The Efficacy of Topical Bacteriophage Semisolid Preparation on Burn Wounds Infected by *Pseudomonas Aeruginosa*

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Abstract

Objective:

Recently, antibiotic resistance of post-burn infections caused by opportunistic pathogens, *Pseudomonas aeruginosa*, became complicated due to its innate and acquired resistance. Bacteriophage therapy containing virulent factors that infect their specific host bacteria can be evaluated as an alternative treatment.In this study,the topical formulation contains lytic phages compared to the antibioticinthe murine model of burn/infected wound healing.

Methods & Materials:

Lytic bacteriophages were extracted from hospital sewage and propagated in broth culture of *P.aeruginosa*(24 hours, 37°C) and subsequently filtered.The collected phages were recultured alongside *P. aeruginosa*. The plaques were observed as clear zones and added to the polyethylene glycol (PEG) base ointment. Twenty-four adult female mice were selected and divided into four groups. Asecond-degree burn wound wascreated on the back of the mice and infected with 100 microliters of 1×10^2 - 3×10^2 CFU/ml *P. aeruginosa* subcutaneously. After 24 hours, each group received one of these interventions: silver sulfadiazine, ointment contains bacteriophage, ointment without bacteriophage (PEG group),or no treatment. Burn wound size, physical activity, and body temperature (rectal) were recorded every other day. On the 10^{th} day, mice were sacrificed through cervical dislocation. Thewound's skin was cut and evaluated histopathologically.

Results:

Significant differences inthe burn wound sizeamongthe bacteriophage groupversus the PEG group, the bacteriophage group versus the no-treatment group, and the antibiotic group versus the PEG group (P= 0.001, P= 0.001, P= 0.002 respectively)were observed. Mice's physical activity was gradually improved in all groups and showed significant differences (P<0.001). Body temperature analyses showed significantdifferences only when day 8^{th} compared with day 2^{nd}, $4^{th,}$ and 6^{th} (P=0.001, P=0.02, P=0.02 respectively). Histopathological results indicated optimal wound healing in both the antibiotic group and bacteriophage group. However, no significant differences were observed in microscopic histopathological criteria in any groups based on Fisher's exact statistical tests.

Conclusion:

Formulated phage ointmenteffectivelyprevents and treats burn wound infection in mice with no allergic reactions.

Introduction

Nowadays, burn wound infections have become a major problem in patients suffering from third-degree burns, and the survival rate depends on the severity of the burn and the types of post-burn infections (1). Burned patients are usually hospitalized for an extended period; consequently, they are susceptible to post-burn infections caused by multidrug-resistance pathogens. Due to the destruction of the stratum corneum layer in burn wounds, immunosuppression and post-microbial infections are considered the leading cause of mortality in burned patients. Burn wound provides large enriched site includes necrotic tissues and protein wound exudate, for bacterial growth (2, 3). On the other hand, in the burn's site, lack of blood circulation interferes with the wound healing process and reduces the efficacy of many delivery routes (1).

Pseudomonas aeruginosa is an opportunistic aerobic Gram-negative pathogen of man and animals. It is the leading cause of many complicated infections such as ear and eye infections, resistant post-burn infections, and reject skin grafts (4). *P. aeruginosa* is resistant to the most efficacious antibiotics and rapidly acquires new resistance mechanisms.

Due to the increased multidrug-resistance bacteria species that develop post-burn infections, obtaining an alternative treatment is necessary. Bacteriophages, the most ubiquitous and ancient organisms on the earth, are small viruses in size 20–200 nm. These microorganisms are known as natural virulent factors that affect their host bacteria with no adverse effect on human or animal cells. Bacteriophage therapy can be considered a suitable alternative therapy versus antibiotics (5, 6) or in combination with antibiotics (7). Bacteriophages are divided into two groups based on their infection mechanism, the first group consists of lytic phages, and the other group is lysogenic phages. Lytic phages are a suitable type of bacteriophage used in the bacteriophage therapy process (8). Bacteriophage therapy provides higher specificity, a narrow spectrum of activity, higher safety, better tolerability, and inexpensive process, while antibiotic therapy showed more resistance and side effects (9). Topical formulations contain lytic phages such as ointments, creams, and lotions can be used to optimize wounds healing. These preparations are easy to apply and remove and provide more stability that doesn't need frequent applications (10).

A few studies have assessed the efficacy of topical bacteriophages administration in burn/ infected wounds (11, 12). In this research, a polyethylene glycol (PEG) ointment base contains phages was prepared, and its therapeutic potential was evaluated to treat burn/ infected wounds caused by *P. aeruginosa* in mice.

Methods And Materials

Phage preparation

Samples of hospital sewage were collected from the third pond of sewage in Ghaem Hospital of Mashhad city and filtered by membrane filter (pore size 0.45 µm).

P. aeruginosa PTCC 1074 (Persian Type Culture Collection, Iran) was inoculated in a sterile tube with 5 ml Tryptic Soy Broth (TSB, HiMedia, India), incubated in a shaker incubator at 37˚C and 180 rpm. Two ml of

the filtrated sewage were added to the tube and incubated overnight in a shaker incubator. After the incubation period, the tube contents were transferred to sterile tubes, centrifuged at 4500 × g for 5 min, and filtered by a membrane filter to remove bacterial residue.

Two plates with soft agar (Tryptic Soy Agar, HiMedia, India) were prepared. A hundred (100) µl of the filtrated suspension was mixed with 100 µl of *P. aeruginosa* overnight suspension, 2 ml of top agar was added. After mixing, transferred to plates containing soft agar and incubated for 24 hours. As a control plate, 100 µl of *P. aeruginosa* overnight suspension was mixed with 2 ml top agar and transferred to a soft agar plate and incubated for 24 hours at 37˚C.

