DEVELOPMENT AND VALIDATION OF A REPRODUCIBLE EXPERIMENTAL MODEL OF DEEP THIRD-DEGREE THERMAL SKIN BURNS IN RATS

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Abstract. Thermal injury represents one of the most prevalent and methodologically significant experimental models in pathology, widely employed for investigating the mechanisms of wound repair, tissue regeneration, and disorders of the hemostatic system. The present study was designed to develop and validate a standardized and reproducible experimental model of deep third-degree skin burns in laboratory rats, enabling comprehensive assessment of morphofunctional alterations during the wound-healing process and evaluation of the efficacy of locally applied hemostatic agents. A controlled contact burn was induced by applying a preheated metal probe to the shaved dorsal skin of anesthetized animals. Morphological and histopathological examinations were performed on days 3, 7, 14, 21, 30, and 60 following thermal injury. The findings revealed rapid development of tissue necrosis within the first 24 hours, followed by pronounced inflammatory infiltration and subsequent formation of granulation tissue. Maximal fibroblast activity and collagen deposition were observed between days 14 and 21, corresponding to the peak of the reparative phase. By day 60, the burn defect was predominantly replaced by fibrotic scar tissue, accompanied by partial re-epithelialization. The proposed experimental model demonstrates high reproducibility and reliability and may serve as an effective platform for studying morphofunctional changes associated with deep thermal burns and for preclinical evaluation of therapeutic strategies aimed at enhancing tissue regeneration.

Keywords: experimental burns, thermal injury, morphofunctional changes, rats, granulation tissue, fibrosis, wound healing.

Аннотация. Термическая травма является одной из наиболее распространённых и методологически значимых экспериментальных моделей в патологии, широко используемой для изучения механизмов заживления ран, регенерации тканей и нарушений системы гемостаза. Настоящее исследование было направлено на разработку и валидацию стандартизированной и воспроизводимой экспериментальной модели глубоких ожогов кожи III степени у лабораторных крыс, позволяющей

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всесторонне оценить морфофункциональные изменения в процессе заживления ран, а также эффективность местного применения гемостатических средств.Контактный ожог вызывали путём воздействия предварительно нагретого металлического зонда на выбритую кожу спины анестезированных животных. Морфологические и гистопатологические исследования проводили на 3, 7, 14, 21, 30 и 60-е сутки после термического повреждения. Полученные результаты показали быстрое развитие тканевого некроза в течение первых 24 часов, за которым следовали выраженная воспалительная инфильтрация и последующее формирование грануляционной ткани. Максимальная активность фибробластов и отложение коллагена наблюдались в период с 14-го по 21-й день, что соответствовало пику репаративной фазы. К 60-м суткам ожоговый дефект преимущественно замещался фиброзной рубцовой тканью с частичной реэпителизацией. Предложенная экспериментальная модель характеризуется высокой воспроизводимостью и надёжностью и может служить эффективной платформой для изучения морфофункциональных изменений при глубоких термических ожогах, а также для доклинической оценки терапевтических стратегий, направленных на усиление регенерации тканей.

Ключевые слова: экспериментальные ожоги, термическая травма, морфофункциональные изменения, крысы, грануляционная ткань, фиброз, заживление ран.

Annotatsiya. Termal shikastlanish patologiya sohasida eng keng tarqalgan va metodologik jihatdan muhim eksperimental modellardan biri bo‘lib, yara bitishi mexanizmlarini, to‘qimalarning regeneratsiyasini hamda gemostaz tizimi buzilishlarini o‘rganishda keng qo‘llaniladi. Ushbu tadqiqot laboratoriya kalamushlarida terining chuqur III-darajali kuyishini standartlashtirilgan va qayta takrorlanadigan eksperimental modelini ishlab chiqish hamda validatsiyalashga qaratilgan bo‘lib, yara bitishi jarayonida yuzaga keladigan morfofunksional o‘zgarishlarni har tomonlama baholash va mahalliy qo‘llaniladigan gemostatik vositalarning samaradorligini aniqlash imkonini beradi. Kontaktli kuyish oldindan qizdirilgan metall zond yordamida narkoz ostidagi hayvonlarning qirilgan orqa terisiga ta’sir ettirish orqali chaqirildi. Morfologik va gistopatologik tekshiruvlar termal shikastlanishdan keyin 3, 7, 14, 21, 30 va 60-kunlarda o‘tkazildi. Tadqiqot natijalari dastlabki 24 soat ichida to‘qima nekrozining tez rivojlanishini, undan keyin esa yaqqol yallig‘lanish infiltratsiyasi va granulyatsion to‘qima hosil bo‘lishini ko‘rsatdi. Fibroblastlarning maksimal faolligi va kollagen to‘planishi 14–21-kunlar oralig‘ida kuzatilib, bu reparativ fazaning eng yuqori bosqichiga to‘g‘ri keldi. 60-kunga kelib, kuyish nuqsoni asosan fibroz chandiq to‘qima bilan

