Reviewer #1:

Question #1: Fig. 1D: any significant differences among the different concentrations?

Response*: We appreciate the reviewer's comment. There is no significant difference between groups with a P-value of 0.197 based on ANOVA test. This information is added to the revised manuscript first paragraph on page 12 and figure legend (Figure 1D) on page 22.*

Question #**2**: Fig. 1F: Any significant difference between Released EPO and the other groups?

Response*: Indeed, there is a highly significant difference between released EPO and media and HA scaffold with a P-value of 0.003. The statistical analysis results are added to the revised figure legend (Figure 1F) on page 22.*

Question #**3.** May want to carry out statistical analyses for A, B, E. etc.

Response*: Agree. Statistical analysis results are added to the revised Figure 2 on Page 23.*

Question #**4.** Abstract: reduce should be reduced (to be consistent with the past tense had in the same sentence).

Response: *The abstract (on page 2) has been revised as suggested by the reviewer.*

Reviewer #2

Question #**5.** Page 13. Results and Discussion is a new section. Need to create the new sub-section numbers accordingly.

Response: *The new sub-section numbers have been revised accordingly.*

Question #6. Besides the recognition by CD44 receptors, HA is a natural polysaccharide. Will it be recognized by other sugar receptors on the cell membrane?

Response: *This is an excellent question. Although our and several published studies have shown that HA can interact with chondrocytes via CD44 receptors, there are insufficient results to exclude the participation of other receptors. Such a statement is included in the revised "Results and Discussions" section in the last paragraph on page 12.*

Question #7. Figure 1(C). The scale bar is missing.

Response*: The scale bar has been added to the revised Figure 1C on page 22.*

Question #8. Figure 1(E). How many repeated groups for drug release studies? It seems a burst release of EPO. The authors have to discuss more details in the manuscript. Such release profile is good for recruiting cells? If not, how to improve the drug release rate from the HA scaffolds in the future?

Response: *The sample number of drug release study is 3 samples per group. This information is added to the legend of the Figure 1E (page #21). This early burst release of EPO is essential to the creation of a cytokine gradient and subsequent cell recruitment. It should be noted that the EPO release rate slowed down after 24 hours and only 30% of the remaining EPO was released during the following 3 days. The EPO release rate from the HA scaffolds can be further delayed via chemical conjugation of the EPO to the HA microscaffolds. Such a statement is included in the revised "Results and Discussions" section on page 12.*

Question #9. Figure 1(F). Is EPO stable in a physiological condition?

Response: *Yes. EPO is stable in a physiological condition. The stability of EPO is confirmed by the fact that EPO released from microscaffolds retain its bioactivity to recruit human MSCs in vitro. This statement is added to the 3rd paragraph on page 12.*

Question #10. All figures. Because the authors reported the data using Mean ± SD, how many repeating samples should be addressed?
Response: *The sample numbers for different test groups were added to the legend of the all Figures.*

Reviewer #3
Question #11. Many important detail information are missing, including methods for migration assay, cytotoxicity test, surgical procedure, and so on. Such missing information cause unnecessary confusion and misunderstanding in regard to data interpretation.
Response*: Thanks for the reviewer's comment. The detail for cell migration, cytotoxicity test and surgical procedure have been added to the revised "Materials and Methods" section on page 6 and 7.*

Question #12. a. In regard to Fig. 1D, please describe detail methods for cytotoxicity test. How cell viability is over 100% for multiple data points?
Response*: The >100% value of cell viability might be attributed to the proliferative effect of HA microscaffold on chondrocytes. Such a statement is added to the revised "Results and Discussions" section at the end of first paragraph on page 12.*

Question #13. b. Similarly, migration assay is not clear, including time, dose, control group, addition of FBS, initial cell number, etc, which are important contributing factors to result.
Response: *More details about cell migration method have been added to the revised "Materials and Methods" section on page 7.*

Question #14. There is no single error bar in the release profile in Fig. 1E. One data point was used for each time point? Then the authors must increase sample numbers.
Response: *We are sorry for the confusion. The graph has been modified to clearly show the error bar as shown on page 22.*

Question #15. There is no single scale bar in any of histology images.
Response: *Scale bars are added to the revised Figure 1, 4, 5, 6 and 7.*

Question #16. In overall, description of data is insufficient and discussion is very limited. Please include more comprehensive descriptions and discussion.
Response*: Thank you for your comment. Additional descriptions and discussions are added in the revised "Results and Discussions" section on pages 12-17 (the highlighted parts).*

Question #17. CF647 is a product name and not a correct term to express. You should clarify it as a product by providing a supplier and its correct product name.
Response: *The information is added in the revised "Materials and methods" section on page 5. "CF647A amine dye were supplied from Biotium, Inc. (Fremont, CA).".*