After the incubation period, plates were examined, and the plates that contained bacteriophages were more transparent than the control plates. To provide a suitable titer of bacteriophages for addition to the PEG ointment, phage suspension was serially diluted into 12 dilutions (10^{-1} to 10^{-12}), and each dilution was cultured. Phage plaques were counted by the colony counter device. After washing the surface of the plate with the broth culture medium, 1.8 ml phage suspension was mixed with 10% v/v glycerol and stored at -70˚C.

Peg Ointment

Semisolid formulation of phage containing ointment (PEG 400/PEG 4000 = 8:4 (g)) as the main treatment, and formulation of PEG 400/PEG 4000 = 9:3 (g) without phage as carrier control, were prepared. In each formulation, phages were incorporated at 10^7 PFU/ml concentration in the base with trituration using a geometric dilution procedure to get a homogeneous mass.

For examining lytic phage stability in PEG ointments, an agar lawn plate of *P. aeruginosa* PTCC 1074 was prepared, and five serial dilutions of ointment were syringed onto the surface of the lawn. Plates were incubated for 24 h at 37˚C. The lytic ability of phages was indicated as clear zones on the surface of the agar plate. Ointment bases were also tested for thermo-stability and pH changes. The ointments were stored at 4˚C, 20–25˚C, and 40˚C, and their properties and pH changes were recorded at 0, 2. 4 months (13).

Animals And Primary Supportive Treatments

Animals were selected from the Animal Laboratory of Mashhad University of Medical Sciences, School of Pharmacy. Ethical terms and conditions were considered based on the Ethics Committee rules, and the punctual analgesic process was considered. Animals received a sufficient amount of food and water and were sacrificed by a painless scientific method, cervical dislocation. This research was completed after receiving an ethical authorization code: IR.MUMS.PHARMACY.REC.1397.063, from the Ethical Committee of Mashhad University of Medical Sciences.

Twenty-four female adult mice weighing 20–30 g were selected and divided into four groups (6 mice in each group). They were sedated by IP injection of ketamine (100 mg/kg)/xylazine (10 mg/kg) (14). The hair was clipped from the backs of sedated mice and then shaved.

An electric kettle and a plastic template were used to provide and conduct hot steam water on the back of the mice. Each mouse was directly contacted with a conduction steam tube for 10 seconds to induce a 15 × 15 mm burn wound on the shaved backs of the mice. Then all mice were received 0.5 ml dextrose saline serum IP to prevent hypovolemic shock. For analgesia effect, 0.25 mg/ml acetaminophen was dissolved in mice's drinking water (15). Half an hour later, 100 µl of 1×10^2 – 3×10^2 CFU/ml *P. aeruginosa* inoculum was injected subcutaneously to induce infection (16). Then each mouse was placed in a separate cage with a special tank for food and water to eliminate the risk of contact other mice's skin. The room temperature was regulated appropriately for the animals. All animals received 75 IU vitamin A, 20 IU vitamin $D_{3,}$ and 0.5 ml D/S serum for the next three days as primary supportive treatments.

Main Treatment Protocols

Twenty-four hours later, each group of mice received one of these treatments for ten days:

Group 1 (bacteriophage group): mice were received PEG base ointment (PEG 400/PEG 4000 = 8:4) containing 10^7 PFU/ml bacteriophage and applied to the infected burn wounds once a day.

Group 2 (positive control group): mice were received silver sulfadiazine ointment applied to the infected burn wounds once a day.

Group 3 (negative control group): mice received no treatment.

Group 4 (PEG ointment group): to ascertain that the carrier ointment won't have any therapeutic effect or interfere with the treatment process, mice were received PEG ointment (PEG 400/PEG 4000 = 9:3) that contained no bacteriophage once a day.

Skin Irritation Assessment

Eighteenhealthy mice were randomly assigned into the three groups; no ointment was applied to group I. In groups II and III, half a gram of PEG ointment and PEG+phage ointment were evenly and gently applied on the back shaved skin area of six mice in each test group one day before the experiment. The untreated skin area of the other six mice served as the control. During 24 hours, the test areas were examined for signs of irritation (the progress of the rash, inflammation, swelling, scaling, and abnormal tissue growth).

Morphological assessments

Burn wound size, body temperature (rectal), and physical activity were examined and recorded every other day. Burn wound's change percentage was calculated based on this formula: Burn wound's changes percentage (x) = [(burn wound's size in day 1st – burn wound's in day x)/ burn wound's size in day 1st)]×100 In this formula,the percentage of the positive change indicatesa reduction of the burn wound's size, and the percentage of the negative change indicatesan increasein the burn wound's size. Burn wound's surface (mm2) in all images measured through Digimizer software and calculated by Meeh formulation(17). Mice's physical activity scores are shown in Table1and scored based on Kumariet al.study(15).

Histopathological assessments

On the 10^{th} day, mice were sacrificed by the cervical dislocation method.The whole burn wound site was removed and immediately fixed in 4% formaldehyde (v/v) prepared in PBS (0.01 M, pH 7.2), followed by routine histological processing through dehydrated in graded alcohol, cleared in xylene, embedded at 58°C–60°C in paraffin, microtomy with 4-5 µm cuts, and hematoxylin & eosin staining.

Histopathological evaluation and scoring were done as the method described by Tkalcević*et al.*(18).