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almashgan bo‘lib, qisman qayta epitelizatsiya kuzatildi. Taklif etilayotgan eksperimental model yuqori darajada qayta takrorlanish xususiyati va ishonchliligi bilan ajralib turadi hamda chuqur termal kuyishlar bilan bog‘liq morfofunksional o‘zgarishlarni o‘rganish, shuningdek to‘qima regeneratsiyasini kuchaytirishga qaratilgan terapevtik strategiyalarni doklinik baholash uchun samarali platforma bo‘lib xizmat qilishi mumkin.

Kalit so‘zlar: eksperimental kuyishlar, termal shikastlanish, morfofunksional o‘zgarishlar, kalamushlar, granulyatsion to‘qima, fibroz, yara bitishi.

INTRODUCTION

Thermal burns constitute a significant medical and experimental challenge due to their complex pathophysiology, which encompasses extensive tissue necrosis, inflammatory responses, microvascular disturbances, and prolonged impairment of wound healing. Experimental modeling of thermal injuries in laboratory animals is essential for elucidating the mechanisms underlying tissue damage and regeneration, as well as for preclinical evaluation of novel pharmacological and local therapeutic interventions. Among small laboratory animals, rats are extensively employed in burn research owing to their well-characterized physiological and reparative responses, ease of handling, and suitability for detailed morphological and histological investigations. The development of a standardized, reproducible, and controllable burn model is a critical prerequisite for reliable assessment of morphological, functional, and biochemical processes occurring at different stages of wound healing, as well as for objective evaluation of hemostatic and reparative agents. The present study focuses on the creation of a controlled and ethically justified experimental model of deep third-degree thermal skin burns in rats. The proposed methodology enables precise regulation of burn depth and surface area, reduces experimental variability, and minimizes the risk of secondary infection. Systematic analysis of morphofunctional changes during successive stages of wound healing was performed to validate the reliability and applicability of the model for future experimental and pharmacological studies.

Aim. The aim of this study was to develop a reproducible and ethically justified experimental model of deep third-degree thermal skin burns in laboratory rats under controlled conditions. The model was designed to enable systematic evaluation of morphofunctional alterations in the skin and underlying tissues throughout the wound-healing process and to provide a reliable experimental platform for assessing the efficacy of local hemostatic and reparative agents.

MATERIALS AND METHODS

Experimental Animals

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The study was conducted on 30 clinically healthy white outbred male rats weighing 180–220 g. All experimental procedures complied with institutional animal care and ethical standards.

Anesthesia and Preparation

Before burn induction, the animals were anesthetized with intraperitoneal sodium thiopental (40 mg/kg). The dorsal region was shaved and cleaned with 70% ethanol to ensure uniform skin exposure.

Burn Induction Procedure

A brass metal probe (diameter 2 cm, weight 80 g) was preheated in water to 96–98°C and applied to the dorsal skin for 10 seconds under controlled pressure to produce a full-thickness (third-degree) burn covering approximately 10% of the total body surface area. The burned region was left open to the air without any immediate treatment to observe natural wound progression.

Post-Burn Care

Animals were housed individually in sterile cages and observed daily. Standard feed and water were provided ad libitum. To prevent secondary infection, wound areas were treated with 0.05% chlorhexidine solution once daily.

