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IN-VIVO EFFICACY STUDY OF RESVERATROL ENCAPSULATED BIOMIMETIC SKIN GRAFT FOR BURN TREATMENT

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ABSTRACT

Severe burn injuries remain a major clinical challenge due to prolonged inflammation, excessive oxidative stress, delayed extracellular matrix remodeling, and impaired skin regeneration. This study evaluated the therapeutic potential of a drug-loaded porous microparticle composite graft (gel-drug-PMS) for enhancing burn wound healing in a hot metallic rod-induced third-degree burn rat model over 28 days. Animals were treated with gel-drug-PMS, gel-drug, or left untreated (control), and wound healing was assessed by visual observation, percentage wound closure, body weight, feed consumption, and collagen Type I content. The gel-drug-PMS-treated group demonstrated superior healing throughout the study. By Day 28, wound closure reached 76%, compared with 70% in the gel-drug group and 60% in the untreated controls. Visual examination revealed faster wound contraction, earlier granulation tissue formation, and improved re-epithelialization in animals receiving the composite graft. In addition, gel-drug-PMS significantly improved body weight recovery and feed consumption, indicating attenuation of the burn-induced hypermetabolic response and enhanced systemic recovery. Collagen Type I levels were markedly higher in the composite graft-treated group, suggesting enhanced fibroblast activity, extracellular matrix deposition, and dermal remodeling. The improved therapeutic performance of gel-drug-PMS is attributed to the synergistic effects of sustained antioxidant release, the extracellular matrix-mimicking porous scaffold, and bioactive peptide-functionalized microparticles, which together promote cellular infiltration, angiogenesis, collagen synthesis, and tissue regeneration while reducing oxidative stress and inflammation. Overall, the developed gel-drug-PMS composite graft significantly accelerated burn wound healing and improved both local tissue regeneration and systemic recovery compared with the gel-drug formulation and untreated controls. These findings highlight its potential as a promising bioactive wound dressing for the treatment of full-thickness burn injuries and support further preclinical and clinical investigations for translational burn care applications.

KEYWORDS: Resveratrol, Biomimetic Skin Graft, Burn Wound Healing, *In Vivo* Evaluation, Controlled Drug Delivery.

INTRODUCTION

Burn injuries remain one of the most devastating forms of trauma, causing extensive destruction of the skin and underlying tissues.[1] Deep third-degree burns involve complete loss of both the epidermis and dermis, resulting in severe impairment of the skin's barrier function, increased susceptibility to infection, excessive fluid loss, delayed healing, and permanent scar formation.[2] Conventional treatment strategies, including skin grafting and topical wound dressings; often fail to restore the complex architecture and biological functions of the native skin.[3] Consequently, there is a growing interest in the development of biomimetic tissue-engineered scaffolds capable of supporting cellular regeneration, accelerating wound healing, and promoting functional restoration of damaged skin.[4]

Successful repair of deep burn wounds depends largely on rapid dermal regeneration, extracellular matrix (ECM) remodeling, neovascularization, and controlled inflammation. An ideal wound dressing should therefore provide a three-dimensional microenvironment that mimics the natural extracellular matrix, supports adhesion and proliferation of skin cells, maintains adequate moisture, absorbs wound exudates, and delivers therapeutic molecules in a sustained manner.[5] Biomimetic composite grafts have emerged as promising candidates for burn wound management because they combine structural support with bioactive components capable of directing the healing process.

Oxidative stress is one of the major factors responsible for delayed burn wound healing.[6] Thermal injury generates excessive reactive oxygen species (ROS), leading to lipid peroxidation, protein oxidation, DNA damage, prolonged inflammation, and impaired fibroblast function.[6] Therefore, incorporation of antioxidants into biomaterial-based wound dressings represents an effective strategy to minimize oxidative damage and improve tissue regeneration.

Antioxidant-loaded composite grafts not only neutralize free radicals but also promote collagen synthesis, enhance angiogenesis, and facilitate re-epithelialization.[7]

Resveratrol, a naturally occurring flavonoid found in various fruits and vegetables, possesses potent antioxidant, anti-inflammatory, antimicrobial, and wound-healing properties. Numerous studies have demonstrated that resveratrol effectively scavenges reactive oxygen species, inhibits lipid peroxidation, modulates inflammatory cytokines, and stimulates fibroblast proliferation and collagen deposition. These biological activities make resveratrol an attractive therapeutic agent for burn wound management.[8] However, its poor aqueous solubility and hydrophobic nature significantly limit its incorporation into hydrophilic polymeric systems such as gelatin, thereby reducing its therapeutic applicability.[9] Nanoprecipitation has emerged as an effective approach to overcome this limitation by producing resveratrol nanoparticles with improved dispersibility, stability, and drug loading efficiency within hydrogel-based formulations.[10]

Gelatin is a biodegradable, biocompatible, and highly hydrophilic natural polymer extensively utilized in wound healing applications because of its excellent moisture-retaining ability, low immunogenicity, and similarity to collagen, the principal structural protein of skin.[11] Gelatin hydrogels provide a favorable environment for cell attachment, migration, and proliferation. However, gelatin alone possesses limited mechanical strength and lacks sufficient biological cues required for efficient dermal regeneration. Therefore, incorporation of porous biomimetic scaffolds and bioactive molecules can substantially enhance its regenerative potential.[12]

Porous microparticle scaffolds serve as artificial extracellular matrices by providing interconnected pore networks that facilitate cell infiltration, nutrient diffusion, oxygen transport, vascularization, and extracellular matrix deposition. Coupling these microparticles with cytomodulin, a bioactive signaling molecule capable of regulating cellular proliferation and differentiation, further enhances their regenerative capacity.[13] The combination of cytomodulin-coupled porous microparticles with gelatin hydrogel creates a biomimetic composite scaffold that closely resembles the native dermal microenvironment and supports tissue regeneration.

Based on these concepts, the present study aimed to develop and evaluate a resveratrol-loaded biomimetic composite graft consisting of gelatin hydrogel incorporated with cytomodulin-coupled porous microparticles for the treatment of deep third-degree burn wounds. Resveratrol nanoparticles were prepared using the nanoprecipitation technique to facilitate their incorporation into the gelatin matrix. Composite grafts containing different concentrations of resveratrol (2.5, 5, and 10 mg/mL) were formulated, and the optimized formulation was selected based on physicochemical characteristics (Design and *In-Vitro* Characterization of Resveratrol Encapsulated Porous Biomimetic Skin Graft for Burn Treatment: *in press*).

The optimized resveratrol-loaded composite graft was comprehensively characterized for its morphological architecture using scanning electron microscopy (SEM), cytomodulin coupling efficiency using the NanoOrange® protein assay, micro-shrinkage temperature by hot-stage microscopy, antioxidant activity through DPPH free radical scavenging assay, fluid uptake capacity, in vitro drug release, and degradation behavior under physiological conditions. These evaluations were performed to determine the structural integrity, thermal stability, antioxidant potential, swelling characteristics, controlled drug release profile, and biodegradation properties of the developed scaffold.

Among the formulations investigated, the composite graft containing 10 mg/mL resveratrol exhibited optimal physicochemical and biological characteristics. Nanoprecipitation enabled successful incorporation of hydrophobic resveratrol into the gelatin matrix, producing nanoparticles with an average particle size of approximately 602.5 nm (PDI 0.564). The composite graft demonstrated excellent thermal stability at physiological temperature, preserved the antioxidant activity of resveratrol with an SC50 value (6.8 µg/mL) comparable to pure resveratrol (6.0 µg/mL), exhibited approximately 50% fluid uptake suitable for exudate management, possessed an interconnected porous architecture, and achieved approximately 83% cytomodulin coupling efficiency.

MATERIAL AND METHOD

Evaluation of biomimetic composite skin graft

In Vitro Drug Release Study of Resveratrol-Loaded Graft

The *in vitro* release profile of resveratrol from the developed biomimetic graft was evaluated using phosphate-buffered saline (PBS, 100 mM, pH 7.4) to simulate physiological conditions. Three formulations containing different concentrations of resveratrol (0.25%, 0.5%, and 1% w/w) were investigated to assess the influence of drug loading on the release behavior.[14]

For the release study, freshly prepared graft formulations were transferred into individual glass vials and allowed to undergo complete gelation at room temperature for approximately 1 hour to ensure proper network formation. After gelation, 5 mL of PBS was carefully added to each vial, ensuring that the graft was completely immersed in the release medium. The vials were then placed in a reciprocal shaking water bath maintained at 37 ± 0.5°C with a shaking speed

of 100 rpm to mimic physiological temperature and provide gentle agitation for uniform diffusion of the drug into the release medium.

At predetermined time intervals, 2 mL of the release medium was withdrawn from each vial for analysis and immediately replaced with an equal volume of fresh pre-warmed PBS to maintain sink conditions and a constant release volume throughout the experiment. The collected samples were filtered, when necessary, before quantitative analysis.

The amount of resveratrol released into the dissolution medium was determined using a validated high-performance liquid chromatography (HPLC) method.[15] Chromatographic analysis was performed at a detection wavelength of 308 nm, which corresponds to the maximum absorbance of resveratrol under the optimized analytical conditions. The cumulative percentage of drug released at each sampling point was calculated from the measured concentrations and plotted against time to evaluate the release kinetics and sustained-release characteristics of the graft formulations.

Studies of composite skin graft in burn model

Development of Skin Burn Model in Wistar Rats

The *in vivo* burn wound healing study was conducted following approval from the Institutional Animal Ethics Committee (IAEC), Pinnacle Biomedical Research Institute (PBRI), Bhopal (Registration No.: 1824/PO/RcBi/S/2015/ CCSEA; Protocol Approval No.: PBRI/IAEC/08-05-2026/017). All experimental procedures involving animals were performed in strict accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India, to ensure ethical handling and welfare of the experimental animals.

A total of 36 healthy male albino Wistar rats, weighing between 220 and 250 g, were selected for the burn wound healing study. The animals were acclimatized to laboratory conditions before the experiment and housed under standard environmental conditions, including a controlled temperature (22–25°C), relative humidity (50–60%), and a 12-hour light/dark cycle. Standard laboratory pellet diet and drinking water were provided ad libitum throughout the study.

The animals were randomly divided into three experimental groups, with 12 rats in each group. For evaluation of wound healing at different intervals, three animals from each group were sacrificed at each predetermined time point for histological and biochemical analyses.

