nature portfolio

Peer Review File

A dynamically phase-adaptive regulating hydrogel promotes ultrafast anti-fibrotic wound healing

Corresponding Author: Professor Zhenglin Li

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The manuscript reports a healing phase-adaptive regulating hydrogel with hierarchically delivering performance for programmed modulation of chronically infected wounds. Overall, the framework of the article is relatively complete, and the topics discussed are of research significance. However, it will be more suitable to be accepted after major revision in the following aspects:
1. What is the loading capacity of CeO2 nanoparticles and F/R MCs in the hydrogel and how about the local retention and systemic metabolism of nanoparticles in vivo? Did the CeO2 nanoenzyme continue consuming ROS during the entire healing process?
2. The evidence of phase-adaptive regulation by the hydrogel should be further provided. Did the CeO2 and F/R MCs release simultaneously upon the hydrogel applied to the wound sites? Otherwise, they should be administered separately.
3. Did the PLGA MCs retain at the wound area during the healing process? How about the degradation rate of the PLGA particles and the release kinetics of loaded bFGF and c-Jun siRNA from PLGA?
4. Further explanation of why the F/R gel accelerated the proliferation of various cell lines including 3T3 and HUVEC.
5. Many researches have been reported on scar-free wound repair using multi-functional hydrogel. The author should highlight the innovation of this work compared to other existing literature in the introduction section.
6. Throughout the entire manuscript, the presented data did reflect a positive effect on accelerating scar repair, and the author should add relevant experiments to illustrate the rapid scar-free wound healing.
7. In Figure 5d, the number and size of cells in live/dead staining images seem not consist with that in the fluorescence images of DCFH-DA probe and HPF probe.
8. A general vision of how the hydrogel would be applied clinically would help the reader better appreciate the contributions. Diverse components were incorporated into the system and stimulating events work in concert to help with wound healing.

Reviewer #2

(Remarks to the Author)

Schiff base-crosslinked hydrogels have garnered significant attention due to their biocompatibility, injectability, self-healing properties, and responsiveness to environmental stimuli, making them well-suited for biomedical applications, particularly wound healing. The present study introduces an advanced version of a Schiff base-crosslinked hydrogel (F/R gel) containing εPL-modified oxygen-deficient nanoceria and basic fibroblast growth factor (bFGF)/c-Jun siRNA co-loaded PLGA microcapsules (F/R MCs). This formulation aims to accelerate scarless wound healing.
The study demonstrates that the new hydrogel maintains comparable antibacterial, anti-biofilm, antioxidant, and anti-inflammatory properties to the control gel (without F/R MCs) while also achieving faster and scarless wound healing. The F/R gel outperformed the control gel and a commercial wound gel in both MRSA-infected chronic wounds (mouse model) and rabbit ear hyperplasia wounds.
The authors also discuss the regulatory mechanisms underlying the fast and scarless wound healing seen with this new hydrogel. The study addresses a significant gap in the current understanding of wound healing and scar prevention, providing a promising solution to the medical need for effective scarless healing. Therefore, the manuscript is recommended for publication following minor revisions, as outlined below.

Revision Comments:
1. The amount of basic fibroblast growth factor (bFGF) and c-Jun siRNA used in the assays needs to be clearly specified. The sequence of c-Jun siRNA should also be provided.
2. The resolution of images in Figure 6f should be improved for better interpretation of the data.
3. In Figure 7D, showing immunofluorescence images of TGF-β, c-Jun, CK-19, and β3-tubulin, please include quantitative statistical analysis of the results across the different groups. Update the images with higher resolution for better visual presentation.
4. It appears that F/R MCs alone do not exhibit c-Jun blocking in the mouse model, even though c-Jun gene silencing was confirmed in fibroblasts (Figure 7f). Please provide an explanation for this discrepancy. It seems likely that c-Jun siRNA works synergistically with other components of the hydrogel to achieve c-Jun blocking. Please elaborate.
5. It's interesting to note that collagen I expression in the F/R gel group is significantly reduced, while collagen III expression shows the opposite trend. Given that c-Jun is reported to directly bind to the promoters of COL1A1 and COL3A1 and inhibit their transcription, could these results be related to c-Jun activity? How would you interpret the differential regulation of collagen types I and III in this context?
6. In Figure 8a, to further support your hypothesis that bFGF and c-Jun siRNA stimulate fibroblast proliferation while modulating distinct fibroblast subpopulations for anti-fibrotic wound healing, you should perform FACS experiments to analyze the proportions of fibroblast subtypes across all control groups. Especially the differences in fibroblast subpopulations between scarred wound healing (Beifuji/ blank Gel) and scarless wound healing (F/R gel) groups.

Once addressed, the manuscript would be well-suited for publication.

Reviewer #3

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #4

(Remarks to the Author)
The manuscript introduces an innovative phase-adaptive hydrogel with dynamic properties to promote rapid and scar-free healing of chronic wounds. It effectively addresses challenges such as multidrug-resistant infections, oxidative stress, and excessive fibrosis in wound healing by leveraging a hierarchical drug release mechanism. The study presents a robust combination of experiments, from material synthesis and in vitro assessments to in vivo testing on wound models, showing substantial promise for clinical translation. However, the manuscript requires revisions to enhance clarity, address technical gaps, and improve experimental rigor. Below are detailed comments for revisions.
1. The introduction, although comprehensive, should place more emphasis on how this hydrogel design compares to existing wound healing technologies. A clearer statement of novelty should be added.
2. The abstract and introduction provide an ambitious overview of the hydrogel's design and functions, but certain key terms, such as "phase-adaptive" and "hierarchical drug release" are not fully explained in their initial introduction. It is essential to clarify what is meant by "phase-adaptive regulating functions" and how they specifically contribute to the wound healing process at a molecular level.
3. While the manuscript provides a comprehensive analysis of the hydrogel's components (e.g., εPL, CeOv nanozyme, siRNA, and bFGF), the mechanistic elucidation of their interactions within the dynamic wound microenvironment can be further refined. The transition from bacterial clearance to immunomodulation and scar inhibition through c-Jun suppression necessitates a more robust mechanistic linkage between various phases of healing.
4. The characterization of the mechanical properties of the hydrogel, including its stiffness and adhesiveness, should be more comprehensively linked to its in vivo performance. Were any assessments performed to determine the hydrogel's adherence to the wound surface under physiological conditions? Could this influence its efficacy in promoting wound healing?
5. The drug release profile (Fig. 2k) needs more clarification. The current graph suggests a step-wise release, but the dynamics of drug diffusion from the gel need to be explained more comprehensively. The manuscript would benefit from including data on the stability and degradation of the hydrogel matrix in vitro.
6. Angiogenesis is briefly discussed but not thoroughly evaluated. The manuscript mentions the promotion of neoangiogenesis; however, the angiogenic effects of bFGF should be explored in greater depth. Were there any specific assays done to quantify the degree of vascularization in the wound sites?
7. The mouse wound healing model illustrates the efficacy of the hydrogel in comparison to a commercial gel (Beifuji). However, additional control groups are necessary to isolate the contributions of each component of the hydrogel. For example, assessing the effects of F/R gel without c-Jun siRNA could elucidate its specific role in scar reduction. Furthermore, it is recommended to enhance the presentation of wound healing data, particularly through quantification of angiogenesis markers such as CD31 and VEGF.
8. There are some grammatical issues, particularly with sentence structure in the introduction and methods sections. A thorough proofreading is necessary to ensure the text is polished for submission.
9. The supplementary information includes crucial experimental details (e.g., synthesis, characterization). However, some key results, such as the antioxidant assays, would be more impactful if moved to the main manuscript.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have addressed related issues.