Granulation tissue was described as follows:

i. Immature granulation tissue: loose granulation tissue (macrophages, fibroblasts) with emerging vessels
ii. Mature granulation tissue: fibroblasts and sparse extracellular matrix proteins forming layers and vessels running perpendicular
iii. Fibrosis: extracellular matrix proteins (mainly collagen) dominating the granulation tissue, fewer fibroblasts, and vessels.

The score for each wound was given in a semi-quantitative manner with grades of 1–3 for each of the three granulation tissue categories.

1. Wound bed partially covered with granulation tissue
2. Thin granulation tissue over the whole wound bed
3. Thick granulation tissue over the whole wound bed.

Other features added to this study include:

-Grading of soft tissue necrosis (subcutaneous fat and skeletal muscle):

1. Severe necrosis involved more than half of subcutaneous soft tissue
2. Moderate necrosis involved 10-50% of subcutaneous soft tissue
3. Minimal to mild necrosis involved less than 10% of subcutaneous soft tissue

-Inflammatory score

1. Severe inflammation consists of extensive and condensed inflammatory cells
2. Moderate between 1 and 3
3. Minimal to mild inflammation, sparse inflammatory cells

-Reepithelializationscore:

1. Incomplete
2. Complete, thin epithelialization
3. Complete, near-normal epithelialization

Statistical analyzes

Data were analyzed using Stata 14 and SPSS 25 software through repeated measure analyses, chi-squared, and Fisher's exact with Bonferroni correction. P-value < 0.05 considered significant.

Results

Lytic phage related results

Clear zones created on the plate's surface confirmed that the bacteriophages were in the lytic phases (Fig. 1).

To ascertain whether bacteriophages formulated in the ointment can cause *P. aeruginosa* lysis or not, five serial dilutions of ointment were prepared and then syringed on the same plates' surface *P. aeruginosa* PTCC 1074 and incubated for 48 h at 37˚C. After the incubation period, the plate's surface was evaluated, and clear zones were observed, indicating the lysis of the host bacteria by phages formulated into the ointment. This stability test showed that lytic bacteriophages had the most efficacy in the first 24 hours, which are shown as the clearest zone on the surface of the agar plate (Fig. 2).

pH Measurements

pH changes in PEG-based ointments (PEG 400/PEG 4000 = 8:4 (g) and PEG 400/PEG 4000 = 9:3 (g)) at different temperatures are shown in Table 2 for four months. The results indicate no significant differences ($P < 0.05$) in pH changes at different temperatures (4, 25, 40˚C).

Animals

Mice's baseline characteristics are presented in Table 3. Although burn wound size shows no significant statistical differences between the three groups, examining the mean quantities declared over 10% mean differences between the three groups. Hence the burn wound's size on the 1st day was considered a

confounding factor, and data were evaluated through repeated measure ANCOVA. Further analyzes were done after this factor adjustment.

In burned/infected mice treated with a daily topical application of phage, protection as a 90.9% survival rate was detected compared to 45.5% survival in the untreated control group on the first post-wounding day ($P > 0.05$). On the second day onwards, daily topical application of phage ointment on the burned and infected area still resulted in significantly higher survival rates of 100% compared to 83.3% survival observed in the untreated control group and antibiotic-treated group (83.3%) till the 10th days past the treatment period.

Skin irritation assessment

Mice showed no irritation signs after treatment with ointments of phage. The treated skin was intact; no inflammation and redness compared to the untreated mice.

Wound Healing Evaluation

After considering burn wound area as a confounding factor, statistical test repeated measure ANCOVA displayed significant differences between 4 topical treatment groups ($F (3,104) = 6.91$, $P < 0.001$). However, no significant differences showed between the various time measurements ($F(4,106) = 0.40$, $P = 0.81$). Post-hoc comparison among the groups after Bonferroni correction indicated significant differences only when the phage group compared to the ointment-based group, the phage group versus the negative control group, and the antibiotic group compared to the ointment-based group ($P = 0.001$, $P = 0.001$, $P = 0.002$, respectively).

The percentage of wound size in different days compared with the first day was also recorded, and the results are in Table 4. After considering the burn wound area as a confounding factor, the statistical test repeated measure ANCOVA didn't show any significant differences between 4 topical treatment groups ($F (3,104) = 2.66$, $P = 0.052$). But no significant differences showed between the various time measurements ($F (4,104) = 0.15$, $P = 0.96$).

Figure 3 shows burn wound changes procedure during different days of treatment. Based on this Figure, in the phage and the antibiotic group, the mean of burn wound percentage altered with time and could be interpreted as proportional burn wound healing. In the PEG group, the percentage was not remarkably changed. In the negative control group that received no treatment, the burn wound even progressed during the treatment period and could not detect the wound skin's healing process.

Fisher's exact statistical test indicated no significant differences in physical activity between treatment groups ($P = 0.58$). Mice's physical activity was gradually improved, and its differences were statistically significant ($P < 0.001$).

Body temperature changed in various time measurements compared to the first day and between different groups (Table 5). After considering burn wound area as a confounding factor, statistical test repeated measure ANCOVA didn't show any significant differences in mean and SD of body temperature between the four topically treated groups ($F(3,104) = 1.71$, $P = 0.17$). Still, it indicated significant changes between the various time measurements ($F(4,104) = 3.35$, $P = 0.007$). Post-hoc comparison after Bonferroni correction indicated that significant alterations were taken when day 8th compared with day 2nd, day 4th, and day 6th ($P = 0.001$, $P = 0.02$, $P = 0.02$, respectively).