Before induction of burn injury, each rat was anesthetized by intraperitoneal administration of ketamine (60 mg/kg body weight) and xylazine (5 mg/kg body weight) to achieve adequate analgesia and muscle relaxation. The dorsal surface of each animal was carefully shaved using an electric clipper, and the exposed skin was cleaned to remove loose hair. A defined area on the back was marked to standardize the burn size among all animals.

A standardized full-thickness thermal burn wound was created using a preheated cylindrical metallic rod. The metal rod was heated to approximately 100°C over an open flame burner and then gently applied to the marked dorsal skin for 70 seconds, producing a reproducible deep burn injury.[16] This procedure was performed according to the method described by Guo et al. (2011), with slight modifications. After burn induction, the animals were monitored until complete recovery from anesthesia and subsequently maintained under standard laboratory conditions with free access to food and water.

The experimental animals were assigned to the following treatment groups:

Group I (Control, Untreated): Burn wounds were left untreated and covered only with Tegaderm® followed by Micropore® adhesive dressing to protect the wound from contamination.[17]

Group II (Gel-Drug Treated): Burn wounds were treated with a gelatin-based hydrogel containing resveratrol, after which the wounds were covered with Tegaderm® and Micropore® dressing.

Group III (Gel-Drug-PMS Treated): Burn wounds were treated with a gelatin-resveratrol formulation incorporated with cytomodulin-coupled porous microparticles (PMS). Following application of the graft, the wound area was covered with Tegaderm® and secured using Micropore® dressing to maintain the dressing in position and provide a moist wound healing environment.

The treatment was continued according to the experimental protocol, and wound healing was assessed at predetermined intervals by evaluating wound contraction, tissue regeneration, histopathological changes, and other relevant healing parameters.

Table 1: Groups of animal to study efficacy study of the composite skin graft.

Group type	Treatment	
Group I: Control (untreated)	Burn animal covered with Tegaderm® and Micropore®	4 time points × 3animals at each time points = 12 animals
Group II: Gel-drug (Treated)	Burn animal treated with gelatin-Resveratrol and further covered with Tegaderm® and Micropore®	4 time points × 3animals at each time points = 12 animals
Group III: Gel-drug-PMS (Treated)	Burn animal treated with gelatin-Resveratrol-cytomodulin couples porous microparticles (PMS) and further covered with Tegaderm® and Micropore®	4 time points × 3animals at each time points = 12 animals
	Total number of animals =	36 animals

Evaluation of Burn Wound Healing in the Developed Animal Model

Following the induction of thermal burn injury, all animals were immediately resuscitated by subcutaneous administration of physiological saline solution (10 mL/kg body weight) to compensate for fluid loss associated with burn trauma and to maintain hemodynamic stability. The animals were then closely monitored during the post-burn recovery period and maintained individually under standard laboratory conditions with unrestricted access to food and water. A period of three days was allowed for the establishment of a full-thickness (third-degree) burn wound before initiating treatment.

On the third day after burn induction, the respective treatment formulations were carefully applied to the wound area according to the designated experimental groups. Following application of the graft or gel formulation, the wound was covered with Tegaderm®, a semi-permeable transparent dressing, and further secured with Micropore® adhesive tape to maintain the dressing in place, minimize contamination, and provide a moist environment favorable for wound healing. Animals were housed individually throughout the study to prevent damage to the dressings and to avoid interference with the healing process due to grooming or biting by cage mates.

The progress of wound healing was evaluated at predetermined intervals on Day 3, Day 7 (1st week), Day 14 (2nd week), and Day 28 (4th week) following treatment. At each observation period, the wounds were carefully examined and photographed for documentation, and healing parameters were assessed using standardized procedures.

The percentage reduction in wound area was measured to determine the rate of wound contraction and tissue regeneration. Changes in body weight were recorded throughout the experimental period as an indicator of the general health status and systemic recovery of the animals after burn injury. In addition, feed intake (feed ratio) was monitored to evaluate the nutritional status and physiological well-being of the animals, as severe burns often lead to reduced appetite and altered metabolic activity.

To assess the quality of tissue repair at the molecular level, collagen type I expression was analyzed in the healed granulation tissue collected from each experimental group at the designated time points. Since collagen type I is the principal structural protein responsible for restoring the tensile strength and integrity of healed skin, its expression served as an important biomarker for extracellular matrix remodeling and the maturation of newly formed tissue.

Comparative analysis of wound contraction, body weight, feed consumption, and collagen type I expression among the treatment groups provided a comprehensive assessment of the therapeutic efficacy of the developed graft formulations in promoting burn wound healing.

A. Measurement of Wound Closure

The rate of wound healing was assessed by periodically measuring the reduction in wound area throughout the experimental period. Wound closure was evaluated at predetermined time intervals to determine the effectiveness of the different treatment formulations in promoting tissue regeneration and wound contraction.

At each observation point, the margins of the wound were carefully outlined on a sterile transparent tracing sheet by gently tracing the wound boundary without disturbing the healing tissue.[18] The traced outlines were then transferred onto graph paper, and the wound area was determined in square millimetres (mm^2) by counting the enclosed grid squares. This standardized method enabled accurate and reproducible measurement of changes in wound size over time.

In addition to the tracing method, direct wound measurements were obtained on Days 4, 7, 14, and 28 after burn induction using a sterile metallic ruler. The maximum length and width of each wound were measured under aseptic conditions to minimize the risk of contamination. Since burn wounds in rats generally assume an elliptical configuration due to the anisotropic mechanical properties of the skin, the wound area was calculated by considering the wound as an ellipse.[19]

The wound area (A) was calculated using the following equation (Huang *et al.*, 2008):

$$\text{Wound area } (cm^2) = \frac{1}{4}[\text{long axis (cm) X short axis (cm)}] \times \pi \qquad (1)$$

The percentage wound closure was measured using a standard planimetry method. At predefined study time points, the wound area was traced on a transparent graph sheet and expressed as the percentage of surface area reduction (equation 5):[20]

$$\text{Percentage wound closure} = (A_0 - A_t)/A_0 \times 100 \qquad (2)$$

where, A_0 is the original wound area and A_t is the area of wound at the respective study time points. Photographs of the healing wound tissues were also captured for visual observation at preset time points.

A greater percentage of wound contraction indicated faster wound healing and more effective tissue repair. The calculated values were recorded for all experimental groups and compared at each observation period to evaluate the burn wound healing efficacy of the developed graft formulations.

B. Assessment of Body Weight Changes (%)

Changes in body weight were monitored throughout the experimental period as an important indicator of the overall health status, metabolic condition, and recovery of the animals following burn injury.[21] Severe burn trauma is known to induce a hypermetabolic and catabolic state characterized by increased energy expenditure, protein degradation, dehydration, and reduced food intake, all of which contribute to a significant loss in body weight during the initial phase of wound healing.

Immediately after burn induction, the animals typically exhibited a gradual decline in body weight due to physiological stress, tissue damage, fluid loss, and increased metabolic demands associated with the inflammatory response.[22] As the healing process progressed and the animals recovered, body weight gradually increased toward normal values, reflecting improved nutritional status, reduced systemic stress, and successful tissue repair.

The body weight of each animal was measured using a calibrated digital weighing balance prior to burn induction (baseline) and subsequently at regular predetermined intervals throughout the study period. All measurements were recorded under identical experimental conditions to minimize variability. The percentage change in body weight was calculated by comparing the body weight recorded at each observation point with the initial body weight measured before burn induction.

The percentage change in body weight was calculated using the following equation:

$$\text{Body weight change } (\%) = \frac{W_t - W_0}{W_0} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where, W_0= Initial body weight of the animal before burn induction (g)

W_t= Body weight of the animal at a specific observation day (g)

The recorded body weight data were compared among the experimental groups to evaluate the systemic effects of the treatment formulations. Animals demonstrating a faster recovery of body weight were considered to have improved physiological recovery, better nutritional status, and enhanced burn wound healing. Thus, body weight served as an important complementary parameter for assessing the overall therapeutic efficacy of the developed gelatin-based graft formulations in the management of burn injuries.

For burn wound healing studies, body weight is commonly recorded on Day 0 (before burn induction), Day 3, Day 7, Day 14, and Day 28, and the percentage change is calculated relative to the initial body weight. This provides an indication of the animal's systemic recovery and overall health during the healing process.

C. Assessment of Feed Consumption (%)

Feed consumption was monitored throughout the experimental period as an important physiological parameter to evaluate the nutritional status, metabolic recovery, and general health of the animals following burn injury.[23]

Thermal burns are known to induce systemic stress, inflammation, and a hypermetabolic response, which often results in reduced appetite and decreased food intake during the initial stages of wound healing. Therefore, changes in feed consumption serve as an indirect indicator of the severity of injury as well as the effectiveness of the therapeutic intervention.

Following induction of the standardized burn wound using a preheated metallic rod, the experimental animals were randomly allocated into their respective treatment groups. Each animal was housed individually to allow accurate measurement of daily food intake and to eliminate variations caused by group feeding. A predetermined weighed quantity of standard laboratory pellet diet was provided to each animal at regular intervals throughout the study.

At each observation period, the remaining feed was collected and weighed using a calibrated electronic balance. The amount of feed consumed by each animal was determined by calculating the difference between the weight of feed offered and the weight of feed remaining after the feeding period. Feed intake was recorded at predetermined time intervals corresponding to the wound healing assessment schedule.

The percentage change in feed consumption was calculated using the following equation:

$$Percent\ change\ in\ feed\ consumption = \frac{Feed\ consumption\ at\ time\ t - Initial\ feed\ consumption}{Initial\ feed\ consumption} \times 100 \ldots\ldots\ldots (4)$$

Where:

Feed Consumption at Time t = Amount of feed consumed at the specified observation day (e.g., Day 7, 14, 21, etc.)

Initial Feed Consumption = Amount of feed consumed before burn induction or at Day 0 (baseline)

The recorded feed consumption data were compared among the experimental groups to assess the influence of the different treatment formulations on post-burn recovery. An increase in feed intake during the healing period was considered indicative of improved physiological status, reduced systemic stress, enhanced metabolic recovery, and successful wound healing.