Reviewer #2

(Remarks to the Author)
The additional experiments provided are thorough and the data strengthen their conclusions. The quality of revised text has significantly improved. At this stage, I have no further concerns. The manuscript is well-suited for publication.

Reviewer #3

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #4

(Remarks to the Author)
This manuscript has been revised according to previous comments,and I think it can be accepted for publication without change.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The manuscript reports a healing phase-adaptive regulating hydrogel with hierarchically delivering performance for programmed modulation of chronically infected wounds. Overall, the framework of the article is relatively complete, and the topics discussed are of research significance. However, it will be more suitable to be accepted after major revision in the following aspects:

Our Response: Thanks for the reviewer's positive comments and professional suggestions on our work. According to these suggestions, we have tried our best to revise the manuscript, and our responses point by point according to the reviewer's comments are listed as below.

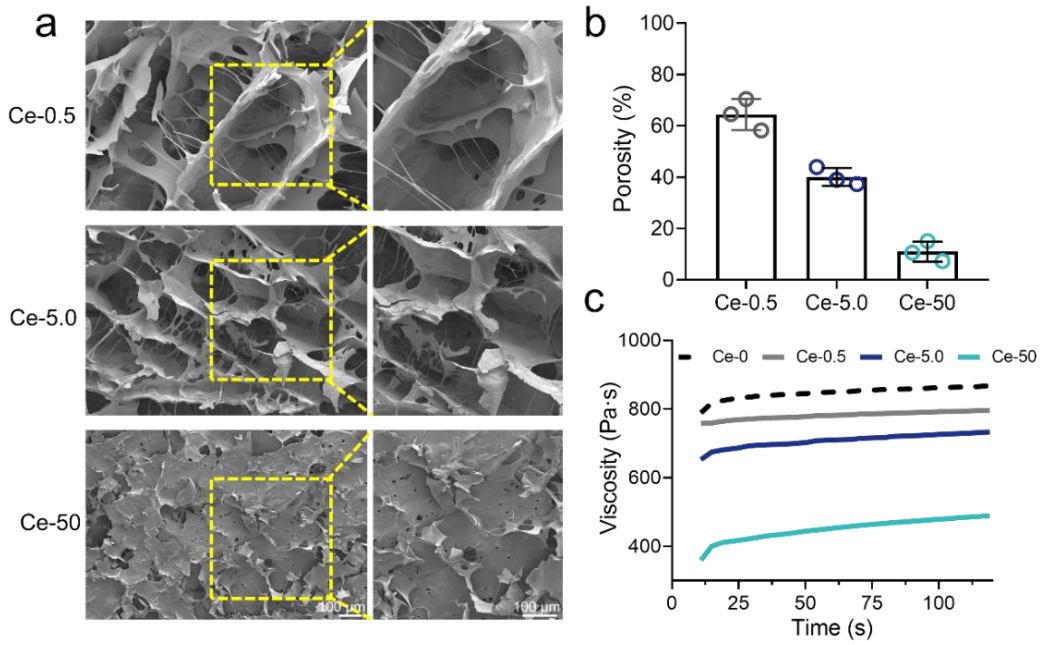
1. What is the loading capacity of CeO_2 nanoparticles and F/R MCs in the hydrogel and how about the local retention and systemic metabolism of nanoparticles in vivo? Did the CeO_2 nanoenzyme continue consuming ROS during the entire healing process?

Our Response: Many thanks to the reviewer for insightful comments. Following your suggestions, we have added the following experiments:

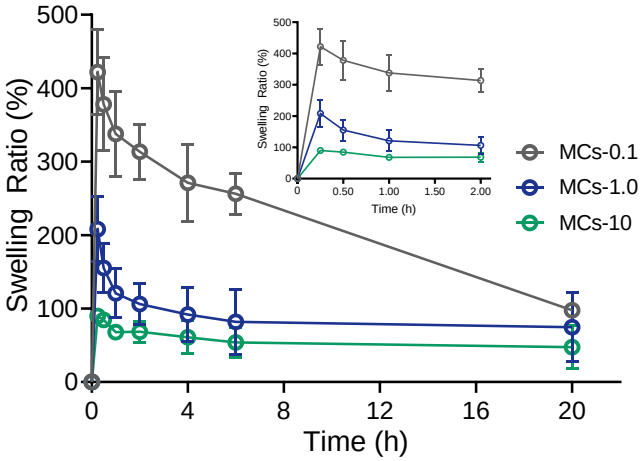
(1) Loading capacity of εPL-CeO_v and F/R MCs in the hydrogel

In our work, we selected F/R gel formula with loading amount of εPL-CeO_v at 5.0 mg/mL and F/R MCs at 1.0 mg/mL respectively for the follow-up cell/animal experiments. Actually, the loading capacity of εPL-CeO_v or F/R MCs in F/R gel could be easily adjusted based on their initial inputs during preparation. For optimizing the hydrogel's loading capacity, we conducted the following exploratory experiments:

Firstly, incremental concentrations of εPL-CeO_v nanoparticles at 0.5, 5.0 and 50 mg/mL were used to prepare hydrogels, which were named as Ce-0.5 gel, Ce-5.0 gel and Ce-50 gel, respectively. SEM analysis showed that the gel's internal network and porosity were markedly affected by εPL-CeO_v amount, in which higher concentration of nanoparticles resulted in denser network structure with reduced porosity (Supplementary Fig. 17a-b). Therefore, the loading of εPL-CeO_v could change F/R gel's crosslinking degree. Moreover, we tested the viscosity changes of hydrogels by rheometer, and found that the loading of εPL-CeO_v nanoparticles reduced the gel's viscosity, especially for the Ce-50 gel sample with a sharply decreased value (Supplementary Fig. 17c). When consistently fixing the loading concentration of εPL-CeO_v at 5.0 mg/mL, we prepared three F/R gel samples encapsulated with 0.1, 1.0, and 10 mg/mL F/R MCs, which were named as MCs-0.1 gel, MCs-1.0 gel and MCs-10 gel, respectively. Hydrogel swelling test was performed by measuring mass change of the gels in PBS (pH = 7.4) solution, and the results indicated a rapid swelling characteristic for all gels within the first 15 min (Supplementary Fig. 18). Specially, as the loaded F/R MCs' concentration increased, the swelling ratio significantly decreased. As network structure, porosity and swelling property are important parameters that affect hydrogel's biological functions, we combined these performance tests with the *in vitro* antioxidant activity (Fig. 4b-f) and c-Jun gene silencing data on fibroblasts (Fig. 1f), and finally determined εPL-CeO_v at 5.0 mg/mL, F/R MCs at 1.0 mg/mL as the applicable loading concentrations in F/R gel for subsequent experiments.



Supplementary Figure 17. (a) SEM, (b) the porosity, and (c) the viscosity of hydrogels loaded with different concentrations of εPL-CeO_v (unit: mg/mL).



Supplementary Figure 18. The swelling ratio changes of hydrogels loaded with different concentrations of F/R MCs (unit: mg/mL).

We have added these new data and more description on this issue in our revised manuscript as follows:

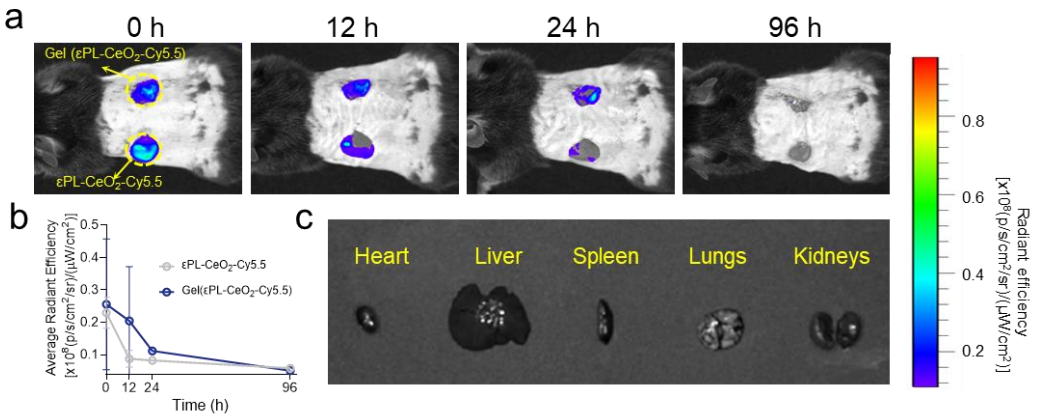
Page 7: "*Moreover, the loading capacity of F/R gel for εPL-CeO_v and F/R MCs had important influences on gel's network morphology and mechanical property, which revealed that an increased loading amount significantly reduced the gel's pore structure, viscosity, and swelling properties (Supplementary Fig. 17-18).*"

(2) Local retention at wound site, *in vivo* systemic metabolism, and ROS scavenging stability of εPL-CeO_v nanoparticles

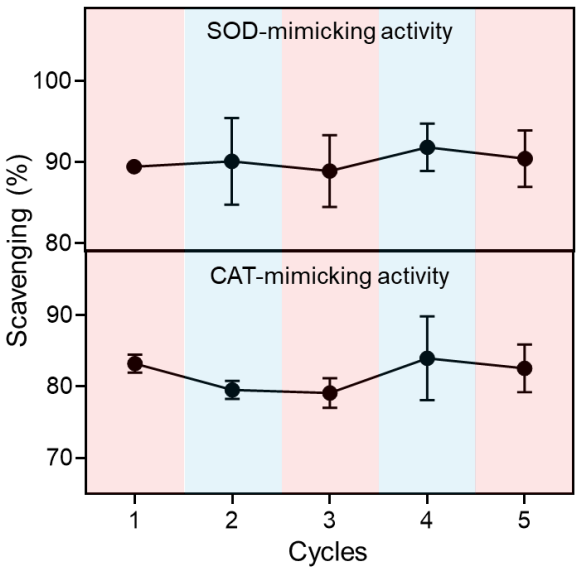
To evaluate the retention effect of εPL-CeO_v at wound site, Cy5.5 dye was firstly used to label the nanoparticles (denoted as εPL-CeO_v-Cy5.5), and then a fluorescent gel (Gel (εPL-CeO_v-Cy5.5))

was prepared by using εPL-CeO_v-Cy5.5. Subsequently, we performed *in vivo* fluorescence imaging experiment to observe the local retention of free εPL-CeO_v-Cy5.5 or Gel (εPL-CeO_v-Cy5.5) at wound sites. As shown in Supplementary Fig. 40a, εPL-CeO_v-Cy5.5 possessed a longer retention time (> 24 h) at wound site when loaded in the gel, while free εPL-CeO_v-Cy5.5 group's fluorescence signal was almost lost within 24 h. Specially, the average local fluorescence intensity of Gel (εPL-CeO_v-Cy5.5) group was about 2.3 times higher than that of εPL-CeO_v-Cy5.5 group at 12 h, and 1.3 times higher at 24 h (Supplementary Fig. 40b). Hydrogel loading successfully realized sustained-release effect and significantly improved retention effect of εPL-CeO_v nanoparticles at the wound site, which was largely unaffected by the tissue exudate loss or the natural movement of mice. In addition, we dissected the vital organs of mice after 96 h for fluorescence imaging, and the results showed that almost no nanoparticles migrated to accumulate in the systemic organ tissues, implying no systemic metabolic burden and potential *in vivo* toxicity.

In addition, to verify long-term ROS scavenging efficiency, we investigated the ROS consuming activity of εPL-CeO_v during five-reuse cycles. As shown in Supplementary Fig. 27, there was no significant decrease in the removal efficiency after repeated cycles. Although these additional tests indicated stable ROS scavenging ability of εPL-CeO_v nanoparticles, here we could speculate that the ROS consuming time and efficiency were closely related to the retention effect of εPL-CeO_v at local wound site. The *in vivo* retention experiments (Supplementary Fig. 40) clearly indicated that the CeO_2 nanoenzyme mainly remained in the early inflammatory stage (< 96 h) rather than the entire healing process to exert its ROS scavenging effect, which was very beneficial to the rapid healing of wounds.