Histopathological Results

Histopathological results of burn wound tissue samples are shown in Table 6. Some of the qualitative data regarding histological evaluation according to Table 6 at day ten has been depicted in Figs. 4–7. The worst wound healing condition in all examined criteria found in the negative control group, while the antibiotic and the phage groups showed better wound healing parameters (Table 6). However, no significant differences were detected in histopathological criteria, based on Fisher's exact test.

Discussion

This paper is the first to investigate PEG-based hydrophilic ointments on a murine model of burn/infected wounds to the best of our knowledge. The current study indicates that semisolid bacteriophage preparation could promote the wound healing process as a topical agent and represent an attractive approach for treating burn wounds.

Post-burn infections became a severe life-threatening problem that the severity of the burn and type of infections determine the survival of patients. Shortly, most patients get infected with opportunistic bacteria from multidrug-resistance types and cause complicated infections. *P. aeruginosa*, an inherently multidrug-resistant pathogen, is known as one of the leading causes of life-threatening post-burn infections (19). In this study, lytic bacteriophages of *P. aeruginosa* were extracted and used against this pathogenic bacterium. As declared before, first, it was ensured about the bacteriophage plaques on the plate's surface based on their clear zones that proved the lytic phase formation (Fig. 1).

Bacteriophage therapy can consider as an alternative treatment for resistant infections due to its specificity for different bacterial species and its safety for the eukaryotic cells (1, 20). Phages can be bactericidal at low concentrations because of their self-replication property and their ability to increase exponentially in the presence of the bacterial host. Therefore, only a small number of phages can be sufficient to control bacterial infection (20) as our data determined a low effective bacteriophages concentration in wound healing. Merabishvili *et al.* study indicated that therapeutic bacteriophage preparations should have active particles in the range of 10^6-10^7 PFU/ml concentration (21). Since bacteriophages should have a high titer in the body in contact with suitable host bacteria, we considered 10^7 PFU/ml as an effective therapeutic dose.

Meanwhile, decreasing the blood circulation in the burn site causes many delivery routes to be less effective; we prefer to apply bacteriophages as a topical ointment. It is well known that topical delivery routes directly contact the antibacterial agent and the wound surface (1). Chang *et al.* studied different topical formulations. Traditionally gauze soaked in phage preparation being applied, but this structure is associated with some difficulties, such as phage may get stuck in the gauze, and the release of phages may be incomplete. Spray devices can also be used, but phages may get stuck in the device, too. Phages formulated in semisolid forms such as gels or creams or PEG ointment bases were more stable and reduced the limitations of the liquid formulations. Semisolid formulations could protect and hydrate the skin and be a suitable carrier for phages. They were easy to apply, washable with water, and the slightest irritation observed with them. However, alcohol may inactivate some phages, and water-based semisolid formulations are preferred over organic solvent-based ones. It was also determined that non-ionic vehicles have minimally intervened with phage releasing profile (22). Brown *et al.* came to a similar conclusion. They found that non-ionic carriers resulted in optimal recovery and more lytic phage stability than anionic or cationic vehicles. Ionic nature, specially anionic-based vehicle, could easily cause inactivation (23). Another study showed that preservatives might interfere with the phage releasing profile (24).

The aforementioned studies led us to use PEG ointments with no added preservatives as the most suitable carrier that minimally interfered with the phage structure or its releasing process. To reach the most effective formulation, the ointment was prepared every day without any preservatives. It was proved that lytic bacteriophages remain active and stable in PEG ointment bases. Moreover, topical phage application makes it less likely to be removed by the immune system. To ascertain that the carrier ointment won't have any therapeutic effect or interfere with the treatment process, a negative control group of mice received just PEG ointment once a day.

Holguin *et al.* showed mice with phage receiving immediately, or 45 min after inducing infection, survived. However, because many bacteria remain in the lesion, immunological response and skin mastocytosis happened, and the wound didn't recover completely. These findings were also confirmed histopathologically. In mice treated 24 h or 48 h after infection, lesions recovered very well (25). These results lead us to initiate treatment 24 h after inducing infection.

Herein, we showed that the topical formulation of phage is as effective as topical silver sulfadiazine (an FDA-approved product), which is in line with previous studies; Kumari *et al.* examined the efficacy of topical treatment with phage kpn5 compared to the natural product aloe vera and honey (12). Also, the same investigator reported the efficacy of topical phage kpn5 formulation compared to the topical gentamicin and silver nitrate (2). They concluded that hydrogel-based phage kpn5 showed significant efficacy in wound healing than natural or FDA-approved products.

The current results showed that phage combined with the PEG yields a similar protection level and wound healing obtained with standard antibiotic, silver sulfadiazine. We also indicated the lysis of the host

bacteria by phages formulated into the ointment (Fig. 2). It appears that the lytic phage was able to enter into the wound, thus eradicate infections.

Skin wound healing in murine models comprises skin contraction, which occurs by secondary intention, granulation, and reepithelialization (26, 27). In staining with hematoxylin/eosin, stained sections, PEG + phage treated group showed less inflammatory cells and better reepithelialization, as compared to the untreated control. Then it would be reasonable to suggest that topical application of phage promotes wound healing by effectively modulating cells involved in inflammatory and proliferative phases of healing. There is now a well-established suggestion that the principal effect of phages is anti-inflammatory and downregulating immune hyperactivity, which gives reason to hope for phage uses in medicine further than their well-known antibacterial action (28).