D. Determination of Collagen Type I Content

The collagen type I (Col I) content in the wound healing tissue was quantified using a commercially available Rat Collagen Type I (Col I) ELISA Kit (Cusabio Biotech Co. Ltd., China) according to the manufacturer's recommended protocol. Briefly, tissue samples collected from the wound site were processed to obtain the required protein extract for analysis (24). The ELISA microplate supplied with the kit was pre-coated with a monoclonal antibody specific for rat collagen type I.

A series of collagen type I standards and appropriately prepared tissue samples were dispensed into the designated wells of the microplate. Subsequently, a biotin-labeled antibody specific for collagen type I was added to each well, followed by the addition of horseradish peroxidase (HRP)-conjugated avidin. The plate was incubated under the recommended conditions to facilitate the formation of the antibody–antigen–enzyme complex.

Following incubation, the wells were washed thoroughly to remove any unbound reagents. Thereafter, 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution was added to each well. In the presence of HRP, the TMB substrate underwent enzymatic oxidation, resulting in the development of a blue-colored product. The intensity of the color produced was directly proportional to the amount of collagen type I present in the sample.[25]

The enzymatic reaction was terminated by adding sulfuric acid stop solution, which converted the blue color to yellow.

The absorbance of each well was then measured at 450 ± 2 nm using a microplate reader. A standard calibration curve was constructed using the absorbance values of the collagen type I standards, and the collagen type I concentration in the tissue samples was calculated by interpolating their optical density (OD) values from the standard curve. The collagen content was expressed according to the manufacturer's recommended units and used to evaluate the extent of extracellular matrix deposition and wound healing.

Reagent preparation

Wash buffer- Wash buffer concentrate (20 ml) was diluted into deionized or distilled water to prepare wash buffer (500 ml).

Standard- Standard vials were centrifuged at 6000-10000rpm at 30s. Standard was reconstituted with sample diluents (1 ml). This reconstitution produced stock solution (5000 pg/ml) then standard was allowed to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions.

Biotin antibody- Vials were centrifuged before opening and was diluted to the working concentration using biotin antibody diluent (1:100).

HRP-avidin- Vials were centrifuged before opening and it was diluted to the working concentration using HRP-avidin diluent (1:100).

Prior to analysis, all tissue samples were diluted 20-fold using the sample diluent supplied with the ELISA kit by mixing 15 µL of sample with 285 µL of sample diluent. Subsequently, 100 µL of standards, blanks, and diluted samples were dispensed into the designated wells of the pre-coated ELISA microplate. The plate was covered with an adhesive film and incubated at 37°C for 2 hours to allow collagen type I present in the standards and samples to bind to the immobilized capture antibodies.

Following incubation, the liquid contents were carefully discarded from each well. One hundred microlitres (100 µL) of the prepared biotin-conjugated antibody working solution was then added to each well. Since the biotin-antibody solution may become slightly turbid during storage, it was first allowed to reach room temperature and mixed gently until a homogeneous solution was obtained before use. The plate was covered and incubated at 37°C for 1 hour to facilitate the formation of the antibody–antigen complex.

After incubation, the contents of each well were aspirated, and the wells were washed thoroughly with 200 µL of wash buffer. The wash buffer was allowed to remain in the wells for approximately 2 minutes before being discarded by inverting the plate and gently tapping it on absorbent paper. This washing step was performed to remove any unbound antibody and minimize background interference.

Next, 100 µL of horseradish peroxidase (HRP)-conjugated avidin working solution was added to each well. The microplate was again covered and incubated at 37°C for 1 hour, allowing the HRP-conjugated avidin to bind specifically to the biotinylated detection antibody. Following this incubation, the washing procedure was repeated to eliminate excess enzyme conjugate.

Thereafter, 90 µL of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution was added to each well. The plate was incubated at 37°C for 10–30 minutes in the dark until a distinct blue color developed, particularly in the wells containing the highest concentrations of the standard solutions. The enzymatic reaction was terminated by adding 50 µL of stop solution to each well, resulting in a color change from blue to yellow.

The optical density (OD) of each well was measured within 30 minutes after stopping the reaction using a microplate reader set at 450 nm. The concentration of collagen type I in the tissue samples was subsequently calculated by comparing the absorbance values of the samples with those obtained from the standard calibration curve generated using the known collagen standards.

Statistical analysis

One-way ANOVA followed by Tukey test was applied for comparison among the different groups using Sigma Stat 3.5 software for windows. Difference was considered statistically significant at level of $p < 0.05$.

RESULTS AND DISCUSSION

Evaluation of composite skin graft

***In vitro* drug release**

Resveratrol-loaded composite grafts were prepared by incorporating resveratrol at three different concentrations (2.5 mg/mL, 5 mg/mL, and 10 mg/mL) into a gelatin matrix, followed by chemical cross-linking with glutaraldehyde to obtain a stable polymeric network capable of providing controlled drug release. The in vitro drug release profile was evaluated over a period of 5 days to investigate the release characteristics of the different formulations.

The cumulative percentage of resveratrol released from the composite grafts after 5 days was 87%, 85%, and 75% for the formulations containing 10 mg/mL, 5 mg/mL, and 2.5 mg/mL of resveratrol, respectively. These findings indicate that the formulation containing the highest drug loading exhibited the greatest cumulative release during the study period. Furthermore, mass balance analysis demonstrated that the percentage of drug retained within the graft after completion of the release study was 11.8%, 13.2%, and 21.1% for the 10 mg/mL, 5 mg/mL, and 2.5 mg/mL formulations, respectively, confirming that the majority of the encapsulated drug had been released while only a small fraction remained entrapped within the polymeric matrix.

An ideal wound dressing for burn management should provide an adequate concentration of antioxidant at the wound site immediately after application to effectively neutralize the excessive reactive oxygen species (ROS) and free radicals generated during the acute inflammatory phase of burn injury. The rapid availability of the antioxidant minimizes oxidative stress, reduces secondary tissue damage, and protects the surrounding viable tissue. In addition to the initial release, the dressing should maintain a sustained release of the therapeutic agent over an extended period to ensure continuous antioxidant activity, thereby preventing delayed oxidative injury and promoting tissue regeneration throughout the healing process.

Among the tested formulations, the composite graft containing 10 mg/mL resveratrol demonstrated the most desirable release characteristics by providing an 87% cumulative release over 5 days, thereby ensuring both an effective initial release and prolonged sustained delivery of the antioxidant. This controlled release profile is advantageous for maintaining therapeutically effective concentrations of resveratrol at the burn wound site during the critical early stages of healing, where oxidative stress is maximal, while also supporting the subsequent phases of tissue repair and regeneration. The sustained release behavior of the optimized formulation is illustrated in figure 1.

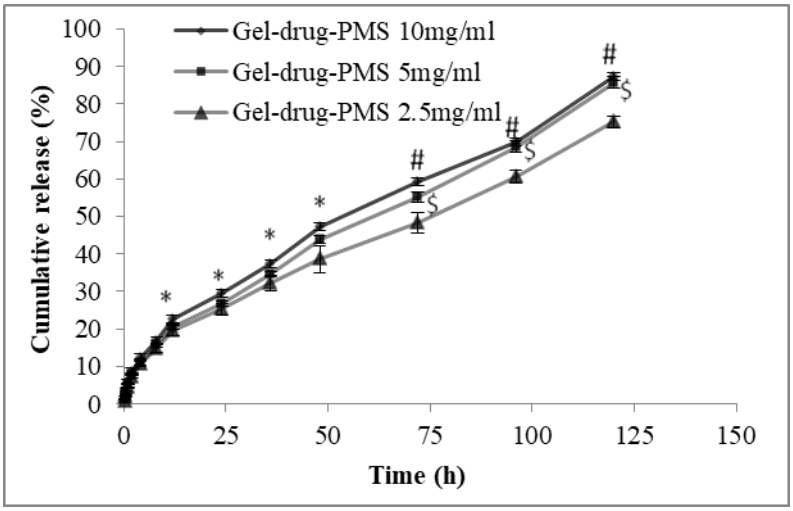

Figure 1: *In vitro* drug release profile of resveratrol from composite skin graft (n=3).

*p<0.05 vs loading 2.5 mg/ml, #p<0.05 vs loading 2.5 and 5 mg/ml and $p<0.05 vs 2.5 mg/ml

Studies of composite skin graft in burn model

Development of skin burn model on wistar rat

Thermal burn injury is characterized by extensive tissue destruction and the excessive production of reactive oxygen species (ROS), resulting in a cascade of oxidative stress, inflammation, and cellular dysfunction. Following a severe burn, activated neutrophils, macrophages, and damaged mitochondria generate large quantities of superoxide anions, hydroxyl radicals, hydrogen peroxide, and reactive nitrogen species. These reactive molecules initiate lipid peroxidation, protein oxidation, DNA damage, and mitochondrial dysfunction, thereby extending tissue injury beyond the initial zone of thermal damage. Recent studies have demonstrated that uncontrolled oxidative stress not only delays wound healing but also contributes to systemic inflammatory response syndrome (SIRS), immune dysregulation, sepsis, and multiple organ dysfunction syndrome (MODS), which remain major causes of morbidity and mortality in patients with extensive burns. Consequently, therapeutic strategies aimed at reducing oxidative stress through antioxidant supplementation have gained considerable attention in burn wound management.

Several experimental and clinical investigations have reported the beneficial effects of both systemic and topical antioxidants in promoting burn wound healing. Polyphenolic antioxidants such as resveratrol, curcumin, quercetin, epigallocatechin gallate (EGCG), and vitamin E have been shown to scavenge excessive free radicals, suppress lipid peroxidation, inhibit pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6, and enhance endogenous antioxidant defense mechanisms through activation of the Nrf2/HO-1 signaling pathway. Among these compounds, resveratrol has received significant attention because of its potent antioxidant, anti-inflammatory, antimicrobial, and pro-angiogenic properties. In addition to neutralizing reactive oxygen species, resveratrol promotes fibroblast

proliferation, collagen synthesis, extracellular matrix remodeling, and neovascularization while reducing apoptosis and inflammatory cell infiltration. These biological effects collectively accelerate tissue regeneration and improve the quality of wound healing.