Supplementary Figure 40. Retention effect of εPL-CeO_v at the wound sites. (a) Representative images of εPL-CeO_v-Cy5.5 and Gel (εPL-CeO_v-Cy5.5) in mouse wounds at different time points. (b) Average radiant efficiency of mouse wound sites at different time points (n = 3). (c) Representative images of fluorescence intensity in the isolated tissues at 96 h.



Supplementary Figure 27. ROS scavenging stability test of εPL-CeO_v nanoparticles during 5 cycles (n = 3).

Based on the reviewer's comments, we have added these data and more discussions in our revised manuscript as follows:

Page 10: "*Besides, there was no significant decrease in the removal efficiency after five cycles, indicating that εPL-CeO_v had excellent ROS scavenging stability (Supplementary Fig. 27).*"

Page 13: "*By observing the retention effect at wound site by small animal live imaging technology, we found that the hydrogel encapsulation provided prolonged retention time in the wounds, while the administered dose during treatment was not sufficient to cause obvious accumulation in major organs (such as heart, liver, spleen, lung, and kidney) (Supplementary Fig. 40-41).*"

2. The evidence of phase-adaptive regulation by the hydrogel should be further provided. Did the CeO_2 and F/R MCs release simultaneously upon the hydrogel applied to the wound sites? Otherwise, they should be administered separately.

Our Response: We appreciate the reviewer for this good comment. Hierarchically delivering property is one of the biggest design highlights in our F/R gel system for healing phase-adaptive regulating function on anti-scar wound healing. We constructed F/R gel *via* the formation of Schiff base bond and electrostatic interaction mainly between cationic εPL and HA-CHO, accompanied by encapsulating εPL-CeO_v and F/R MCs. Upon administration to chronic wounds, (1) the F/R gel underwent fast dissociation firstly triggered by weakly acidic microenvironment, and at this point, the low-molecular εPL or ultrasmall εPL-CeO_v released immediately to exert antibacterial and immunomodulatory functions; (2) limited by the much larger size and slower degradation characteristic of PLGA microcapsules, F/R MCs' release and disintegration were delayed than εPL or εPL-CeO_v, and hence the loaded bFGF/siRNA could be liberated for facilitating regeneration cascades. To further elucidate such drug release properties, we re-conducted the *in vitro* drug releasing experiments on F/R gel by using two different pH conditions: neutral pH = 7.4 PBS and acidic pH = 4.5 PBS. The results clearly verified the nature of sequential releasing profiles (Fig. 2k), in which εPL-CeO_v released first, then followed by bFGF/siRNA drugs to the external solutions. Moreover, acidic microenvironment (pH = 4.5) triggered faster drug release rates especially for εPL-CeO_v nanoparticles, which was consistent with the acid sensitivity of Schiff base bond. In addition,

the faster degradation of F/R gel in acidic environment was evidenced again in Supplementary Fig. 19. Therefore, these results suggested that εPL-CeO_v and F/R MCs as well as the loaded drugs released in a certain sequence, rather than simultaneously from the F/R gel.

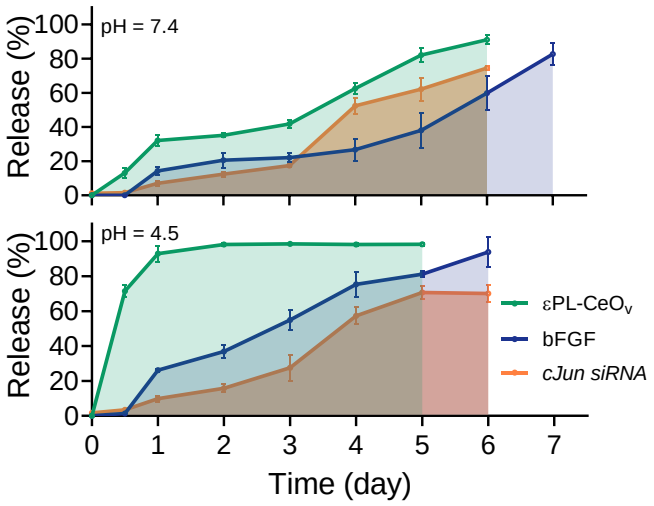
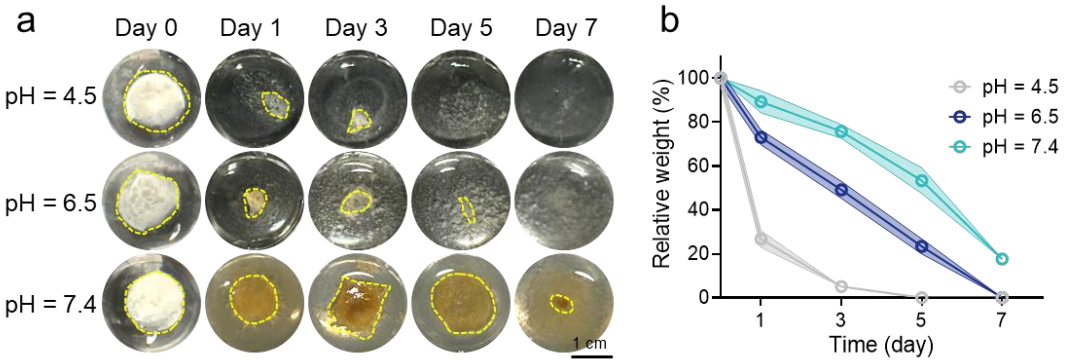


Figure 2. (k) *In vitro* release properties of F/R gel's components in neutral PBS (pH = 7.4) and acidic PBS (pH = 4.5).



Supplementary Figure 19. (a) Representative photographs showing F/R gel's morphology changes after incubated with pH = 4.5, pH = 6.5, or pH = 7.4 PBS. (b) The corresponding weight changes of F/R gel during the 7-day degradation process.

