2018.04.09

Dear Editor,

Sincerest thanks for your response and reviewers’ comments on our manuscript “JoVE57942-Sever Burn Injury Swine Model for Clinical Dressing Assessment”. We sincerely apologise for the great time it has taken us to respond to these comments, and hope that a revised version of the manuscript will still be considered to accept by JoVE. We have modified the paper in response to the extensive and insightful reviewer comments. We have rewritten sections of the manuscript and we hope that this comply with the referee’s remarks. We hope that the revised manuscript may suitable for the publication in JoVE. We are looking forward to hear from you.

Thanking You

Sincerely,

En Meng

……………………………………………….

Dr. En Meng, MD, Ph.D.

Division of Urology, Department of Surgery,

Tri-Service General Hospital,

No.325, Cheng-Kung Road,

Sec.2, Taipei 114, Taiwan.

E.mail: en.meng@gmail.com

Tel: + 886-2-87927169

Fax: +886-2-87927172

Editorial comments: Changes to be made by the Author(s):

**1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.**

Corrected.

**2. Figure 2A: Please provide units on the scale bar.**

Corrected.

**3. Please be consistent with panel labeling for the Figures. Figure 1 has no parentheses but the other Figures have parentheses for the panel labels (A), etc.**

Corrected.

**4. Please combine all panels of one figure into a single image file.**

All panels have been combined in a single image file.

**5. Please mention how proper anesthetization is confirmed.**

Please refer to “4. Anesthesia”.

**6. 6.3: 137 degrees Celsius? Where are the 6 wounds made? What order?**

It should be 137 degree Celsius. Wound creation please refer to “6. Burn Wound Creation”.

**7. 9.2: What temperature for growth?**

Plates were incubated at 37°C. Please refer to “9. Bacterial Growth Experiments of Post Burn Tissues”

**8. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).**

Corrected

**9. Please specify the composition of all solutions/media used.**

The composition of all solutions/media are shown in “MATERIALS”.

**10. Please specify the euthanasia method.**

Please refer to “10. Euthanasia and Tissue Fixation”.

**11. Please move step 11 to the Discussion.**

Step 11 has been incorporated into Discussion.

**12. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.**

Highlighted in yellow. Please refer to “Protocol”.

**13. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.**

Reviewer #1:

Manuscript Summary:

The manuscript describes a method to create burn wounds in a pig model. The authors are evaluating whether a CAPS wound dressing vs. traditional dressings (designated "CMC", Aquacel) will improve wound healing (re-epithelialization and scarring).

**Major Concerns: Burn depth and lack of clinical translation: Burns of >100C for 30 seconds will be full thickness wounds (and acknowledged by the author on line 255). Clinical practice would be to excise the necrotic tissue and apply a graft (if donor sites are available) or an allograft/skin substitute. The proposed model does not mimic clinical practice and therefore makes it less desirable.**

In this study, we are seeking treatments which are differ from clinical practice. Rat, mouse, and rabbit have been used in dermatologic toxicology. However, swine share a number of anatomic and physiologic characteristics with humans in terms of the process of epithelialisation, cellular proliferation, and angiogenesis that make swine potentially a better model for certain procedures and studies comparing with other species. Although there is still a slightly different in burn conditions between human and swine model, this model still facilitates the development of novel treatment for burn injury.

**Analgesia concerns: The authors use ketoprofen as analgesia daily for at least 1 week but it is an NSAID. It is widely known that NSAIDS delay wound healing. A more appropriate analgesic for swine would be buprenorphine SR-LAB which requires administration every 72 hours and will not impact wound healing.**

Ketoprofen should be corrected as transdermal fentanyl patch (50 μg/hour).

**Comparison between treatments: The authors are comparing CAPS vs. "CMC" (Aquacel); however, Figures 2-4 do not show any of the results from the Aquacel group. In the corresponding text of the results section and the figure legends, again only CAPS data is provided. If the authors want to state CAPS is superior to traditional dressings (lines 315- 317), the appropriate data needs to be shown with significantly statistical results.**

Comparison between different dressings was not our intension. Related paragraphs have been rewritten.