Topical delivery of antioxidants has emerged as a particularly attractive approach because it enables the therapeutic agent to be delivered directly to the wound site, thereby maximizing local drug concentration while minimizing systemic exposure and associated adverse effects.[26] Sustained-release biomaterial-based dressings, including hydrogels, collagen scaffolds, gelatin matrices, and biodegradable polymeric systems, have further improved the therapeutic efficacy of antioxidants by maintaining prolonged drug availability throughout the different stages of wound healing. Such delivery systems provide an initial release sufficient to neutralize the burst of reactive oxygen species generated immediately after burn injury, followed by a sustained release that continues to suppress oxidative damage during the inflammatory and proliferative phases of healing.[27]

Experimental burn models have consistently demonstrated that uncontrolled oxidative injury at the burn site can trigger systemic inflammatory responses capable of affecting organs distant from the original wound. Increased vascular permeability, cytokine release, and oxidative stress may subsequently lead to hepatic, renal, pulmonary, and cardiovascular dysfunction, thereby increasing the risk of sepsis and multiple organ failure. Therefore, effective local management of burn wounds not only facilitates cutaneous repair but may also reduce the incidence of systemic complications associated with severe thermal injury.

In the present investigation, a standardized third-degree burn model was established by applying a stainless-steel metallic rod preheated to 100°C to the shaved dorsal surface of anesthetized rats for 70 seconds, producing a reproducible full-thickness thermal injury. Following burn induction, the animals were randomly allocated into three experimental groups (n = 3 per group) according to the treatment administered. The untreated burn group served as the control, whereas the treatment groups received either the gel-drug formulation or the gel-drug-PMS composite formulation applied topically to the wound site throughout the experimental period.

The therapeutic efficacy of the developed formulations was assessed at predetermined intervals on days 4, 7, 14, and 28 after burn induction. General health status was monitored by measuring the percentage change in body weight and percentage change in feed consumption, which reflect the systemic effects of burn injury and post-burn recovery. Wound healing progression was quantitatively evaluated by determining the percentage of wound closure using planimetric analysis. In addition, collagen type I content in the regenerated tissue was estimated using an enzyme-linked immunosorbent assay (ELISA) as an indicator of extracellular matrix deposition and dermal remodeling. These parameters collectively provided comprehensive information regarding the antioxidant efficacy, regenerative potential, and wound-healing performance of the developed topical formulations during the different phases of burn repair.

Evaluation of burn in the developed model

A. Wound closure measurement

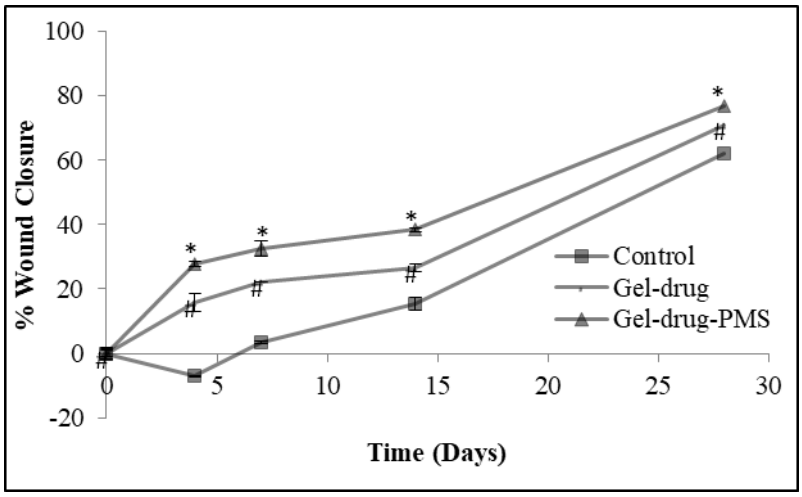

Figure 2: % Change in wound closure of skin with respect to time in different groups (n=3).

Gel-drug-PMS showed significant higher wound closure than gel-drug and control at all time points (*$p<0.05$) and gel drug showed higher wound closure than control at all time points (#$p<0.05$).

Following induction of the full-thickness burn injury, the optimized formulation was applied directly to the wound in its semisolid state immediately after glutaraldehyde (GTA)-mediated gelation for 70 seconds, as shown in figure 3. The rapid *in situ* gelation enabled the formulation to conform closely to the wound surface, producing a stable three-dimensional matrix capable of maintaining intimate contact with the injured tissue. After application, the treated wound was covered with Tegaderm® as a semi-occlusive dressing and secured using Micropore® surgical tape to protect the wound from external contamination, minimize moisture loss, and maintain an optimal wound microenvironment. The animals were then housed individually to prevent wound interference and cross-contamination, and the progression of wound healing was monitored at predetermined intervals throughout the experimental period.

Full-thickness burn wounds are characterized by complete destruction of both the epidermis and dermis, frequently extending into the subcutaneous tissue and, in severe cases, involving the underlying fascia, muscle, or bone.[28]

Unlike superficial wounds, full-thickness burns possess minimal regenerative capacity because of the extensive loss of skin appendages, vascular networks, and resident stem cell populations. Consequently, healing depends largely on inflammatory cell recruitment, angiogenesis, fibroblast proliferation, extracellular matrix deposition, collagen remodeling, and re-epithelialization. Contemporary burn biology recognizes wound healing as a dynamic and highly coordinated process consisting of four interrelated phases: hemostasis, inflammation, proliferation, and remodeling. Although these phases are described separately, they overlap considerably, and numerous cytokines, growth factors, chemokines, and extracellular matrix proteins regulate the transition from one stage to the next. Successful healing therefore requires a tightly regulated balance between inflammatory responses and tissue regeneration.

Immediately after thermal injury, the inflammatory phase is initiated by platelet activation and the release of inflammatory mediators, followed by infiltration of neutrophils and macrophages into the wound bed.[29] These immune cells eliminate necrotic tissue and invading microorganisms but simultaneously generate excessive reactive oxygen

species (ROS), proteolytic enzymes, and pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6.[30] While controlled inflammation is essential for wound debridement, prolonged oxidative stress delays tissue regeneration by impairing fibroblast function, reducing angiogenesis, and degrading extracellular matrix proteins. Therefore, recent studies have emphasized the importance of antioxidant-based biomaterials capable of modulating oxidative stress while simultaneously supporting tissue repair.

During the proliferative phase, fibroblasts migrate into the wound bed and synthesize collagen, fibronectin, and other extracellular matrix components that provide structural support for the regenerating tissue.[31] Concurrently, endothelial cells promote angiogenesis to restore oxygen and nutrient supply, while keratinocytes proliferate and migrate to re-establish epidermal continuity. The subsequent remodeling phase involves replacement of immature collagen with highly organized collagen fibers, contraction of the wound through myofibroblast activity, and progressive improvement in the tensile strength of the repaired tissue.[32] Biomaterial-based dressings that maintain a moist environment while providing sustained release of therapeutic agents have been shown to accelerate each of these biological events and improve the overall quality of wound repair.[33]

As illustrated in figure 2, wound contraction was assessed visually on days 4, 7, 14, and 28 following burn induction. Progressive wound closure was observed in all experimental groups; however, the rate of healing differed markedly depending on the treatment administered. Animals treated with the gel-drug-PMS formulation exhibited significantly faster wound contraction than both the untreated control group and the gel-drug group. By the end of the study (Day 28), approximately 76% wound closure was achieved in the gel-drug-PMS-treated animals, whereas the gel-drug group demonstrated approximately 70% wound closure. In contrast, the untreated control group exhibited only 60% wound closure, indicating delayed healing in the absence of therapeutic intervention, as shown in figure 4. The enhanced wound contraction observed in the treated groups suggests that sustained local delivery of resveratrol effectively reduced oxidative injury, promoted fibroblast activity, and accelerated tissue regeneration.

The superior healing response produced by the gel-drug-PMS formulation compared with the gel-drug formulation indicates that the incorporation of cytomodulin-functionalized porous microparticles provided additional biological benefits beyond antioxidant drug delivery alone. Porous microparticles offer an interconnected architecture that facilitates oxygen diffusion, nutrient transport, cellular infiltration, and vascular in growth while simultaneously serving as a sustained-release reservoir for the encapsulated therapeutic agent. The resulting microenvironment is highly conducive to extracellular matrix deposition and tissue remodeling, thereby accelerating burn wound repair.

Although the precise molecular mechanism of cytomodulin has not yet been fully elucidated, previous studies have suggested that its biologically active and conformationally stable Val–Ala sequence can interact with cell-surface receptors involved in tissue regeneration, particularly members of the transforming growth factor-β (TGF-β) signaling pathway.[34] Activation of TGF-β-mediated signaling is known to stimulate fibroblast proliferation, enhance collagen type I synthesis, promote myofibroblast differentiation, increase actin cytoskeleton organization, and regulate extracellular matrix remodeling. Recent advances in regenerative medicine further indicate that peptide-based bioactive molecules can influence multiple cellular signaling pathways, including Smad, MAPK, and PI3K/Akt, thereby enhancing cell migration, angiogenesis, matrix deposition, and re-epithelialization.[35] Therefore, the improved wound closure observed with the gel-drug-PMS formulation is likely attributable to the combined effects of sustained antioxidant release from resveratrol, the structural support provided by the porous microparticle matrix, and the

regenerative activity of cytomodulin, which together create a favorable microenvironment for rapid skin regeneration and functional tissue repair.

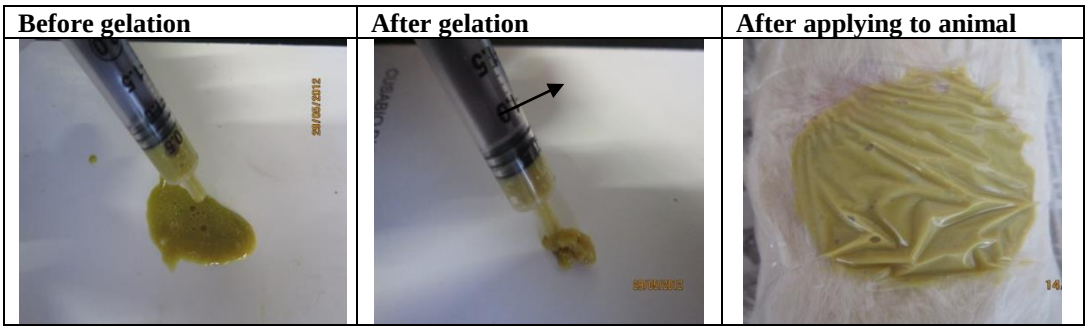

Figure 3: Visual presentation of application of skin graft before and after gelation.