Following the reviewer's comments, we have added the new data and more discussions on this part in our revised manuscript as follows:

Page 8: "*As shown in Fig. 2k, when incubated in neutral PBS, εPL-CeO_v released first from the gel, in which ~ 91.13% was detected to release into external solutions at day 6. With gel degradation, release of bFGF and siRNA drugs became gradually detectable. By day 7, the cumulative release amount of bFGF was close to 85%, while the siRNA drug reached ~ 74.48%. Moreover, acidic microenvironment (pH = 4.5) triggered faster drug release rates especially for εPL-CeO_v, which was consistent with the acid sensitivity of Schiff base bond. In addition, the accelerated breaking of Schiff-base hydrogel network in weakly acidic environment was further demonstrated by the in vitro hydrogel degradation experiments (Supplementary Fig. 19), which was more favorable for the*

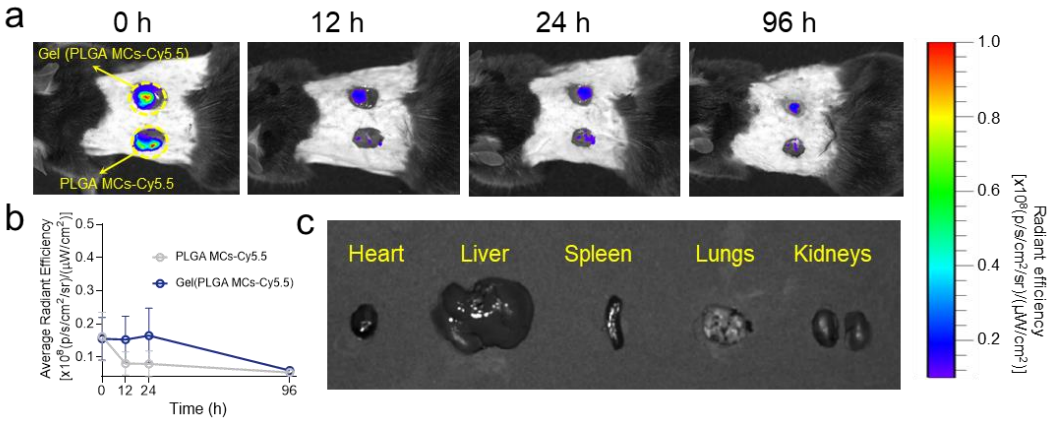
release of εPL-CeO$_v$ and F/R MCs from F/R gel. We also compared the drug release rates of free F/R MCs and F/R gel, and found that the encapsulation of MCs in hydrogel matrix significantly delayed the release of bFGF and siRNA drugs, regardless of the solution's pH values (Supplementary Fig. 20)."

3. Did the PLGA MCs retain at the wound area during the healing process? How about the degradation rate of the PLGA particles and the release kinetics of loaded bFGF and c-Jun siRNA from PLGA?

Our Response: Thanks to the reviewer for insightful comments. Based on the reviewer's suggestions, we have added the following relevant experiments:

(1) Retention of PLGA MCs at wound site

Cy5.5-labeled PLGA was formulated into microspheres (denoted as PLGA MCs-Cy5.5). The microspheres were incorporated into hydrogel to prepare fluorescent Gel (PLGA MCs-Cy5.5). The retention of PLGA MCs at the wound site was monitored by using *In Vivo* Imaging System. As shown in Supplementary Fig. 41a, fluorescence signals in Gel (PLGA MCs-Cy5.5) group persisted at the wound site for more than 96 h, which was much higher and longer than that in free PLGA MCs-Cy5.5 group. For instance, the local average fluorescence intensity of Gel (PLGA MCs-Cy5.5) group at 24 h was ~ 2.1 times higher than that of PLGA MCs-Cy5.5 group (Supplementary Fig. 41b). Besides, almost no accumulated PLGA MCs were detected in the vital organs of mice after gel treatment. Therefore, these data indicated that the hydrogel system significantly extended the retention time of PLGA MCs at the wound area during the healing process.



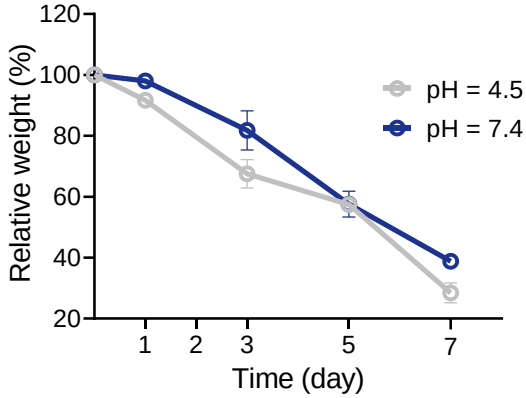
Supplementary Figure 41. Retention experiments of PLGA MCs-Cy5.5 at the wound site. (a) Representative images of PLGA MCs-Cy5.5 and Gel (PLGA MCs-Cy5.5) in mouse wound areas at different time points. (b) Representative images of fluorescence intensity in the isolated tissues at 96 h. (c) Average radiant efficiency of mouse wound sites at different time points (n = 3).

Based on the reviewer's comments, we have added the data and more descriptions in our revised manuscript as follows:

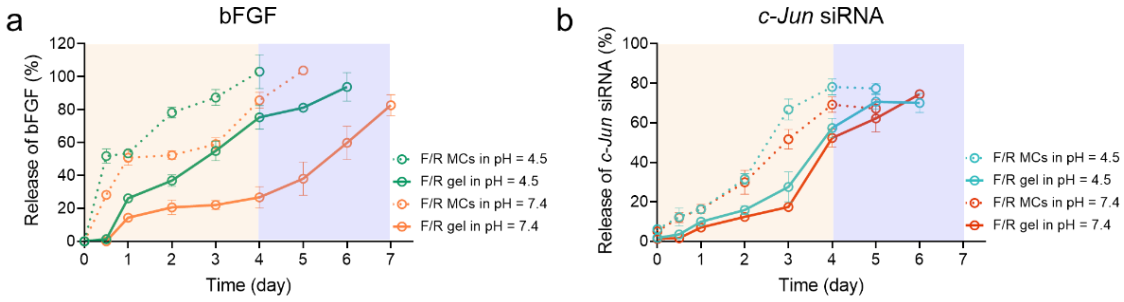
Page 13: "*By observing the retention effect at wound site by small animal live imaging technology, we found that the hydrogel encapsulation provided prolonged retention time in the wounds, while the administered dose during treatment was not sufficient to cause obvious accumulation in major organs (such as heart, liver, spleen, lung, and kidney) (Supplementary Fig. 40-41).*"

(2) Degradation rate of PLGA particles, and release kinetics of bFGF and c-Jun siRNA

We evaluated the degradation rate of PLGA MCs by incubating in neutral (pH = 7.4) and acidic (pH = 4.5) PBS mediums, and found that PLGA MCs' degradation rate was slightly faster in the weakly acidic environment, however there was no significant difference between the two groups (Supplementary Fig. 10). Within 7 days, PLGA MCs degraded ~ 61.2% in neutral PBS and ~ 71.6% in acidic PBS, respectively. Subsequently, we also evaluated the drug release behavior of bFGF and c-Jun siRNA from F/R MCs (Supplementary Fig. 20). Overall, sustained release profiles were observed for both bFGF and c-Jun siRNA from the microspheres. Up to 85% of bFGF and 70% of c-Jun siRNA were released on day 4. In addition, we also found that the encapsulation of MCs in F/R gel matrix significantly delayed the release of bFGF and siRNA drugs, regardless of the solution's pH values.