In this study, we determined the efficacy and safety of semisolid phage preparation. When topically applied daily on a burn wound area resulted in significantly higher survival rates of 100% comparing to 83/3% survival in untreated control mice over ten days of treatment. At the end of the experiment (day 10th) the percent survival in the PEG-treated group was less (83.3%) than survival seen in the group treated with PEG + phage (100%). The mice in all treatment groups did not show any sign of irritation when observed for 24 hrs before the experiment and a period of ten days. Although we determined the therapeutic potential of phages combined with no adverse reaction as a topical agent, it seems unlikely that phage therapy will ever replace antibiotics; however, it is a clear potential for it to be used as a complementary treatment. It is suggested that, in the future, semisolid phage preparation may be used along with the antibiotics to assess its potential when used in combination in treating burn wounds.

Conclusion

The results indicated that the prepared PEG ointment-loaded bacteriophage causes histopathological improvement with no allergic reactions and could be an effective alternative to silver sulfadiazine for burn wounds. The results of the bacteriophage therapy method can be compared with the antibiotic therapy method based on the research findings, especially in cases that the pathogens are resistant to the antibiotic. This method can be considered as an inexpensive therapeutic approach due to the source of bacteriophage extraction. Future research should concentrate on establishment the involved mechanism making phages effective in promoting burn wound healing. More experimental researches are also required to assess the best treatment duration and determine the proper formulation.

Declarations

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethics approval

Ethical approval for this study was obtained from Mashhad University of Medical Sciences Ethics Committee (ID: 960278).

Consent to Participate

Not applicable.

Consent for publication

Not applicable

Availability of data and material

The data sets used and/or analyzed during the current study are available from the corresponding author on request.

Code availability

Not applicable

Authors' Contributions

Piranaghl H collected data, analyzed the results, drafted the manuscript, and approved the final version of the manuscript.

Taherzadeh Zh, designing the project, analyzing the results, revising and approving the final version of the manuscript.

Golmohammadzadeh Sh, designing the project, analyzing the results, revising and approving the final version of the manuscript.

Soheili V, analysis of the results, revising and approval of the final version of the manuscript.

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Jalali SM, collecting data, revising, and approval of the final version of the manuscript.

Fazly Bazzaz BS, supervising and designing the project, analyzing the results, revising and approving the final version of the manuscript.

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Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