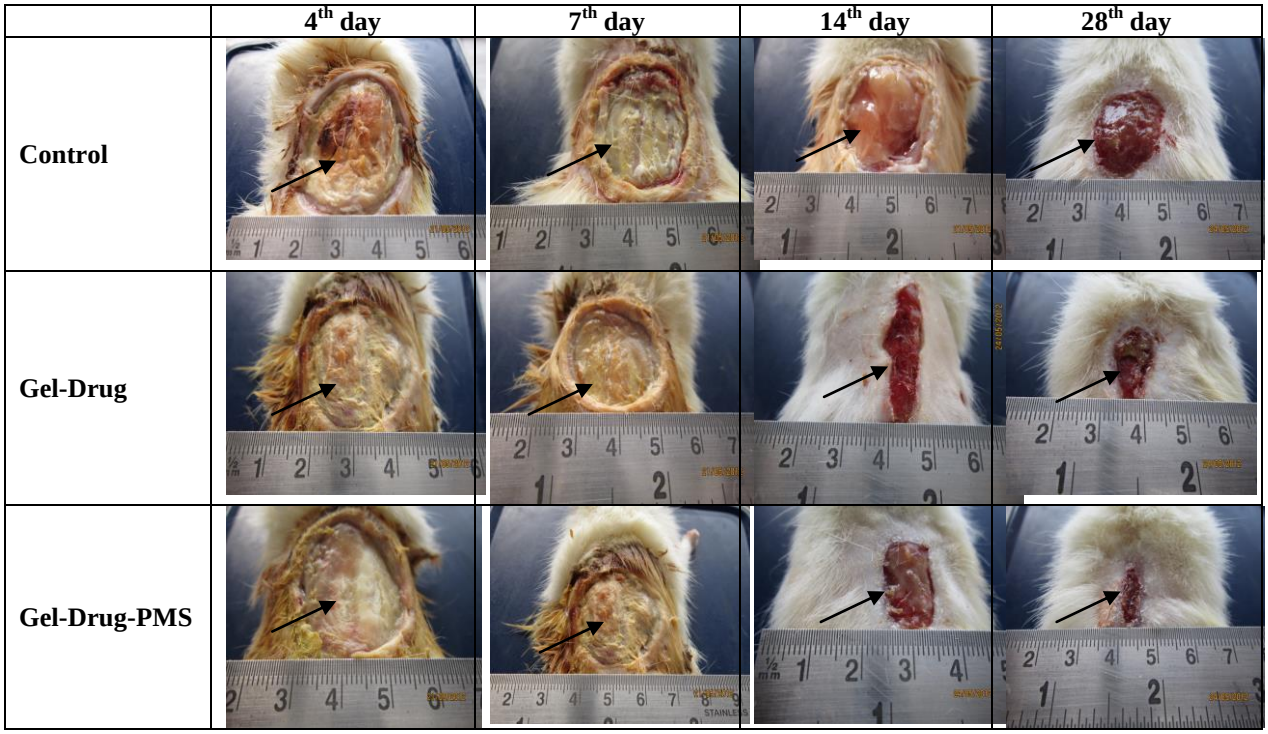

Figure 4: Visual images of wound closure at different time points in control and treated animals.

B. Body Weight Change (%)

Body weight is considered an important indicator of the general health status and metabolic recovery of experimental animals following severe burn injury.[36] In the present study, body weight was monitored at predetermined time intervals throughout the treatment period, and the percentage change in body weight was calculated to evaluate the systemic effects of burn injury as well as the therapeutic efficacy of the developed formulations. Monitoring body weight provides indirect information regarding nutritional status, energy metabolism, severity of the hypermetabolic response, and overall recovery during wound healing.

As shown in figure 5, animals in all experimental groups exhibited a reduction in body weight during the early post-burn period, particularly on Days 4 and 7. This initial weight loss is consistent with the physiological response to severe thermal injury and reflects the acute inflammatory and hypermetabolic phases of burn healing. However, from Day 14 onward, body weight gradually increased in all groups, indicating progressive recovery. The increase in body

weight was most pronounced in animals treated with the gel-drug-PMS formulation, whereas the gel-drug-treated group demonstrated an intermediate response. The untreated control group exhibited the least improvement in body weight throughout the observation period.

Severe thermal injury is well known to induce one of the most profound hypermetabolic and hypercatabolic states observed in clinical medicine. Recent studies have demonstrated that major burns initiate a sustained stress response characterized by excessive secretion of catecholamines, cortisol, glucagon, and inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). These mediators markedly increase resting energy expenditure, oxygen consumption, protein turnover, gluconeogenesis, and lipolysis. Consequently, extensive degradation of skeletal muscle proteins and depletion of lean body mass occur to meet the increased metabolic demands associated with tissue repair and immune activation. As a result, burn patients and experimental animals commonly exhibit significant body weight loss during the initial days following injury.

In addition to hypermetabolism, burn-induced oxidative stress further contributes to metabolic imbalance by impairing mitochondrial function, reducing ATP synthesis, increasing reactive oxygen species (ROS) production, and promoting cellular apoptosis. Persistent oxidative stress and prolonged inflammation delay wound healing, suppress appetite, decrease nutrient utilization, and exacerbate muscle wasting. Therefore, therapeutic approaches capable of simultaneously reducing oxidative damage and promoting tissue regeneration are expected to improve both local wound healing and systemic metabolic recovery.

The superior body weight recovery observed in the gel-drug-PMS-treated group may be attributed to the combined therapeutic actions of sustained resveratrol release and the regenerative effects of the cytomodulin-functionalized porous microparticle system. Resveratrol is widely recognized for its potent antioxidant and anti-inflammatory activities, which reduce oxidative stress by scavenging reactive oxygen species and activating endogenous antioxidant defense pathways, particularly the Nrf2/HO-1 signaling pathway. Furthermore, resveratrol suppresses activation of the NF-κB signaling pathway, thereby reducing the production of pro-inflammatory cytokines and limiting secondary tissue damage. These effects collectively improve the wound microenvironment, preserve viable tissue, and accelerate progression from the inflammatory phase to the proliferative phase of wound healing.

The incorporation of porous microparticles further enhances therapeutic efficacy by providing sustained local drug delivery, maintaining prolonged antioxidant activity, and facilitating cellular infiltration, angiogenesis, and extracellular matrix remodeling. In addition, cytomodulin has been reported to stimulate fibroblast migration, collagen synthesis, and tissue regeneration through modulation of growth factor-mediated signaling pathways. These combined actions promote faster wound closure, reduce prolonged inflammation, and decrease the overall metabolic burden associated with tissue repair.

Improved wound healing ultimately reduces the prolonged hypermetabolic state that characterizes severe burn injury.

As tissue regeneration progresses, inflammatory activity declines, nutrient utilization becomes more efficient, and anabolic processes gradually predominate over catabolic pathways. Consequently, treated animals regain lean body mass and exhibit progressive increases in body weight. The significantly greater percentage body weight gain observed in the gel-drug-PMS-treated animals therefore reflects not only enhanced local burn wound healing but also improved

systemic metabolic recovery following thermal injury. These findings are consistent with recent reports demonstrating that antioxidant-based biomaterial dressings capable of sustained therapeutic delivery effectively attenuate burn-induced hypermetabolism, preserve muscle mass, and accelerate functional recovery in experimental burn models.

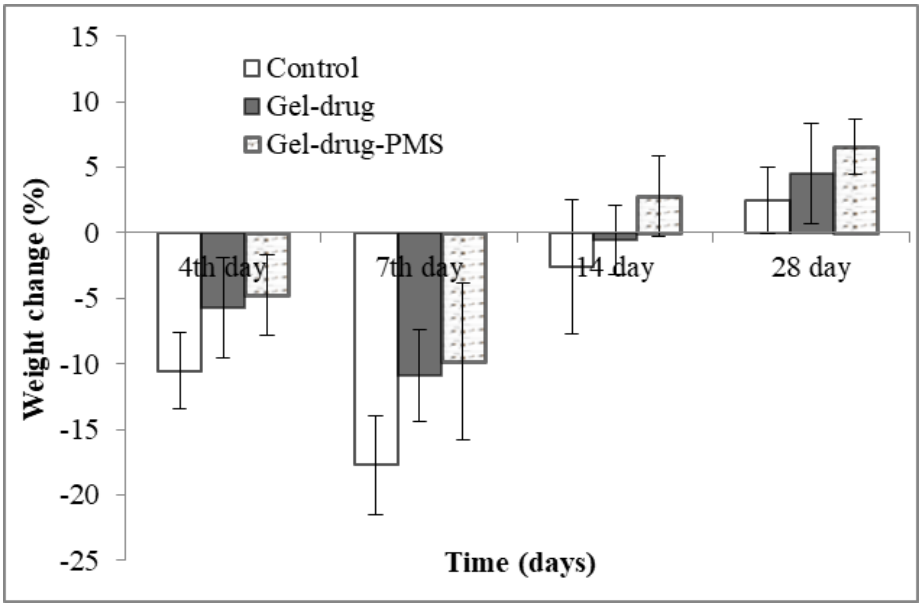

Figure 5: Comparison of % weight change of animals before and after treatment.

C. Change in feed consumption (%)

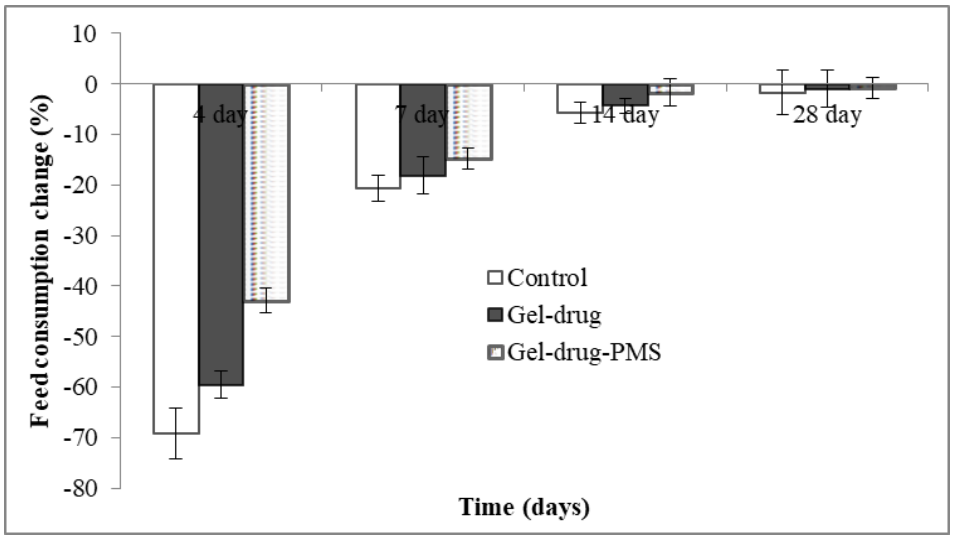

Figure 6: Comparison of % Feed change of animals before and after the treatment.