Supplementary Figure 10. *In vitro* degradation of PLGA microspheres



Supplementary Figure 20. *In vitro* release properties of (a) bFGF and (b) c-Jun siRNA from F/R MCs and F/R gel.

The above new data have been added in our revised manuscript with the following descriptions:

Page 6: "*Degradation tests revealed a slow-rate disintegration of the microcapsules in physiological solutions (Supplementary Fig. 10), and the loaded drugs (bFGF and c-Jun siRNA) displayed sustained release profiles from F/R MCs (Fig. 2k).*"

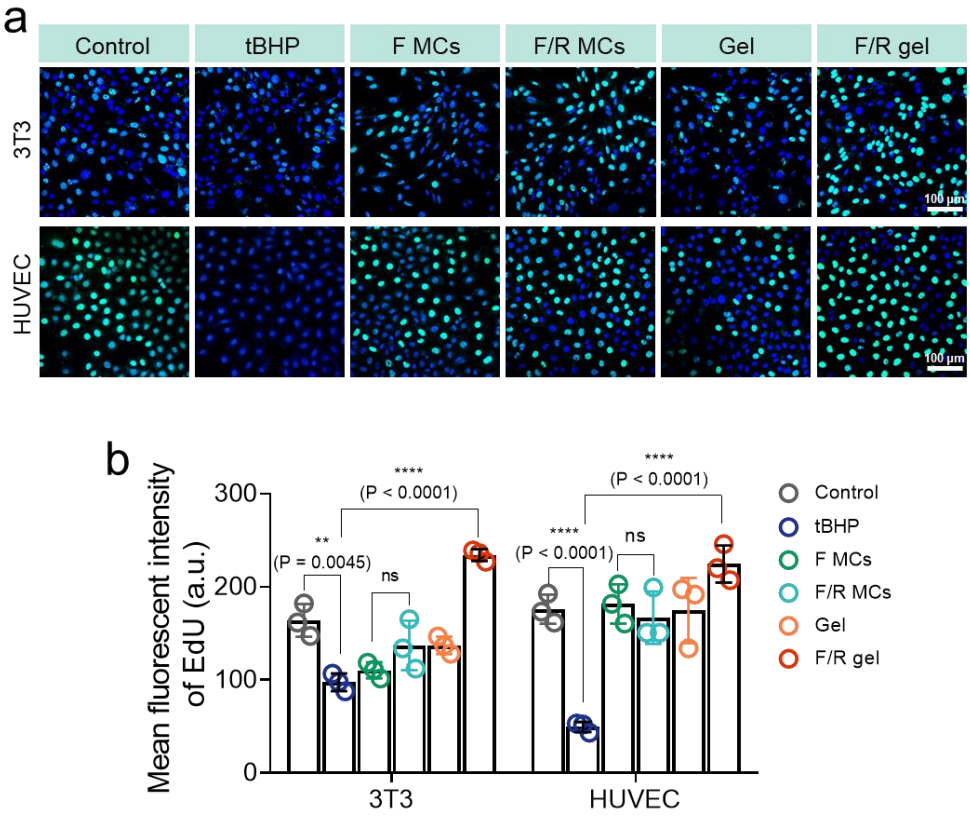
Page 8: "*We also compared the drug release rates of free F/R MCs and F/R gel, and found that the encapsulation of MCs in hydrogel matrix significantly delayed the release of bFGF and siRNA drugs, regardless of the solution's pH values (Supplementary Fig. 20).*"

4. Further explanation of why the F/R gel accelerated the proliferation of various cell lines including 3T3 and HUVEC.

Our Response: We thank the reviewer for this constructive suggestion. We observed a significant

increase of cell viability (Supplementary Fig. 32) after 3T3 or HUVEC cells treated with F/R gel. The proliferative effect of F/R gel on these cell lines were mainly derived from the ROS scavenging effect and the released bFGF. Upon tBHP stimulation, the loaded εPL-CeO_v nanozyme eliminated excessive toxic ROS species and relieved cellular oxidative stress, thus providing the optimal microenvironment to promote cell proliferation. According to previous studies, bFGF can regulate cell proliferation, differentiation, migration, angiogenesis and other biological processes by binding to cell surface receptors (Chuhuang Chen., et al. *Circulation* 101, 171-177, 2000; Bruno, Edward, et al. *Blood* 82, 430-435, 1993; V. Lindner, et al. *J. Clin. Invest*. 85, 2004-2008,1990). Importantly, the proliferation effect of bFGF on cells has been widely studied based on activating different intracellular molecular mechanisms, such as the MAPK/ERK signaling (T. Makino, et al. *Br. J. Dermatol*. 162, 717-23, 2010.) and PI3K/Akt signaling pathways (Feifei Huang, et al. *Stem. Cell. Res. Ther.* 12, 468, 2021.). Thus, bFGF has a wide range of applications in regenerative medicine field (Jialong Chen. et al. *Adv. Compos. Hybrid. Mater.* 5, 1111–1125, 2022.).

Here in our study, we also have added more experiments on cell proliferation of F/R gel, in which EdU staining assay was carried out on 3T3 and HUVEC cells. It was found that the number of EdU-positive cells decreased significantly after tBHP stimulation, while the F/R gel promoted the most cell proliferation when compared with other groups (Supplementary Fig. 33). Moreover, there was no significant difference of EdU fluorescence intensity between the F MCs group and the F/R MCs group, indicating proliferative effect of bFGF loaded in the MCs. In addition, the presence of εPL-CeO_v in F/R gel scavenged cellular oxidative stress, and largely enhanced the role of F/R gel for promoting cell proliferation.



Supplementary Figure 33. (a) Fluorescence images of EdU staining assay performed on 3T3 cells and HUVECs. (b) The corresponding statistical analysis (n = 3).