Question #18. In Fig. 2D, 0 mg/ml HA microscaffolds show no background image but it shows blue color starting 0.02 mg/m. Does it suggest an autofluorescence of HA microspheres itself? If so, the authors should figure out a strategy to distinguish the autofluorescence signal from CF647 derived 'red' signal that starts showing at 0.5 mg/ml.
Response: *We are sorry for the confusion. The background color is removed from the images. The colors represent different degrees of intensity ranging from 5,000 (blue) to 40,000 (red). Intensity bar is added to the revised Figure 2D.*

Question #19. If no autofluorescence, to be proved by additional experimental evidence, please explain why there is blue color given the CF647 is supposed to be red.
Response: *Again, we apologize for the confusion. The pseudo-color is assigned to reflect the light intensity, not the real fluorescence color. Intensity bar has been added to the revised Figure 2D.*

Question #20. There is a serious disparity between 2F and 2D. Why 2F only show spotty blue/red point but 2D show whole explants red?
Response: *Thanks for the excellent comment. The disparity between Figure 2F and 2D was caused by the fact that both sets of images were set at different fluorescent intensity ranges. To resolve such confusion, the intensity bars are added to both Figures.*

Question #21. For Fig. 2E, please provide detail methods to measure intensity.
Response*: The intensity measurement method was described in a previous publication. The reference and some information about the intensity measurement method is included in the revised "Materials and methods" section on page 8.*

Question #22. Missing details in animal procedure are critical. The surgical location, size, and depths should be provided. The authors cited two references but the surgical procedure and animal species used in ref 20 and 21 are very different. Which methods were adopted? You must provide details used in this study.
Response*: We appreciate the reviewer's comment. A detailed animal procedure is added into the revised "Materials and methods" section on page 9.*
"Under general anesthesia (ketamine + xylazine injection and isoflurane inhalation), with the New Zealand White Rabbits in a prone position, the primary contact region of the medial femoral condyle was approached at an angle of 135 degree of flexion. A full-thickness cartilage defects (3 mm) were created in the weight-bearing area of the medial femoral condyle with a dermal biopsy punch and manual debridement to expose the subchondral bone plate. Each specimen then was undergone microfracture. Microfracture holes were created within each full-thickness chondral defect using 0.9-mm Kirschner wire tapped into the subchondral bone (~3 mm) until bleeding from the hole is apparent. By doing this, the subchondral bone was perforated to generate a blood clot within the defect. Once the defect was filled with different groups of HA microscaffolds, the patella was reduced; the joint capsule was closed with interrupted sutures.".

Question #23. As related, as shown in Fig. 3, the selection of area for micro-CT analysis are very different in between samples. Different defect location was selected for each animal? Then why?

***Response**: Since the samples were analyzed at different times, the samples were placed at different angles. To avoid such confusion, the images were removed from the revised Figure 3.*

Question #24. For micro-CT, selection of defect areas and small surrounding volume, followed by reconstructed in 3D will be more beneficial to interpret the outcome than analyzing whole volume.

***Response:** Good point. We monitored cartilage regeneration for 26 weeks and there was significant regeneration in many of the defect sites. To avoid selecting the wrong defect areas, we decide to evaluate the whole joint and BVF. In fact, such measurement methods have been used in many previous works [1, 2]. Fortunately, as shown in our results, we are able to show the effect of the different treatments by measuring BVF. Such a statement is added to the revised page #10.*

References

1. *Li, Y.-L., H. Zhao, and X.-B. Ren, Relationship of VEGF/VEGFR with immune and cancer cells: staggering or forward? Cancer Biology & Medicine, 2016. **13**(2): p. 206.*
2. *Lu, J., et al., Increased recruitment of endogenous stem cells and chondrogenic differentiation by a composite scaffold containing bone marrow homing peptide for cartilage regeneration. Theranostics, 2018. **8**(18): p. 5039.*

Question #25. What is the scientific rationale to use bone marrow MSCs instead of synovial MSCs. Recent research outcome indicates synovial MSCs are key endogenous cell source for joint healing.

***Response:** We agree with the reviewer's comment. Bone marrow MSCs were used in this study sto simulate progenitor cell responses following cartilage injury since microfracture procedure was used to create the cartilage defect and such a procedure can create an opening for the immigration of bone marrow MSCs from bone marrow. Such information is added to the revised "Materials and Methods" on page 6.*

Question #26. Overall, image quality of Fig. 4 is too low to determine the accuracy of staining regarding co-localization of DAPI and FITC.