Feed consumption is an important physiological parameter that reflects the nutritional status, metabolic condition, and overall health of experimental animals following severe burn injury.[37] Thermal trauma induces profound systemic alterations that affect appetite, gastrointestinal function, energy metabolism, and nutrient utilization. Therefore, monitoring changes in feed intake during the post-burn period provides valuable information regarding the severity of the hypermetabolic response and the effectiveness of therapeutic interventions. In the present study, feed consumption was recorded at predetermined time intervals, and the percentage change in feed consumption was calculated to assess the recovery of animals receiving different treatments.

As shown in figure 6, burn injury resulted in a marked reduction in feed consumption in all experimental groups during the early post-burn period. The decrease in food intake was most evident during the initial days following thermal injury, reflecting the acute systemic response associated with severe burns. However, feed consumption gradually improved as the healing process progressed. Animals treated with the gel-drug-PMS formulation consistently exhibited the highest feed intake throughout the experimental period, whereas the gel-drug-treated group demonstrated an intermediate improvement. In contrast, the untreated control group showed the lowest recovery in feed consumption up to Day 28, indicating persistent metabolic stress and delayed recovery.

The reduction in food intake immediately after burn injury is a well-recognized physiological response observed in both experimental animals and burn patients. Recent studies have demonstrated that severe burns trigger a sustained hypermetabolic and hypercatabolic state mediated by activation of the sympathetic nervous system, hypothalamic-pituitary-adrenal axis, and systemic inflammatory pathways.[38] Excessive release of catecholamines, cortisol, glucagon, and inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), profoundly alters appetite regulation and energy homeostasis. These mediators influence hypothalamic centers responsible for hunger and satiety, resulting in anorexia, reduced voluntary food intake, and impaired gastrointestinal motility during the acute phase of burn injury.

In addition to neuroendocrine alterations, extensive oxidative stress generated after thermal injury further contributes to appetite suppression and metabolic dysfunction. Activated neutrophils, macrophages, and damaged mitochondria produce excessive reactive oxygen species (ROS), leading to lipid peroxidation, mitochondrial dysfunction, ATP depletion, and cellular injury. These changes increase resting energy expenditure while simultaneously reducing feeding behavior, thereby creating a negative energy balance. Consequently, burn-injured animals experience rapid depletion of glycogen stores, enhanced proteolysis, accelerated lipolysis, and progressive loss of lean body mass. The resulting protein-energy deficit delays collagen synthesis, angiogenesis, fibroblast proliferation, and immune cell function, ultimately impairing wound healing.[39]

Recent burn care literature emphasizes that early nutritional support is one of the most critical components of burn management because adequate caloric and protein intake helps attenuate the hypermetabolic response, preserve lean body mass, maintain immune competence, and support tissue regeneration. Restoration of normal feeding behavior therefore serves as an indirect indicator of metabolic stabilization and successful recovery from burn injury. Improved appetite generally reflects reduced systemic inflammation, normalization of endocrine responses, improved gastrointestinal function, and enhanced overall well-being.

The significantly greater feed consumption observed in animals treated with the gel-drug-PMS formulation suggests that the developed composite dressing effectively reduced both local tissue damage and systemic metabolic disturbances. The sustained release of resveratrol from the gelatin matrix provides prolonged antioxidant protection by scavenging excessive reactive oxygen species and enhancing endogenous antioxidant defense mechanisms through activation of the Nrf2/HO-1 pathway. Simultaneously, resveratrol suppresses activation of the NF-κB signaling pathway, thereby decreasing the production of pro-inflammatory cytokines responsible for prolonged inflammation and metabolic dysfunction. Reduction of oxidative stress and inflammatory burden accelerates the transition from the inflammatory phase to the proliferative phase of wound healing, thereby reducing the overall physiological stress associated with burn injury.

Furthermore, the incorporation of cytomodulin-functionalized porous microparticles provides additional regenerative benefits by promoting fibroblast proliferation, collagen deposition, extracellular matrix remodeling, and angiogenesis.[40] The porous architecture facilitates oxygen diffusion, nutrient transport, and cellular infiltration, creating a favorable microenvironment for tissue regeneration. Accelerated wound repair reduces the duration of the hypermetabolic state, allowing metabolic processes to gradually shift from catabolism toward anabolism. As systemic inflammation subsides and tissue repair progresses, gastrointestinal function improves, appetite returns, and nutrient utilization becomes more efficient, resulting in increased voluntary feed intake.

The intermediate improvement observed in the gel-drug-treated group indicates that sustained antioxidant delivery alone was beneficial in reducing oxidative damage and promoting healing. However, the superior performance of the gel-drug-PMS formulation demonstrates the additional regenerative advantage provided by the cytomodulin-coupled porous microparticle system, which enhanced tissue repair beyond that achieved by antioxidant therapy alone.

Overall, the increased feed consumption observed in the gel-drug-PMS-treated animals indicates improved metabolic recovery following severe thermal injury. Restoration of normal feeding behavior is closely associated with attenuation of burn-induced hypermetabolism, reduced inflammatory stress, preservation of body protein stores, enhanced immune function, and accelerated wound healing. These findings are in agreement with recent experimental and clinical studies demonstrating that antioxidant-loaded biomaterial dressings capable of sustained therapeutic release not only accelerate local wound repair but also improve systemic nutritional status and metabolic recovery after burn injury. Therefore, the enhanced feed consumption observed in the gel-drug-PMS group further supports the therapeutic potential of the developed composite graft for the management of full-thickness burn wounds.

D. Collagen content determination

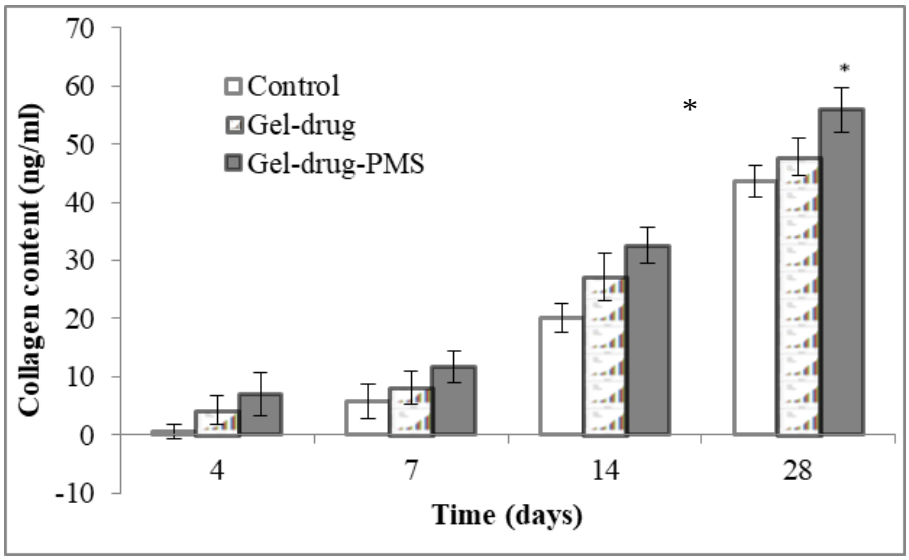

Figure 7: Collagen content (ng/ml) of granulation tissue taken out from control and both treated group (n=3), *p<0.05 vs control.

Wound healing is a highly coordinated and dynamic biological process through which damaged tissue is restored structurally and functionally as close as possible to its pre-injury state. Contemporary wound healing research recognizes this process as consisting of four overlapping phases: hemostasis, inflammation, proliferation, and

remodeling.[41] Successful progression through these phases depends upon several factors, including the severity and depth of the injury, the extent of microbial contamination, tissue oxygenation, nutritional status, immune competence, vascular supply, and the patient's overall metabolic condition. In full-thickness burn wounds, the complete destruction of the epidermis and dermis results in extensive extracellular matrix (ECM) damage, making collagen synthesis and matrix remodeling essential for successful tissue regeneration.

The proliferative phase of wound healing is characterized by the formation of granulation tissue**,** which consists primarily of proliferating fibroblasts, newly formed capillaries, inflammatory cells, extracellular matrix proteins, and a hydrated ground substance.[42] Mesenchymal stem cells and fibroblast precursor cells migrate from the wound margins into the injured area in response to chemotactic growth factors such **as** transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF).

Activated fibroblasts subsequently proliferate within the wound bed and synthesize extracellular matrix components, including collagen, fibronectin, elastin, hyaluronic acid, and proteoglycans, which collectively provide the structural framework required for tissue regeneration.

Among these extracellular matrix components**,** collagen, particularly Type I collagen, is the most abundant structural protein in normal skin and is considered the principal determinant of wound strength and mechanical stability.[43]

Type I collagen constitutes approximately 80–90% of the collagen present in mature dermis, providing tensile strength, elasticity, and structural integrity to the regenerated tissue. During early wound healing, fibroblasts initially synthesize Type III collagen, which forms a temporary extracellular matrix. As healing progresses into the remodeling phase, Type III collagen is gradually replaced by the stronger and more highly organized Type I collagen, resulting in increased tensile strength and improved functional restoration of the skin. Therefore, collagen Type I is widely accepted as one of the most reliable biochemical markers for evaluating wound maturation and the quality of tissue repair.

Recent studies have demonstrated that collagen deposition is a tightly regulated process involving a balance between collagen synthesis by fibroblasts and collagen degradation by matrix metalloproteinases (MMPs). Under normal physiological conditions, tissue inhibitors of metalloproteinases (TIMPs) regulate MMP activity, ensuring appropriate extracellular matrix remodeling. However, severe burn injury disrupts this balance by generating excessive reactive oxygen species (ROS) and inflammatory cytokines that stimulate MMP expression while suppressing fibroblast proliferation and collagen synthesis. Consequently, prolonged oxidative stress delays extracellular matrix deposition, weakens granulation tissue formation, and compromises wound tensile strength. Therefore, therapeutic strategies capable of reducing oxidative stress while stimulating fibroblast activity have become increasingly important in modern regenerative medicine.

