We appreciate all your comments to make our manuscript better. We have done our best to answer your comments and revise our manuscript as your recommendations. Please do not hesitate if there is any query.

1. Formatting:  
-Please use “hr” as the abbreviation for hours.

*We have used “hr” in the whole manuscript.*

-Please remove all references to the video in the manuscript.  
*We will remove all references to the video in the manuscript.*2. Grammar:  
- Long abstract – “process” should be “processes”  
*We have changed “process” to “processes” in long abstract.*

-2.7 – There’s a typo involving parentheses near Triton X-100.

*We have corrected the typo involving parentheses near Triton X-100.*

-2.8, 4.2 – Should be “Incubate cells” since they have not lysed yet.  
*We have changed “incubate lysates” to “inclubate cells” as you recommend.*

-5.2 – “immediately” should only be used once in the sentence

*We have removed one of them in this sentence.*

-5.3 – “microscopy” should be “microscope”  
*We have changed “microscopy” to “microscope”.*

-6.1 – “on the coverslip”  
*We have removed these protocols as the reviewer #2 recommends.*

-7.7 – Should be “flow cytometer”  
*We have changed the “flow cytometry” to “flow cytometer”.*3. Additional detail is required:  
-3.5 – Shouldn’t the referenced section be section 4 rather than steps in section 2?  
*We have changed the referenced section from section 2 to section 4.*

-4.5 – What voltage is used? How long are gels run? What is the voltage/temperature/run time of the transfer?

*We have added the voltage/temperature/run time of the transfer in the protocols 2.11 and 4.5.*  
-4.6 – Aren’t blots blocked first? What are the antibodies diluted in? How long are the blots incubated?

*We have added all the information in protocol 4.6 as follows.*

*“4.6. Block nitrocellulose filter membranes with 5% skimmed milk in Tris-buffered saline with Tween 20 (TBST) for 1 hr, add primary antibodies against phosphorylated and non-phosphorylated HSP27 (1:1000 dilution), phosphorylated Akt (1:1000 dilution), non-phosphorylated Akt (1:1000 dilution, used as a cell survival marker), Bcl-2-associated X protein (1:1000 dilution, used as a pro-apoptotic protein), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:200 dilution, used as a loading control) in 5% bovine serum albumin (BSA), and incubate the membranes overnight at 4 °C on a shaker.”*

-4.7 – What are the antibodies diluted in? How long are the washes? How are blots developed? Is film used?

*We have added all the information in protocols 2.13-17 and 4.7-11 as follows.*

*4.7) Detect immunoreactive bands using horseradish peroxidase-conjugated goat anti-rabbit antibodies (1:10000 dilution) in 5% BSA after washing 3 times with TBST, 10 min each wash.*

*4.8) Incubate the membrane in the western blotting luminol reagents (6–7ml per 10cm × 5cm membrane) for 1 min at room temperature.*

*4.9) Remove the membrane from reagent solution, remove excess liquid with an absorbent towel, and place in a plastic sheet protector.*

*4.10) Working in a dark room with a safe light, place covered membrane in a film cassette with protein side facing up.*

*4.11) Place X-ray film on top of membrane and expose for 1 min.*

-6.7 – What volume is placed on each coverslip?  
*We have removed these protocols as the reviewer #2 recommends.*

-7.3 – What is the composition of the binding buffer? The materials used for FACS should be included in the materials table (propidium iodide, etc.) unless in a kit, in which case the kit should appear in the materials table.

*We have added the composition of binding buffer in the protocol 7.3 and the FITC Annexin V Apoptosis Detection Kit I for FACS in the materials table.*  
4. Results:  
-Please provide a control western blot to show that siRNA transfection specifically reduced HSP27 production.

*We have provided the control western blot to show that siRNA transfection specifically reduced HSP27 production as figure 1 and described our results in the representative results section.*

-Please define the error bars in 1B (SEM, SD, etc.)  
*We have added “The data are shown as means ± standard deviations” in the legend of figure 2.*

5. Discussion: Please discuss the potential modifications/troubleshooting that can be performed. Also, please expand on the significance with respect to alternative methods, starting with what the alternative methods are.

We have already added the following comment regarding the potential modifications.   
*“Unlike previous studies that used rat HSP27-specific siRNA to transfect vascular smooth muscle cells, we used a siRNA transfection technique to modify gene expression in human CECs to effectively knock-down HSP27-specific gene expression and study HSP27 function. Although there were differences in the target sequence that we used as well as in the cell density, final siRNA concentration, and incubation time, the protocol recommended by the manufacturer was explicitly followed.”*

*In terms of alternative methods, we have added following comment in the discussion.*

*“In terms of alternative methods, HSP27 knock-out mouse may be used to show if HSP27 phosphorylation involves epithelial migration and cell apoptosis, however, it is difficult to monitor the change of HSP27 phosphorylation in mouse model, because its phosphorylation occurs in very short period during epithelial wound healing.”*

**Reviewer #1:**  
*Manuscript Summary:*  
The authors attempted to present a protocol to use heat shock protein 27 (HSP27)-specific small interfering RNA to assess the function of HSP27 during corneal epithelial wound healing.  
  
First of all, in that many revisions were attached in their account, I could guess that repetitive editorial reviews should be carried out before peer review, which made this manuscript close to the perfect. I think that this manuscript was well-written and quite helpful for the other research to perform the research with siRNA for RNA Interference.  
  
Their main findings were also described in their previous work,1 but they did not cite it in their reference. If they cite the original article, it would be more helpful for reader to follow the methodology.  
  
References:  
1. Song IS, Kang SS, Kim ES, et al. Heat shock protein 27 phosphorylation is involved in epithelial cell apoptosis as well as epithelial migration during corneal epithelial wound healing. Experimental eye research 2014;118:36-41.

*We have added our previous work in the reference list as reference #13.*

**Reviewer #2:**  
*Manuscript Summary:*  
Prior to this submission, the authors have published an article in Experimental Eye Research titled " Heat shock protein 27 phosphorylation is involved in epithelial cell apoptosis as well as epithelial migration during corneal epithelial wound healing" (Exp Eye Res 118 (2014) 36-41). This manuscript is principally the extended methodology for RNAi-based investigation of the function of HSP 27 during corneal epithelial wound healing using an in vitro scratching model, which has been described in that paper. Since the complete study has been published in a prestigious ophthalmological journal, the significance of the study is justified.  
  
*Major Concerns:*  
1. Have the authors obtained a permission from Exp Eye Res to publish this paper?

*The permission from Exp Eye Res to reuse figures and excerpt data was obtained to publish this manuscript.*

2. Along with the siRNA transfection assay, they also described in the Protocol Western blot analysis, actin cytoskeleton by immunofluorescence, and flow cytometry analysis of apoptosis. However, they did not show the result of these experiments.  
*The protocol and data description for immunofluorescence to show the actin cytoskeleton were removed in the manuscript. The results of flow cytemetry were added as figure 3.*

*Minor Concerns:*  
In this article the authors didn't mention the source of siRNA they used, nor did they mention the sequence of the siRNA. The information did appear in their paper in Exp Eye Res.

*Because we add our previous work in the reference list, the information regarding the source and sequence of siRNA can be found.*