Sampling and Observation Periods

Tissue samples were collected on days 3, 7, 14, 21, 30, and 60 post-burn. Macroscopic observations included wound color, exudation, and epithelialization. Histological examination was performed on paraffin-embedded sections stained with hematoxylin and eosin and Masson's trichrome.

RESULTS

Macroscopic Findings. Table 1 summarizes the macroscopic dynamics of the burn wound healing process. The first 24 hours were characterized by the formation of a dense necrotic crust with clear demarcation from surrounding tissue. By day 7, inflammatory edema and purulent exudate were observed. Granulation tissue formation became prominent by day 14 and reached maximum development between days 14 and 21.

Table 1.

Macroscopic stages of burn wound healing in rats

Observation day	Macroscopic appearance	Healing phase
3	Necrotic crust formation, edema, hyperemia	Necrosis and inflammation
7	Beginning of demarcation, purulent exudate	Inflammatory phase
14	Active granulation tissue growth	Proliferative phase

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21	Wound contraction, decreased exudate	Early remodeling
30	Granulation replaced by fibrous tissue	Remodeling and scar formation
60	Mature scar, no active inflammation	Final epithelialization

Histological and Morphological Findings. On day 3, histological analysis revealed massive coagulative necrosis with inflammatory cell infiltration consisting of neutrophils and macrophages. Capillary thrombosis and local hemorrhages were frequent.

By day 14, granulation tissue showed intensive fibroblast proliferation and newly formed capillaries. Collagen deposition increased sharply during this period. Between days 21 and 30, fibroblasts differentiated into myofibroblasts, initiating scar contraction. By day 60, dense collagen bundles replaced granulation tissue, forming mature fibrous scars with partial re-epithelialization.

Table 2.

Histological dynamics of tissue repair after deep thermal burn

Day	Predominant cell types	Structural characteristics	Functional interpretation
3	Neutrophils, macrophages	Necrosis, edema, vascular congestion	Inflammation, tissue degradation
7	Macrophages, fibroblasts	Granulation tissue onset	Transition to repair
14	Fibroblasts, endothelial cells	Active angiogenesis, collagen synthesis	Peak reparative phase
21	Myofibroblasts	Wound contraction, collagen cross-linking	Remodeling begins
30	Fibrocytes, keratinocytes	Scar tissue formation, epithelial migration	Late remodeling
60	Fibrocytes	Mature fibrotic scar	Functional closure

DISCUSSION. The present study successfully established a reproducible and standardized rat model for deep third-degree thermal burns. The model simulates the morphological and functional dynamics observed in clinical burn injuries in humans, including necrosis, inflammation, granulation, and fibrotic scarring.

Compared with other existing models, the current approach allows precise control of temperature, contact time, and burn area, resulting in minimal inter-animal variability. Morphological data confirm that the active reparative phase (14–21 days) is characterized by fibroblast proliferation, angiogenesis, and collagen fiber synthesis — key processes in tissue regeneration.

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By 60 days post-injury, the wound is completely covered with fibrotic scar tissue, restoring the skin's barrier function though not its physiological elasticity. These results correspond with earlier studies indicating that deep burns undergo a prolonged and complex remodeling process involving cytokine-mediated fibroblast activation and extracellular matrix deposition.

The proposed model's reliability makes it highly suitable for testing local hemostatic and reparative agents such as fibrin-based gels, collagen scaffolds, and pharmacologically active wound dressings. It also enables biochemical assessments of oxidative stress markers, inflammatory cytokines, and angiogenic factors during different healing phases.

Conclusion. The developed method provides a consistent and ethically sound experimental model for inducing deep thermal burns in rats. It ensures precise control of burn parameters and reproduces characteristic morphofunctional changes observed in clinical practice. The peak reparative phase occurs between days 14 and 21, associated with fibroblast proliferation and collagen synthesis. By day 60, complete scarring and partial epidermal regeneration occur. This model is therefore recommended for preclinical testing of new wound-healing and hemostatic compounds, allowing detailed analysis of morphological and biochemical parameters of skin regeneration.

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