In the present study, collagen Type I content in the regenerated skin tissue was quantitatively determined using a Rat Collagen Type I ELISA Kit, and the results were expressed **as** ng/mL. The collagen concentration of animals treated with the gel-drug-PMS composite graft was compared with those receiving the gel-drug formulation and the untreated control group at different post-burn time points.

As illustrated in figure 7, collagen Type I levels progressively increased throughout the healing period in all experimental groups; however, the magnitude of collagen deposition varied significantly according to the treatment administered. The gel-drug-PMS-treated group demonstrated a significantly greater collagen Type I concentration than both the gel-drug-treated and untreated control groups at every observation period. The gel-drug group also exhibited higher collagen levels than the untreated control, although the increase was less pronounced than that observed with the composite graft. These findings indicate that the developed biomimetic composite graft substantially enhanced extracellular matrix formation and accelerated dermal regeneration during burn wound healing.

The increased collagen deposition observed in the gel-drug-PMS group is likely the result of multiple complementary biological mechanisms. Sustained release of resveratrol effectively reduces oxidative stress by scavenging reactive oxygen species and activating endogenous antioxidant defense systems through the Nrf2/HO-1 signaling pathway.

Simultaneously, resveratrol suppresses activation of the NF-κB pathway, thereby decreasing the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. Reduction of prolonged inflammation creates a favorable microenvironment for fibroblast proliferation, migration, and extracellular matrix synthesis. Numerous recent investigations have demonstrated that resveratrol also promotes angiogenesis, enhances fibroblast survival, stimulates collagen biosynthesis, and accelerates re-epithelialization by modulating signaling pathways including TGF-β/Smad, PI3K/Akt, MAPK/ERK, and SIRT1**,** all of which contribute to improved wound repair.

An additional factor contributing to the superior collagen deposition is the presence of the cytomodulin-functionalized porous biomimetic scaffold. Biomimetic scaffolds are designed to closely resemble the architecture and biochemical characteristics of the native extracellular matrix, thereby providing an optimal substrate for cellular attachment, migration, proliferation, and differentiation. The interconnected porous structure facilitates oxygen diffusion, nutrient transport, waste removal, vascular infiltration, and fibroblast colonization throughout the wound bed.[44] Furthermore, bioactive peptide molecules such as cytomodulin may interact with growth factor receptors, particularly components of the TGF-β signaling pathway, enhancing fibroblast activation and stimulating extracellular matrix production.

Increased expression of α-smooth muscle actin (α-SMA), enhanced fibroblast-to-myofibroblast differentiation, and improved collagen fiber organization have all been associated with accelerated wound contraction and improved tissue remodeling in recent regenerative medicine studies.

Collagen deposition is directly correlated with fibroblast proliferation because fibroblasts represent the primary cellular source of extracellular matrix proteins during wound healing. Consequently, any intervention capable of increasing fibroblast survival and proliferative activity is expected to increase collagen biosynthesis and granulation tissue formation. The significantly higher collagen Type I content observed in the composite graft-treated animals therefore suggests enhanced fibroblast function and more efficient extracellular matrix remodeling. Increased collagen accumulation also contributes to improved mechanical strength, wound contraction, basement membrane reconstruction, and re-epithelialization, ultimately leading to superior structural and functional restoration of the injured skin.

Overall, the present findings demonstrate that the gel-drug-PMS composite graft markedly enhanced collagen Type I deposition compared with the gel-drug formulation and untreated control. The improved collagen synthesis reflects accelerated granulation tissue formation, enhanced extracellular matrix remodeling, and superior dermal regeneration.

These observations are consistent with recent literature demonstrating that antioxidant-loaded biomimetic scaffolds capable of sustained therapeutic delivery significantly stimulate collagen production, promote angiogenesis, regulate growth factor signaling, and improve the quality of burn wound healing. Therefore, the increased collagen Type I content observed in the gel-drug-PMS-treated group provides strong evidence for the enhanced regenerative potential of the developed composite graft in the management of full-thickness burn injuries.

CONCLUSION

The present in vivo investigation demonstrated that the developed drug-loaded porous microparticle composite graft (gel-drug-PMS) significantly enhanced the healing of full-thickness burn wounds compared with both the gel-drug formulation and the untreated control. The therapeutic efficacy of the developed formulation was evaluated using a standardized hot metallic rod-induced third-degree burn rat model over a period of 28 days, which closely mimics the pathological features of severe thermal injury, including extensive tissue necrosis, oxidative stress, prolonged inflammation, delayed extracellular matrix remodeling, and impaired skin regeneration.[45]

Burn wound healing was comprehensively assessed through visual wound examination, percentage wound closure, body weight change, feed consumption, and collagen Type I estimation**,** thereby providing information regarding both local tissue repair and systemic physiological recovery. The gel-drug-PMS-treated group exhibited the most favorable healing response throughout the experimental period. By the end of the study, the composite graft achieved approximately 76% wound closure, compared with 70% in the gel-drug-treated group and only 60% in the untreated control group. Gross visual examination further confirmed faster wound contraction, earlier granulation tissue formation, improved re-epithelialization, and better overall wound appearance in animals treated with the composite graft. These findings indicate that the developed biomimetic dressing effectively accelerated the progression of wound healing through the inflammatory, proliferative, and remodeling phases.

Recent advances in burn wound biology have established that excessive production of reactive oxygen species (ROS) following thermal injury is one of the principal causes of delayed healing.[46] Oxidative stress amplifies inflammatory responses, damages cellular membranes, impairs fibroblast function, suppresses angiogenesis, and disrupts extracellular matrix remodeling. Therefore, current burn management strategies increasingly emphasize antioxidant-based therapies capable of simultaneously reducing oxidative injury and promoting tissue regeneration. The superior wound healing observed in the gel-drug-PMS-treated animals is consistent with these contemporary concepts and suggests that sustained local delivery of the antioxidant effectively attenuated oxidative stress during the critical early stages of burn healing.

The significant improvement in body weight and feed consumption observed in the composite graft-treated animals further indicates that the developed formulation not only accelerated local wound repair but also improved systemic recovery following severe burn injury. Thermal burns are known to induce a prolonged hypermetabolic and hypercatabolic state, characterized by increased energy expenditure, skeletal muscle protein degradation, loss of lean body mass, anorexia, and persistent inflammation. Recent literature has demonstrated that successful attenuation of

oxidative stress and inflammatory signaling contributes to normalization of metabolic function, improved appetite, enhanced nutrient utilization, and preservation of body mass. The greater recovery in body weight and feed intake observed in the gel-drug-PMS group therefore reflects improved physiological status, reduced metabolic stress, and accelerated overall recovery following thermal trauma.

An important finding of the present study was the significant increase in collagen Type I content observed in the composite graft-treated animals compared with both the gel-drug and untreated control groups. Collagen Type I is the predominant structural protein of mature dermal tissue and serves as a reliable biochemical marker of wound maturation and extracellular matrix remodeling. Enhanced collagen deposition indicates improved fibroblast proliferation, extracellular matrix synthesis, angiogenesis, and dermal regeneration. Contemporary studies have demonstrated that successful burn healing depends upon coordinated activation of multiple signaling pathways, including TGF-β/Smad, PI3K/Akt, MAPK/ERK, VEGF, and Nrf2/HO-**1**, which regulate fibroblast migration, collagen biosynthesis, neovascularization, antioxidant defense, and tissue remodeling. The increased collagen content observed in the present study strongly suggests that the composite graft created a favorable regenerative microenvironment that supported efficient extracellular matrix reconstruction and functional skin repair.

The improved therapeutic outcome achieved with the gel-drug-PMS composite graft is likely attributable to the synergistic interaction between its individual components. The sustained release of the encapsulated antioxidant provided prolonged protection against oxidative damage throughout the healing process, while the porous biomimetic scaffold offered a three-dimensional extracellular matrix-like architecture that facilitated cellular attachment, migration, oxygen diffusion, nutrient transport, and vascular infiltration. In addition, the bioactive peptide-functionalized microparticles likely promoted fibroblast activation, collagen synthesis, and tissue remodeling through growth factor-mediated signaling pathways. Such multifunctional biomaterial systems represent an emerging direction in regenerative medicine because they simultaneously provide structural support, controlled drug delivery, and biological stimulation of tissue repair.

Recent developments in wound tissue engineering have shifted the focus from conventional passive wound dressings toward bioactive smart biomaterials capable of modulating inflammation, scavenging reactive oxygen species, stimulating angiogenesis, regulating extracellular matrix remodeling, and promoting complete skin regeneration.[47]

The findings of the present investigation support this evolving therapeutic concept and demonstrate that the developed composite graft possesses several characteristics desirable for an advanced burn wound dressing, including sustained drug release, antioxidant activity, enhanced collagen deposition, improved wound contraction, and better systemic recovery.

Overall, the present study establishes that the drug-loaded porous microparticle composite graft (gel-drug-PMS) provides superior healing efficacy compared with the gel-drug formulation and untreated control in a full-thickness burn model. The formulation effectively accelerated wound closure, improved body weight recovery, restored feed consumption, and significantly enhanced collagen Type I deposition, indicating improved dermal regeneration and extracellular matrix remodeling. Collectively, these findings demonstrate that the developed biomimetic composite graft represents a promising therapeutic platform for the treatment of severe burn injuries and may serve as a potential candidate for future translational studies aimed at clinical burn wound management. Further investigations involving

detailed molecular mechanisms, immunohistochemical analyses, long-term safety evaluation, and clinical validation are warranted to establish its therapeutic applicability in human burn care.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The *in vivo* burn wound healing study was conducted following approval from the Institutional Animal Ethics Committee (IAEC), Pinnacle Biomedical Research Institute (PBRI), Bhopal (Registration No.: 1824/PO/RcBi/S/2015/CCSEA; Protocol Approval No.: PBRI/IAEC/08-05-2026/017). All experimental procedures involving animals were performed in strict accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India, to ensure ethical handling and welfare of the experimental animals.

CONSENT FOR PUBLICATION

All the authors read and approve the final version of manuscript for publication.

AVAILABILITY OF DATA AND MATERIALS

Data will be made available when required.

