Wu & Landén, 2020; Grada *et al*., 2019; Dunn *et al*., 2013). In addition, the ethical aspects involved in wound healing studies on humans make it necessary to resort to other approximate techniques, like using animals in experimental models. These have been used for studies for decades; however, bioethical regulations with respect to their use have become necessary, all related to establishing codes of conduct for the management of laboratory animals. A laboratory animal is any living non-human being, vertebrate or invertebrate, used for experimentation and other scientific purposes (Guillen, 2012).

To determine which experimental animal model comes closest to the morphological characteristics of human skin and healing, the use of some animals bred under laboratory conditions has been allowed, which makes them suitable for research studies. This involves considering animal welfare, which according to some of its precursors, would be the result of the animal-environment interaction,

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Received: 2023-03-02 Accepted: 2023-04-28

but not the environment referring solely to the conditions of the environment, but rather to an environment that includes social conditions (Morales, 2015; Concepción *et al*., 2007). Accordingly, it is fitting to consider what is called the Five Freedoms according to Gimpel (2009): that the animals are not hungry or thirsty, they do not suffer physical distress or pain, do not suffer wounded or diseases, can adjust to their essential normal behavior, and do not suffer fear or anxiety. In addition, it is demanded that the proposal by Russell and Burch in 1959 be declared a universal premise, with the three Rs: Replacement, Reduction, and Refinement, which will minimize animal damage and improve preclinical, experimental practices (Molina *et al*., 2017).

The breeding of animals, with or without genetic variation, under ethical laboratory conditions has made it possible to standardize and control the study variables for the testing of different elements or topicals for wounds, applying group refinement and reduction (Cardozo de Martínez & Mrad de Osorio, 2008). The animals described for experimental models to study structural and functional biology to understand exploratory, mechanistic, or predictive mechanisms include prosimians, cats, dogs, reptiles, pigs, goats, chickens, amphibians, primates, and rodents (rabbits, rats, mice, and guinea pigs), all with some genotypic and/or phenotypic characteristics compatible with human processes. Among these models, the mouse is the best characterized and monitored animal in its different lines (Romero *et al*., 2016).

The guinea pig (*Cavia porcellus*) has the advantage of little variability in the thickness of its skin, resembling human histological characteristics. It is one of the few animals, along with primates and humans, that does not synthesize vitamin C, an important component in stimulating collagen fiber formation in healing. Low levels of this vitamin produce diseases that can lead to the animal's death (Grada *et al*., 2019, Moreta, 2018). The skin of this animal is complex in its embryonic development and stratification, in addition to other characteristics like fetal circulation, cardiac and hepatic anatomy, and others, which would make it advisable for experimental studies on the skin and other areas such as immunology, circulatory physiology, pharmacology, endocrinology. However, their studies are still insufficient compared to other rodents, such as rats and mice (Viana *et al*., 2019). Other advantages of using this herbivore are its timid and docile nature, size and weight suitable for handling, and low maintenance costs.

Commonly called the guinea pig, it belongs to the order: Rodienta, family: Caviidae, genus: Cavia, species: porcellus. The life span of the guinea pig is between 2 and 8 years, reaching adulthood at 4-5 months. Its gestation period varies between 26 and 60 days. It has diurnal and nocturnal habits, likes to live in groups, and shelters in a burrow as a defense mechanism. The adult weighs between 500 and 1500 g, with a size of 20-30 cm; its body temperature varies between 37 and 40 °C, with a heart rate of 94-127 beats per minute. It consumes between 50 and 200 cc of water per day, its nutritional requirement is 60-70 g/kg of body weight per day, and the required environmental temperature is 15-22 °C in vivaria (Grada *et al*., 2019; Moreta, 2018).

Work with animal models must also guarantee monitoring of the behavior regarding indicators that reveal stress and pain through physical characteristics and responses to stimuli. The protocol proposed in this work is classified as moderate severity, since it includes the possibility of causing pain related to an injury, which must be detected and managed with palliative measures. In this sense, the modified Morton and Griffiths rodent monitoring protocol (1985) is frequently used in Chile, which monitors indications of weight loss, appearance, spontaneous behavior, behavior in response to handling, and vital signs. With respect to the type of injury, alternatives have been described, such as excisional would, incisional wound, and burn, with this last one being beneficial for the study of all the healing stages, permitting different depths of involvement of the injured tissue (Masson-Meyers *et al*., 2020; Rhea & Dunnwald, 2020). Thermal injury through dry heat by contact with hot metal at 200 °C provides full-thickness burns to analyze vascularization, granulation, inflammatory cells, and others (Caliari-Oliveira *et al*., 2016).

The aim of this work was to systematize the experimental animal model protocol in guinea pig (*Cavia porcellus*) for studies of healing with and without localized infection, used in the Center of Excellence in Morphological and Surgical Studies (CEMyQ) at the Universidad de La Frontera, Temuco, Chile.