Following the reviewer's suggestion, we have supplemented these new data in our revised manuscript as follows:

Page 12: "*Meanwhile, we observed a significant reduction in the number of EdU-positive cells in tBHP-stimulated group, while F/R gel's treatment elicited an enhanced cell proliferation (Supplementary Fig. 33). No significant difference of EdU fluorescence intensity was found between the F MCs and F/R MCs groups, indicating the proliferative effect of bFGF drug loaded in MCs. In F/R gel group, the presence of εPL-CeO_y also scavenged cellular oxidative stress, and thus provided an optimal microenvironment for cell proliferation.*"

5. Many researches have been reported on scar-free wound repair using multi-functional hydrogel. The author should highlight the innovation of this work compared to other existing literature in the introduction section.

Our Response: We thank the reviewer for the constructive suggestion. We have made a detailed literature research, and added more discussions on the related literatures of scar-free wound repair using multi-functional hydrogels in our revised manuscript:

Page 4-5: "*Currently, as a classical regulatory pathway for fibrosis, transforming growth factor beta (TGF-β) has been adopted as a promising therapeutic target for anti-scarring wound repair29. Recently, Wu et al. developed a photo-crosslinking hydrogel dressing that facilitated scarless wound repair through pulsatile release of TGF-β inhibitors*[30]*. This innovative dressing significantly enhanced skin wound closure and effectively suppressed scar formation in both murine skin wound model and preclinical large animal wound model. In another recent pioneering study, Gu et al. designed a core-shell structured microneedle patch loaded with verteporfin drug as photo-controlled programmed platform to facilitate scarless skin repair by modulating immune microenvironment and blocking Engrailed-1 (En1) activation*[28]*. However, studies on achieving scar-free repair of chronic wounds are very scarce at present, and the known reports are still limited to traditional targets. TGF-β, for example, has pleiotropic effects for wound healing, in which it promotes wound closure by regulating cell migration, proliferation, and extracellular matrix (ECM) deposition in early stages, whereas it causes fibrosis and scar formation in later stages*[30,31]*. Therefore, wound dressings targeting TGF-β should be carefully administered, otherwise normal healing process may be interfered.*"

Also, we have summarized a table for "Comparison of recent key publications related to wound dressings in regenerative medicine and our work" as Supplementary Table 5, and added the following description in our revised manuscript:

Page 21: "*However, there are few suitable therapeutic approaches that simultaneously accelerate wound closure rate and inhibit fibrotic scarring by dynamically modulating regeneration cascades and influencing proportion of fibroblast subtypes during wound management (Supplementary Table 5).*"

6. Throughout the entire manuscript, the presented data did reflect a positive effect on accelerating scar repair, and the author should add relevant experiments to illustrate the rapid scar-free wound healing.

Our Response: We are very grateful to the reviewer for this suggestion. In our animal experiments (both infected mouse wound and rabbit ear wound models), digital photographs clearly displayed a much faster wound closure rate in F/R gel-treated group (Fig. 6b, Fig. 9b), and this was determined

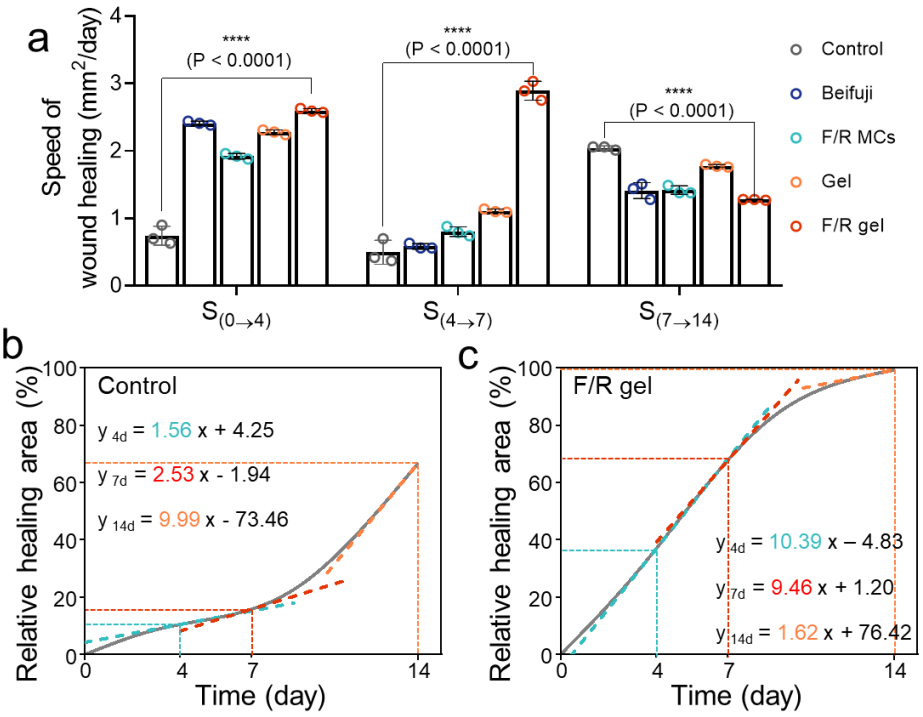
by the smallest wound areas measured on day 4, 7, and 14 (Fig. 6c, Fig. 9c). On the whole, we noticed that F/R gel accelerated wound closure time by approximately 6 to 7 days, which represented a reduction of nearly one-third in total healing duration. For better presentation of **rapid** scar-free wound healing effect, we have reorganized the data of *in vivo* repair effect, which are expressed as wound healing speed as follows:

We define the speed of wound healing as the healed area per unit of time. Let $A_{(t1)}$, $A_{(t2)}$ represent the total healing area at an earlier time point (t1) and at a later time point (t2) respectively, and the healing speed between these two time points can then be expressed as:

$$S_{(t1\rightarrow t2)} = (A_{(t2)} - A_{(t1)})/ (t2 - t1)$$

For detailed clarity, we segmentedly calculated the wound healing speeds during wound healing process, in which Day 4, Day 7, and Day 14 were considered as three important time points for therapeutic evaluation. As shown in Supplementary Fig. 43a, higher healing speeds in the Day (0→4) and Day (4→7) periods were clearly observed in F/R gel-treated group, except for the healing speed in later healing stage (Day 7→14). These data revealed that the wounds in F/R gel group transitioned earlier into proliferative phase than the other groups, while the reduced speed in the later stage avoided excessive proliferation for scarring.