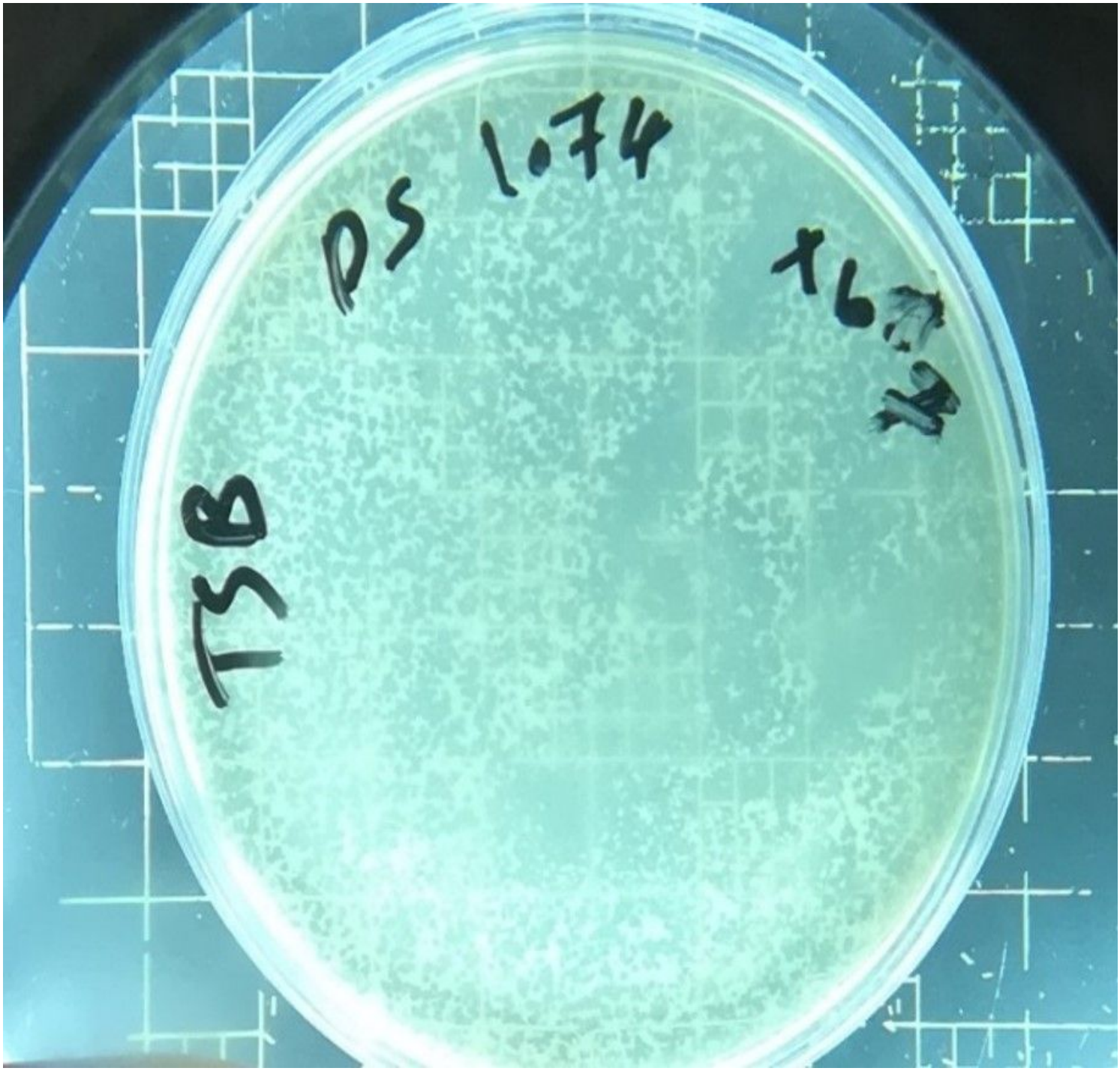

Figure 1

The bacteriophages plaques on the soft agar subculture of Pseudomonas aeruginos

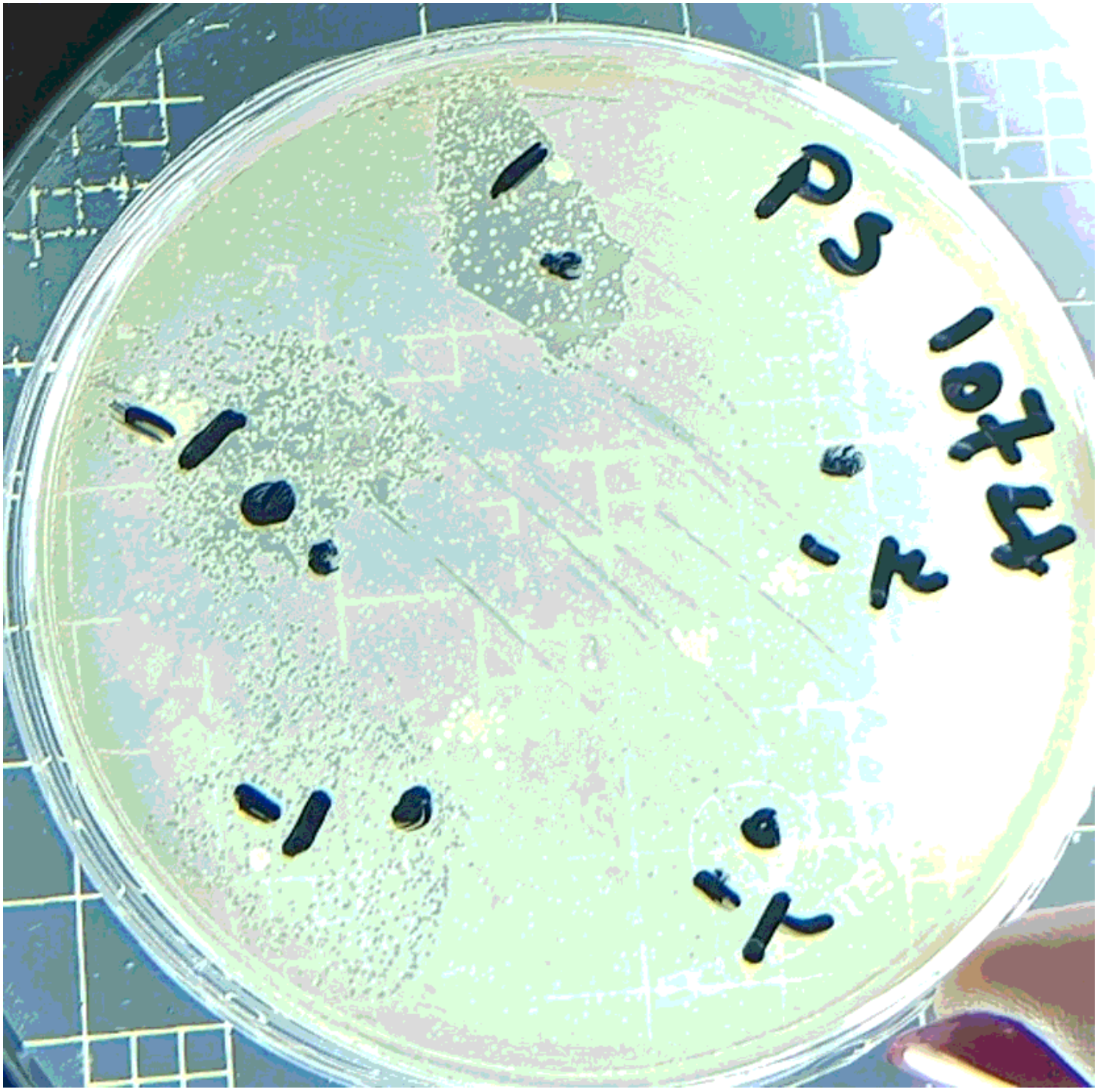

Figure 2

Lysis of Pseudomonasaeruginosa with lytic bacteriophages in PEG ointment PEG:polyethylene glycol