## PROTOCOL

This *in vivo* protocol in guinea pig (*Cavia porcellus*), established for experimental studies in wound healing, consists of 8 stages, which also include the need for localized infection:

### 1. Preparation of the animal model by group

Use 5 guinea pigs (*Cavia porcellus*), reproduced and bred in vivaria, plus 10 %, for each study variable, for a statistically significant number (Mandarim-de-Lacerda & del Sol, 2017). Select specimens of adult age and weight, and

separate them by sex in cages that allow the necessary movement so as not to cause stress and to avoid reproduction. Provide pellet feeding with vitamin C supplements, vegetables, and fruits according to the veterinarian's indications, and water ad libitum. Regulate the room temperature between 15 and 22 °C with natural or artificial 12-12 h day-night light cycles and an environmental humidity of 55-60 %. A 14-day adaptation period is suggested before the intervention. Clean daily and assign an authenticationcode to each animal.

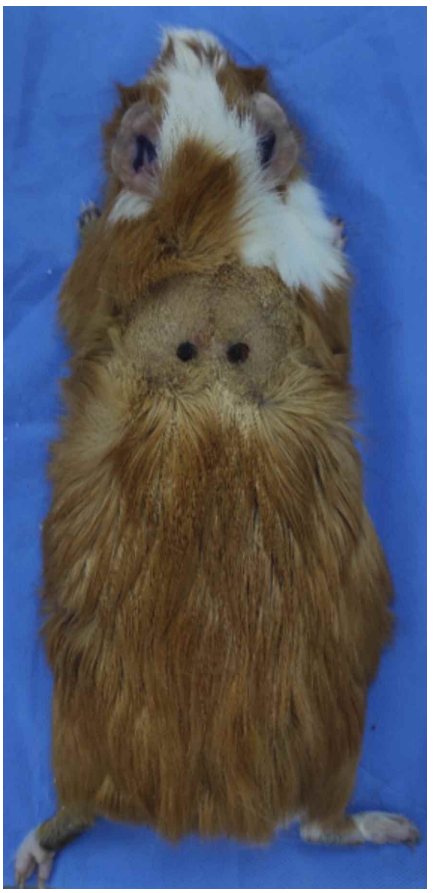

Fig. 1. Bilateral thermal injury, deep thickness (B burn).

## 2. General anesthesia

According to the weight of the animal, apply according to veterinary indication ketamine-xylazine and atropine, a dose of 40 mg/kg of weight, 5 mg/kg of weight, and 0.05 mg/kg of weight, respectively, intraperitoneally, with an induction time of 15 minutes, or gas anesthesia (isoflurane, sevoflurane) depending on the availability of gas anesthesia. While each animal awaits anesthesia, keep it in the ventral decubitus position and monitor the loss of reflexes. In any case, an adequate selection of anesthesia and analgesia protocols must be made in advance.

## 3. Injury

Once anesthetized, perform the trichotomy with clippers in the area to injure, leaving a wide margin in the area of the lesion, wash with antiseptic soap (povidone soap 10 % or chlorhexidine soap 2 %), perform antisepsis of the skin with the same component as the wash, but with antiseptic dye. With the animal anesthetized and in ventral decubitus, locate and mark the medial dorsal area with a non-toxic pencil, paravertebral to the level of the upper limbs, so there is no possibility of scratching, then apply dry heat (200 °C with thermostat) with a round metallic object (marking type), with a contact-pressure time with the animal's skin of 4-6 seconds. This will achieve a burn with deep tissue damage, allowing it to pass through all the stages of healing during wound treatment (Fig. 1). The injuries will have a diameter of 0.8 -1 cm (Fig. 2), which does not represent damage with systemic involvement. The opposite side can be used for a control injury.

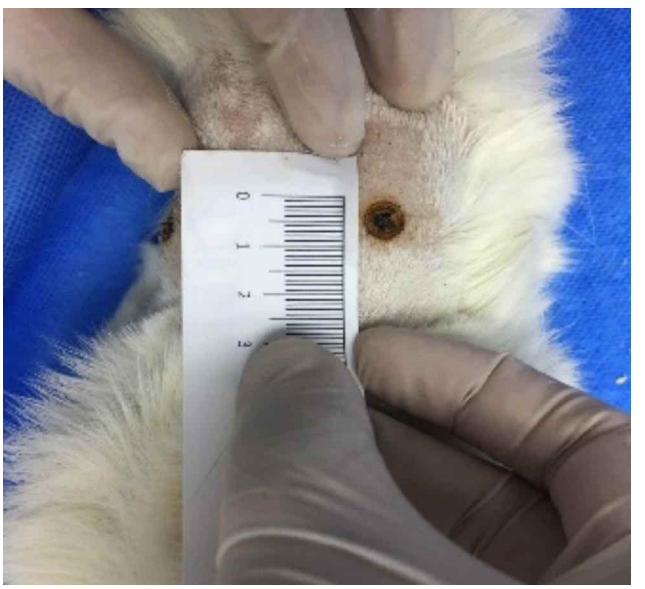

Fig. 2. Lesion diameter 1 cm.

## 4. Recovery from anesthesia

Before passing to recovery, clean the injured area to eliminate antiseptic residue and apply the sterile cover. This phase of anesthetic recovery is monitored by the veterinarian and/or attending technician (anesthetic monitoring must be done continuously through direct assessment), provide a thermoregulated resting unit at 26-28 °C ambient temperature, covers and monitoring of cardiac activity and mobility until the total recovery of the animal, confirmed by its autonomy. The anesthetic recovery period is approximately 1.5 h.