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CONFLICT OF INTEREST

All the authors declare no conflict of interest either financial or personal.

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Author Contributions:

RR conceptualizes the research and methodology of the experiments, DK written original draft of the manuscript. VK performed formal analysis and investigations.

REFERENCES

1. Żwierełło W, Piorun K, Skórka-Majewicz M, Maruszewska A, Antoniewski J, Gutowska I. Burns: classification, pathophysiology, and treatment: a review. International journal of molecular sciences, 2023; 24(4): 3749.
2. Ji S, Xiao S, Xia Z, Burns CBATRo, Trauma Committee C-SMEAoC. Consensus on the treatment of second-degree burn wounds (2024 edition). Burns & trauma, 2024; 12: tkad061.
3. Mahajan N, Soker S, Murphy SV. Regenerative medicine approaches for skin wound healing: from allografts to engineered skin substitutes. Current Transplantation Reports, 2024; 11(4): 207-21.
4. Chen J, Fan Y, Dong G, Zhou H, Du R, Tang X, et al. Designing biomimetic scaffolds for skin tissue engineering. Biomaterials science, 2023; 11(9): 3051-76.
5. Gounden V, Singh M. Hydrogels and wound healing: current and future prospects. Gels, 2024; 10(1): 43.

6. Ukaegbu K, Allen E, Svoboda KK. Reactive oxygen species and antioxidants in wound healing: mechanisms and therapeutic potential. International Wound Journal, 2025; 22(5): e70330.
7. Joorabloo A, Liu T, editors. Recent advances in reactive oxygen species scavenging nanomaterials for wound healing. Exploration, 2024: Wiley Online Library.
8. Bejenaru LE, Biţă A, Belu I, Segneanu A-E, Radu A, Dumitru A, et al. Resveratrol: A review on the biological activity and applications. Applied Sciences, 2024; 14(11): 4534.
9. Salla M, Karaki N, El Kaderi B, Ayoub AJ, Younes S, Abou Chahla MN, et al. Enhancing the bioavailability of resveratrol: combine it, derivatize it, or encapsulate it? Pharmaceutics, 2024; 16(4): 569.
10. Hatami T, de Castro KC, Rodrigues J, Martinez EF, Mei LHI. Resveratrol-loaded injectable hydrogels for enhanced therapeutic efficacy. Journal of Drug Delivery Science and Technology, 2026: 108070.
11. Segneanu A-E, Bejenaru LE, Bejenaru C, Blendea A, Mogoşanu GD, Biţă A, et al. Advancements in hydrogels: a comprehensive review of natural and synthetic innovations for biomedical applications. Polymers, 2025; 17(15): 2026.
12. Szwed-Georgiou A, Płociński P, Kupikowska-Stobba B, Urbaniak MM, Rusek-Wala P, Szustakiewicz K, et al. Bioactive materials for bone regeneration: biomolecules and delivery systems. ACS biomaterials science & engineering, 2023; 9(9): 5222-54.
13. Hao Z-W, Zhang Z-Y, Wang Z-P, Wang Y, Chen J-Y, Chen T-H, et al. Bioactive peptides and proteins for tissue repair: microenvironment modulation, rational delivery, and clinical potential. Military Medical Research, 2024; 11(1): 75.
14. Ciftci F. Release kinetics modelling and in vivo-vitro, shelf-life study of resveratrol added composite transdermal scaffolds. International Journal of Biological Macromolecules, 2023; 235: 123769.
15. Rahaman SM, Chandra A, Biswas R. RP-HPLC method development and validation for simultaneous estimation of curcumin and resveratrol in nano-micelle: dual drug dual form simultaneous estimation. Int J Appl Pharm, 2024; 16(3): 109-18.
16. Sharma R, Yeshwante S, Vallé Q, Hussein M, Thombare V, McCann SM, et al. Rat burn model to study full-thickness cutaneous thermal burn and infection. Journal of Visualized Experiments, 2022; 186.
17. Sikka MP, Bargir JA, Garg S. Modern developments in burn wound dressing. Research Journal of Textile and Apparel, 2025; 29(3): 487-506.
18. Yampolsky M, Bachelet I, Fuchs Y. Reproducible strategy for excisional skin-wound-healing studies in mice. Nature Protocols, 2024; 19(1): 184-206.
19. Alexandra C. Investigation of partial thickness burn injuries with a complex approach: from experimental rat model to meta-analysis of human data: University of Pécs (Hungary), 2023.
20. Bárcenas-García AE, Flores-Merino MV, Azorín-Vega EP, Jimenez-Mancilla NP, Delgadillo-Ramirez A, Oros-Pantoja R. Digital planimetry for assessing wound closure kinetics in a mouse model. JoVE (Journal of Visualized Experiments), 2025(215): e67475.
21. Pavlov S, Babenko N, Kumetchko M, Litvinova O, Mikhaylusov R. Features of metabolism in chronic wound remodelling. Scripta Medica, 2024; 55(1): 53-61.
22. Radzikowska-Büchner E, Łopuszyńska I, Flieger W, Tobiasz M, Maciejewski R, Flieger J. An overview of recent developments in the management of burn injuries. International journal of molecular sciences, 2023; 24(22): 16357.

23. Reed KK, Silverman AE, Abbaspour A, Burger KS, Bulik CM, Carroll IM. Energy expenditure during nutritional rehabilitation: A scoping review to investigate hypermetabolism in individuals with anorexia nervosa. Journal of Eating Disorders, 2024; 12(1): 63.
24. Muñoz-González PU, Lona-Ramos MC, Gutiérrez-Verdín LD, Luévano-Colmenero GH, Tenorio-Rocha F, García-Contreras R, et al. Gel dressing based on type I collagen modified with oligourethane and silica for skin wound healing. Biomedical Materials, 2022; 17(4): 045005.
25. Lin C, Guo X, Mo F, Sun D. Different dimensional copper-based metal–organic frameworks with enzyme-mimetic activity for antibacterial therapy. International Journal of Molecular Sciences, 2023; 24(4): 3173.
26. Fadilah NIM, Phang SJ, Kamaruzaman N, Salleh A, Zawani M, Sanyal A, et al. Antioxidant biomaterials in cutaneous wound healing and tissue regeneration: A critical review. Antioxidants, 2023; 12(4): 787.
27. Khan A, Mostafa HM, Almohammed KH, Singla N, Bhatti Z, Siddique MI. Advances in nanotechnology in drug delivery systems for burn wound healing: a review. Biomedical and Pharmacology Journal, 2025; 18(1): 373-86.
28. Rossella E, Giulio M, Michele M. Burns: classification and treatment. Textbook of plastic and reconstructive surgery: basic principles and new perspectives: Springer, 2022; p. 285-301.
29. Penatzer JA, Srinivas S, Thakkar RK. The role of macrophages in thermal injury. International Journal of Burns and Trauma, 2022; 12(1): 1.
30. Harvanová G, Duranková S, Bernasovská J. The role of cytokines and chemokines in the inflammatory response. Alergologia Polska-Polish Journal of Allergology, 2023; 10(3): 210-9.
31. Diller RB, Tabor AJ. The role of the extracellular matrix (ECM) in wound healing: a review. Biomimetics, 2022; 7(3): 87.
32. Younesi FS, Miller AE, Barker TH, Rossi FM, Hinz B. Fibroblast and myofibroblast activation in normal tissue repair and fibrosis. Nature Reviews Molecular Cell Biology, 2024; 25(8): 617-38.
33. Kaur G, Narayanan G, Garg D, Sachdev A, Matai I. Biomaterials-based regenerative strategies for skin tissue wound healing. ACS Applied Bio Materials, 2022; 5(5): 2069-106.
34. Liu Z, Fang Y. Wound healing and signaling pathways. Open Life Sciences, 2025; 20(1): 20251166.
35. Azadi S, Yazdanpanah MA, Afshari A, Alahdad N, Chegeni S, Angaji A, et al. Bioinspired synthetic peptide-based biomaterials regenerate bone through biomimicking of extracellular matrix. Journal of Tissue Engineering, 2024; 15: 20417314241303818.
36. Wang ZE, Zheng JJ, Feng JB, Wu D, Su S, Yang YJ, et al. Glutamine relieves the hypermetabolic response and reduces organ damage in severe burn patients: a multicenter, randomized controlled clinical trial. Burns, 2022; 48(7): 1606-17.
37. Osborne TJ. Understanding metabolic function after non-severe burn injuries in adults: Murdoch University, 2024.
38. Santelis SJ. The endocrine response to burn injuries: the role of the hypothalamic-pituitary hormones. Burn Care Prevent J., 2022; 2(1): 3-7.
39. Mittal A, Choudhary S, Anshul, Ashwani, Gugulothu D, Chauhan MK. Molecular Nutrition in Wound Healing. Therapeutics and Molecular Nutrition, 2026: 211-46.
40. Abuelella KE, Gaid SN, Abd El-Moniem Amer A. Poly (lactic-co-glycolic acid) in Wound Repair: A Multifunctional Platform for Targeted Drug Delivery and Tissue Regeneration. Bulletin of Pharmaceutical Sciences Assiut University, 2025.

41. Sindhu PE, Taj T, Fakrudheen F, Sahana T, Ahmed MG. Introduction to wound healing: Factors affecting, phases, and principles of wound healing. Nanomaterials for Wound Healing: CRC Press, 2025; p. 1-18.
42. Branch S. The Wound Healing Process. Carrier-mediated Gene and Drug Delivery for Dermal Wound Healing, 2023.
43. Lin P, Zhang G, Li H. The role of extracellular matrix in wound healing. Dermatologic Surgery, 2023; 49(5S): S41-S8.
44. Mukasheva F, Adilova L, Dyussenbinov A, Yernaimanova B, Abilev M, Akilbekova D. Optimizing scaffold pore size for tissue engineering: insights across various tissue types. Frontiers in bioengineering and biotechnology, 2024; 12: 1444986.
45. Bebiano LMB. Bioprinting strategies to engineer biomimetic 3D models of skin burn wounds, 2022.
46. Chen R, Xu H, Li X, Dong J, Wang S, Hao J, et al. Role of oxidative stress in post-burn wound healing. Burns & Trauma, 2025; 13: tkaf040.
47. Li G, Lu B, Weng Y, Ma Z, Zhang X. Engineering bioactive materials for diabetic wound healing. Biomedicine & Pharmacotherapy, 2026; 196: 119141.