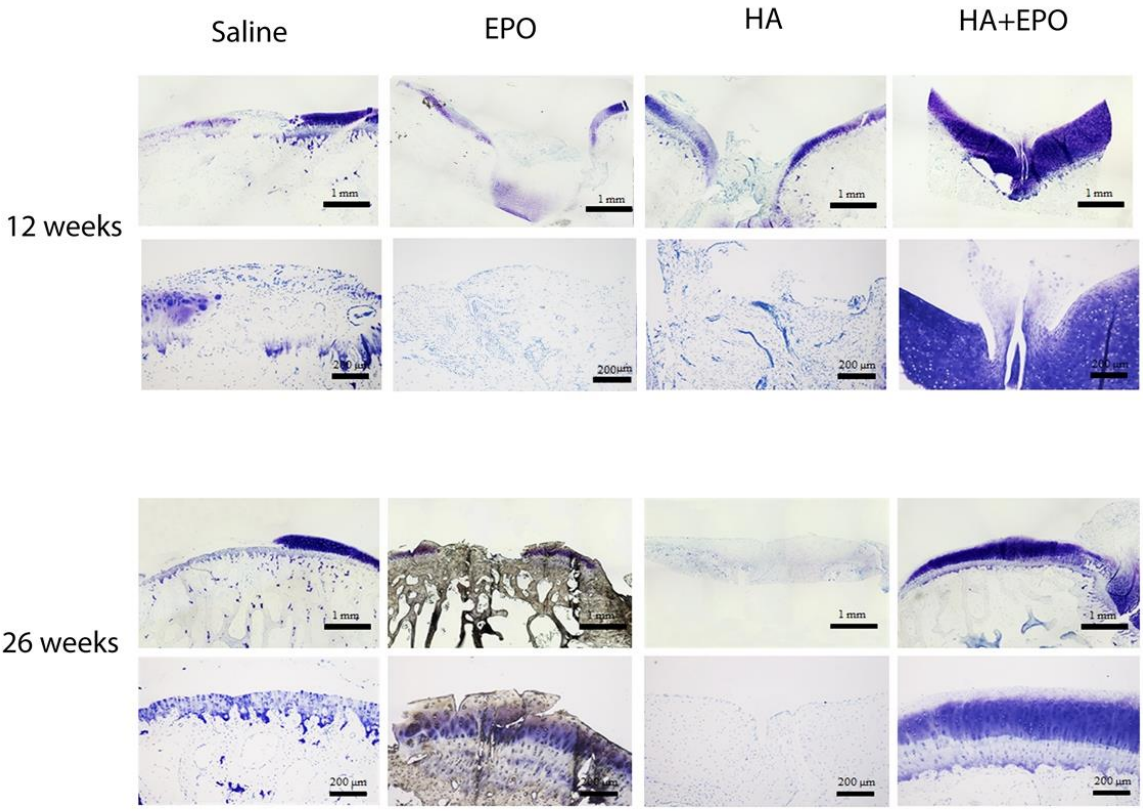
***Response**: The resolution of the images was reduced upon conversion to PDF format. High-quality images will be provided to the publisher during the submission process.*

Question #27. Same as Fig. 5. Image quality is too low.

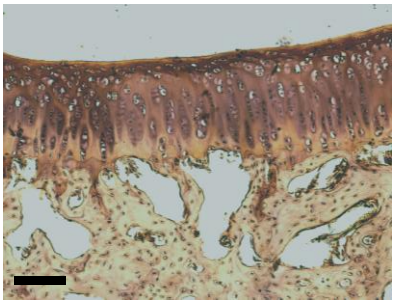
***Response**: The resolution of the images was reduced upon conversion to PDF format. High-quality images will be provided to the publisher during the submission process.*

Question #28. In Fig. 6, histology is unrealistic as none in TB stained slides for some groups. Please provide 1) several different section images from each group and 2) low magnification image showing whole joint to confirm the correctness of histological analysis.

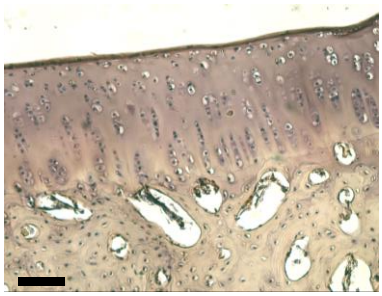
***Response:** We appreciate the reviewer's comments. Images present here are selected images to best represent the whole group. The low magnification images of TB stained slides of 12 and 26 weeks samples are included in the supplementary materials.*


Saline
EPO
HA
HA+EPO
12 weeks
26 weeks
1 mm
200 µm

Question #29. COL-II staining in Fig. 6 mismatch with Fig. 7 SO. COL-II staining seems to be false. Provide the positive and negative controls.
Response: The images of positive and negative controls of COL-II staining are shown below.



Positive control



Negative control