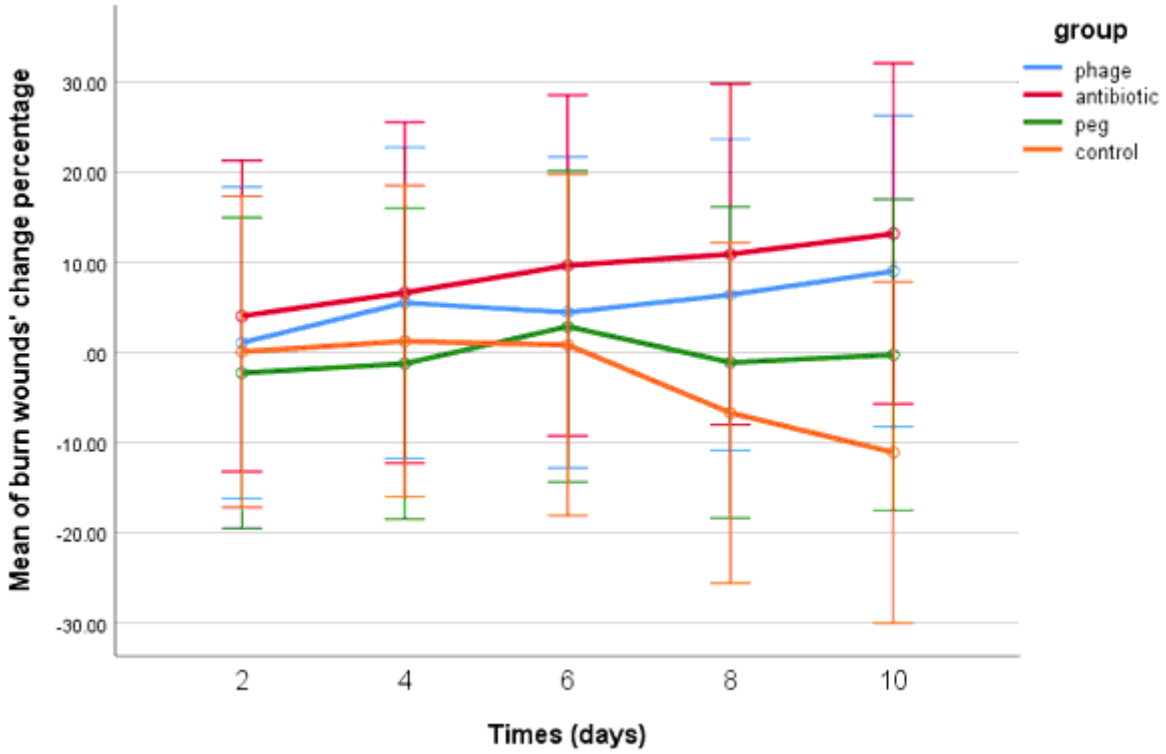

Figure 3

Comparative efficacy of different formulations on wound area contraction in the various timeofmeasurements in control(no treatment), standard (silver sulfadiazine), and treatment groups(PEG+Phges, PEGointment groups). Data are expressed as means ± SD; n= (6). All groups were compared to the control group according to repeated measure ANOVA.

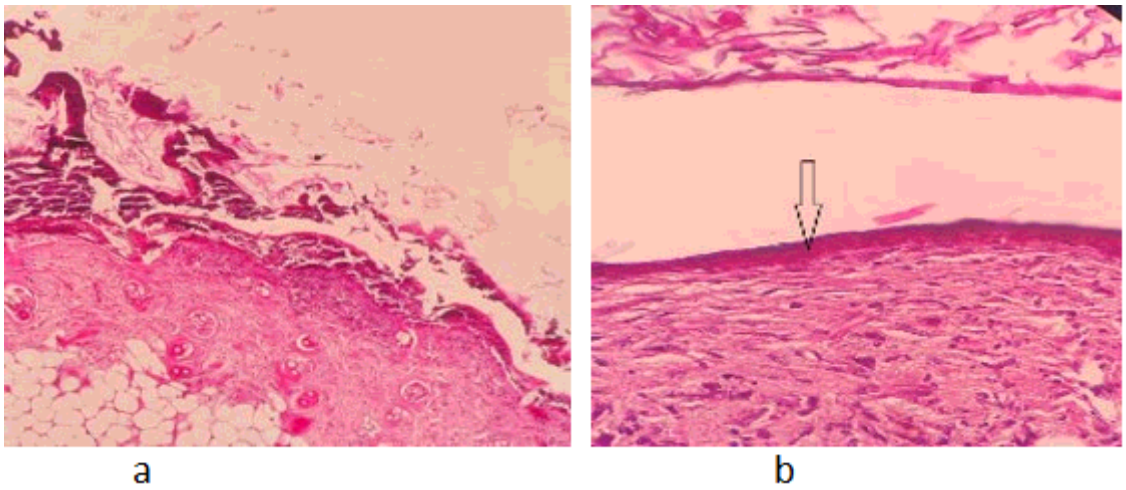

Figure 4

Hematoxylin/eosin staining(×100) of the burn tissue wound site show (A) skin ulcer covered by suppurative exudate and necrotic squamous cells(upper half) and granulation tissue formation in the dermis;(B) Early thin epithelialization (arrow), fibrotic granulation tissue in the dermis and sloughed keratin material in the top. Day 10 post wounding