## 5. Treatments

The treatments allow for macroscopic control. Overall, it is recommended that they be done under basic aspects of the technique that would be done on humans, i.e., mechanical drag with saline solution in the devitalized tissue period (inflammatory stage of healing), and with syringe therapy with a number 21 needle at a distance of 10 cm in the granulation period (granulation or proliferative stage of healing), use debridement by escharotomy, apply the study material as the primary dressing, passive gauze as secondary dressing and elastic cotton bandage as fixation, plus adhesive cloth (Domínguez-Saavedra & Hernández, 2021). The biopsies and frequency of treatments will depend on the objective of the study and elements to be tested; however, considering the care for the animal's behavior, a daily follow-up of the injury is suggested, ideally with a treatment record for each animal that includes the photographic monitoring and measurement of the injury. At this stage, software that can quantify the reduction of the area and perimeter of the injury can be chosen.

## 6. Monitoring of the animal's behavior

This protocol involves an event of moderate severity because it has a surgical procedure and treatments; therefore, they must be monitored daily to detect stress and pain or other behavior that ethically indicates withdrawal of the animal from the study or medical euthanasia when there is a score greater than or equal to 10 points on the Morton and Griffiths animal supervision scale (1985). This protocol does not involve euthanasia; the injuries are small in proportion to the body surface. According to the daily animal behavior assessment, evaluate the need for analgesia, antihistamines, or other drugs.

## 7. Induction of local infection with *Pseudomona aeruginosa*

To induce local infection of the injury, the animal must be isolated for 7 days before the skin injury. Physical review must be done daily to ensure there are no possible sources of sepsis. After the injury, proceed with rest for 24 h, with a sterile cover, to establish the inflammatory process. Then, inoculate with a micropipette (volume 2 - 20 µL), over the escharotomy lesion, with an inoculate of 5 µL, equivalent to $5\times10^5$ CFU of Pseudomona aeruginosa, calculated in exponential growth for 30 min before the inoculate, maintained in suspension in BHI broth (brain heart infusion) at 37 °C. Then, 48 hours after the inoculation, confirm local signs of infection: subtle (Fig. 3) or classic (Serra *et al*., 2015; Valle *et al*., 2005). The inoculated bacteria can come from strains from clinical samples or ATCC. It is advisable to take a culture of the injury.

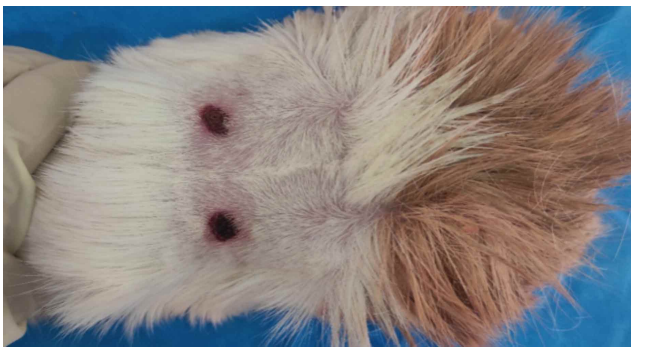

Fig. 3. Subtle signs of infection 24 hours post -bacterial inoculum.

## 8. Biopsies

For biopsies, proceed with local anesthesia only, lidocaine 2 %, 4 mg/kg body weight. Take the total thickness of the skin up to the muscular fascia, use a biopsy punch larger than 10 mm, wash the sample with saline solution, deposit it in phosphate-buffered saline (PBS), and then in buffered formalin (1.27 mol/L of phosphate buffered formalin 0.1M pH 7.2) for fixation and processing. After the biopsy, leave the injured area uncovered for primary closure with a suture that is removed on the seventh day.

## CONCLUSIONS

This *in vivo* guinea pig model offers a realistic representation of the healing process; however, it is necessary to standardize various aspects that can affect the study results, this being themain weakness. The choice of this animal, is an excellent alternative when handling them; its maintenance is low cost, and the injuries caused by deep burn make possible the analysis of several stages of healing in a short time (average 12-15 days) due to the small area of injury, which includes the infection process.

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**RESUMEN:** Los estudios experimentales de cicatrización en humanos son complejos, difícilmente replicables *in vitro*, por lo que se hace necesarias modelos animales que permitan el estudio de las distintas etapas que ella implica. El cobayo (*Cavia porcellus*) resulta ser un modelo cercano a la fisiología humana, incluyendo la falta síntesis de vitamina C precursora de fibras colágenas para la cicatrización. La lesión térmica en este animal, permite estudiar todas las etapas de la cicatrización, mostrando

pocos días en la reparación tisular, tanto en proceso con y sin infección localizada. El objetivo de este trabajo fue sistematizar un protocolo de modelo animal experimental en cobayo (*Cavia porcellus*) para estudios de cicatrización con y sin infección localizada.

**PALABRAS CLAVE: Modelo animal; *Cavia porcellus;* Cicatrización de heridas**

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