**Bacterial quantification: Swabs only collect bacteria on the surface of the wound. Non debrided burn wounds will get infected and the most prevalent bacterial species (e.g> Pseudomonas aeruginosa) can penetrate the eschar which may not all be collected with swabs. A more common approach would be to collect biopsies and report as CFUs/g of tissue.**

Although collection of biopsies is a better method to evaluate the anti-bacterial activity, it significantly changes the wound condition to create other factors which may further affect the study. Besides, the collection of biopsies in different time points in different conditions may increase the number of animals in this study, which is impractical.

**VSS scores: What is the point of performing VSS analysis on days 0, 7, 21? The necrotic eschar wasn't removed which delays the wound healing. Until the wounds are completely re-epithelialized which signifies a transition to the remodeling phase of wound healing, it is pointless to perform any type of scar assessment. Extending the study to 60, 90, or 120 days to allow remodeling to actually occur will provide more insight into scarring.**

The scar scale reflects the progress of wound closure. Rapid wound closure implies a short inflammatory phase, suggesting the dressing facilitate the healing process.

**Minor Concerns: Timeline: Lines 164, 168, and 176 do not coincide. "The dressings were changed every 2 days for 10 days and then twice a week for 6 weeks." The entire experiment was only 42 days. Also how was a swab collected on days 0 and 7 when the dressings were changed every 2 days for 10 days?**

The dressings were changed every 2 days for 10 days and then twice a week until week 6. We did collect samples on day 0 and 7. The dressings were removed for measurements and put back immediately without renewing.

**Wound size: Line 147 states the hot iron creates wounds of 20cm2 but Figure 2 shows a ruler with the diameter of the wounds to be 3 cm which results in a wound size of ~7cm2. This is further confirmed in Figure 3 with a starting wound area depicted of 9cm2. Please reconcile. Image quality: Figure 1 and 4 are too low quality to see details.**

Wound area should be 9 cm^2.

**Terminology issues: Exercise, darning, adhesive plaster, epithelial gap - These terms were used and need further clarification. Please elaborate on their meaning or change the terminology.**

Corrected.

**Pain: line 96-97 states "the present study provide new insights into how a CAPS containing dressing affects pain". No data was provided to show a difference in pain. How can pain be measured in a pig and differentiated between wounds? Was CAPS and Aquacel both used on the same animal?**

Any types of dressing can be applied to the swine model. However, comparison between CAPS and Aquacel was not our intension. Related paragraphs have been rewritten.

Reviewer #2:

**1. There is no novelty in the protocol of experiment to publish in Journal of Visualized experiments. Similar experiments have been carried out in rats and mice "Flexible, micro-porous chitosan-gelatin hydrogel/nanofibrin composite bandages for treating burn wounds" published in RSC advances in 2014. "Honey based hydrogel: In vitro and comparative In vivo evaluation for burn wound healing" published in Nature: Scientific reports in 2017.**

This model may benefit from ensuring that each swine serves as its own control with one wound receiving treatment and the other vehicle control, thereby reducing animal numbers.

**2. Check for spelling, punctuation marks, grammatical errors and sentence formation throughout the article. Eg: In Abstract, line no. 3 "exercise", line no:8 "complementary" In article, line no:49 "composite", line no:57 "scar", line no:69 "trigger-off" are some of the irrelevant words to be replaced.**

Corrected

**3. No reference for protocol supporting material preparation. Try to include reference of your previously published paper.**

Included. Please refer to Ref. 7.

**4. Check for the standard protocol for antibacterial activity. Lack of data supporting antibacterial activity of your scaffold. No error bar and statistical significant data in bacterial growth experiments though you have mentioned in the line 246 as CFU assay was performed in triplicates.**

Corrected.