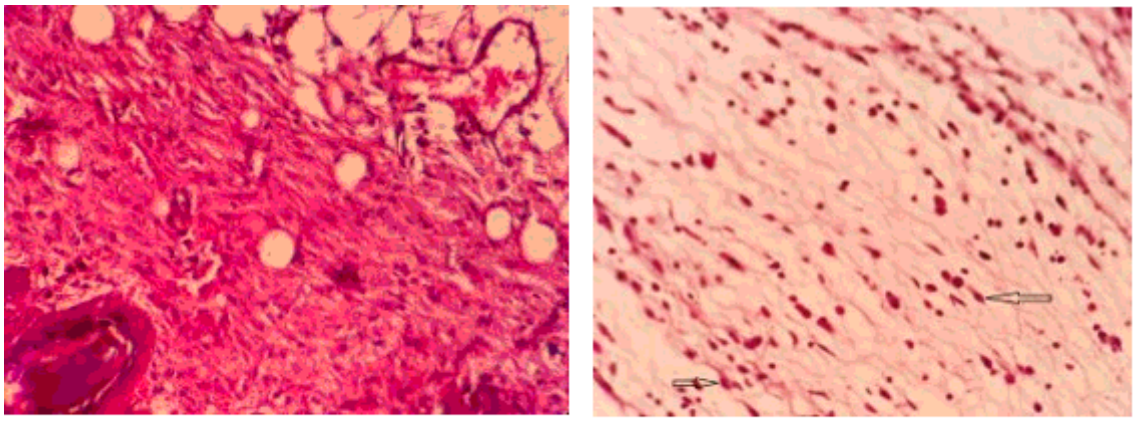

Figure 5

Hematoxylin/eosin staining(×400) of the burn tissue wound sites show (A) Mature fibrotic granulation tissue in hypodermis with the remnant of mature fat cells(lower right);(B) Immature granulation tissue consists of edematous stroma with few numbers of tissue culture –like fibroblasts(arrows), without significant collagen. Day 10 post wounding.

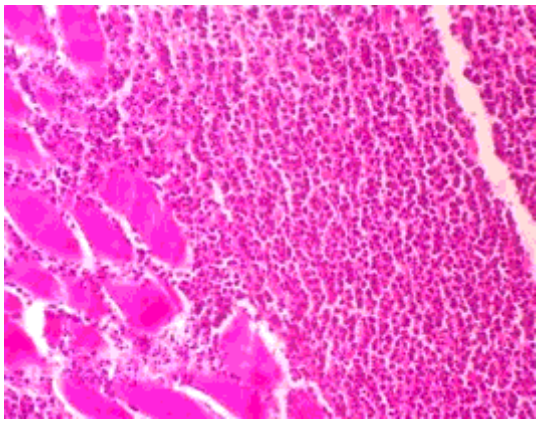

Figure 6

Hematoxylin/eosin staining(×400) of the burn tissue wound sites showed dense suppurative inflammation with an extension between necrotic striated muscle fibers(left third). Day 10 post wounding

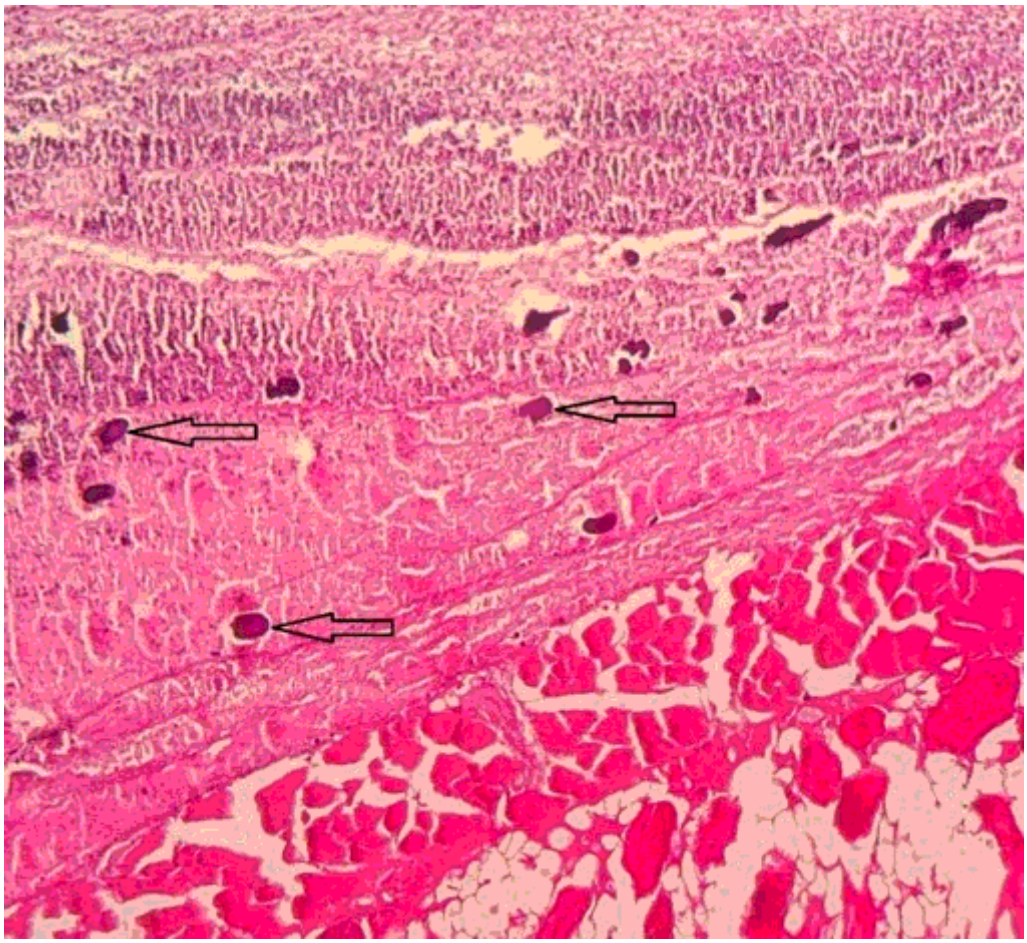

Figure 7

Hematoxylin/eosin staining(×400) of the burn tissue wound sites showed extensive necrosis in the epidermis and dermis replaced by suppurative exudate (upper half, foci of calcification(arrows), and necrotic skeletal (lower half and right); on day 10 post wounding.

Supplementary Files

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- Tables.docx