Also, we fitted the healing curves of the Control and F/R gel group, respectively, and determined the instantaneous healing rate based on the curve tangent line of a specific healing time. The healing rates at Day 4, Day 7, and Day 14 were from the corresponding tangent slopes (Supplementary Fig. 43b-c), and the results indicated a similar trend as Supplementary Figure 43a. Due to the impact of bacterial infection, the wound healing rate in Control group was very low from Day 0 to Day 7. In contrast, F/R gel group demonstrated a rapid and sustained wound healing effect during this period, owing to its excellent antibacterial and ROS scavenging capabilities. Importantly, by regulating *c-Jun* overexpression, the F/R gel group showed a slowly reduced healing rate in the later stage for avoiding ECM over-proliferation, facilitating a smoother transition into the remodeling phase. Moreover, immunofluorescence and PCR results of Ki67 marker (Figure 6f, Supplementary Fig. 48) indicated that F/R gel group had the highest level of Ki67 expression at day 7, which decreased at day 14. Overall, these data clearly suggested that F/R gel could accelerate the healing process but avoid ECM over-proliferation for the remodeling phase, which ensured its rapid scar-free wound healing effect.



Supplementary Figure 43. (a) Average wound healing speeds calculated in the Day (0→4), Day (4→7) and Day (7→14) periods. Fitted curves of relative healing area for the (b) Control and (c) F/R groups, in which the tangent lines reflect instantaneous healing rates on Day 4, Day 7, and Day 14, respectively.

Following the reviewer's suggestion, we have added these data and more discussions in our revised manuscript as follows:

Page 14: "*Detailed analysis on the wound healing rates clearly revealed that F/R gel treatment elicited a potent pro-healing effect in the early healing stage (Day 0⟶7), but showed a moderative healing rate in the later stage for avoiding over-proliferation (Supplementary Fig. 43).*"

Page 15: "*As wounds continued to heal, Ki67 expression gradually decreased on day 14 in F/R gel group (Supplementary Fig. 48) to avoid over-proliferation and transition into remodeling phase, while other groups were still in proliferating at this time.*"

7. In Figure 5d, the number and size of cells in live/dead staining images seem not consist with that in the fluorescence images of DCFH-DA probe and HPF probe.

Our Response: We greatly appreciate for this nice comment. Following this suggestion, we re-checked our original data and correctly unified the scale bars of these fluorescent images. Now, the updated images (Figure 5d) were provided in our revised manuscript.

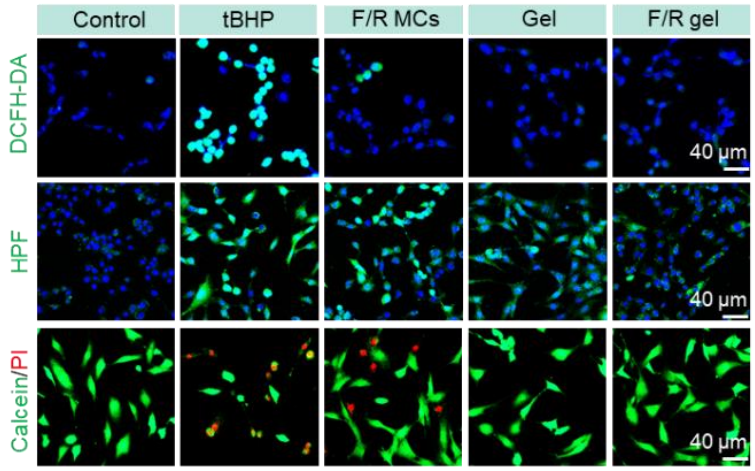


Figure 5. (d) Fluorescence images of DCFH-DA probe, HPF probe, and live/dead cell staining assay on 3T3 cells.

8. A general vision of how the hydrogel would be applied clinically would help the reader better appreciate the contributions. Diverse components were incorporated into the system and stimulating events work in concert to help with wound healing.

Our Response: Thanks to the reviewer for this good suggestion, which will greatly enrich the interpretation of our work. Based on the comment, we have added more discussions on this issue in the "Discussion" part:

Page 21: "*Effective clinical treatment options for chronic wounds are very scarce at present, especially for those caused by severe drug-resistant bacterial infections, and preventing scar formation in the healing skin is even more difficult. The mechanisms of scarring are complex, involving multiple cell types, signaling molecules, and biochemical pathways. While some common wounds can heal spontaneously with a normal skin appearance, chronic wounds are difficult to restore fully functional skin. Moreover, scar treatment after wound healing is usually limited in therapeutic effectiveness and proneness to recurrence. Therefore, to achieve rapid wound healing without scarring, two key issues should be addressed: 1) accelerat wound healing process to ensure repair speed, and 2) inhibit profibrotic pathway and prevent scar formation to ensure repair quality. If these two challenges are tackled separately, the treatment process would require the elaborated use of different drugs at various wound healing stages, which inevitably complicates the approach and hardly achieves ideal curative effect.*"

Reviewer #2 (Remarks to the Author):

Schiff base-crosslinked hydrogels have garnered significant attention due to their biocompatibility, injectability, self-healing properties, and responsiveness to environmental stimuli, making them well-suited for biomedical applications, particularly wound healing. The present study introduces an advanced version of a Schiff base-crosslinked hydrogel (F/R gel) containing εPL-modified oxygen-deficient nanoceria and basic fibroblast growth factor (bFGF)/c-Jun siRNA co-loaded PLGA microcapsules (F/R MCs). This formulation aims to accelerate scarless wound healing.

The study demonstrates that the new hydrogel maintains comparable antibacterial, anti-biofilm, antioxidant, and anti-inflammatory properties to the control gel (without F/R MCs) while also achieving faster and scarless wound healing. The F/R gel outperformed the control gel and a commercial wound gel in both MRSA-infected chronic wounds (mouse model) and rabbit ear hyperplasia wounds.

The authors also discuss the regulatory mechanisms underlying the fast and scarless wound healing seen with this new hydrogel. The study addresses a significant gap in the current understanding of wound healing and scar prevention, providing a promising solution to the medical need for effective scarless healing. Therefore, the manuscript is recommended for publication following minor revisions, as outlined below.

Our Response: We sincerely thank the reviewer for these encouraging and positive comments on our work. According to the suggestions, we have tried our best to revise the manuscript, and our responses point by point according to the comments are listed as bellow.

Revision Comments:

1. The amount of basic fibroblast growth factor (bFGF) and c-Jun siRNA used in the assays needs to be clearly specified. The sequence of c-Jun siRNA should also be provided.

Our Response: We are grateful for the reviewer's kind reminding on these issues. Following the suggestion, we have clarified the amounts of bFGF and c-Jun siRNA concentrations in F/R gel, which were 23 ng/mL for bFGF and 0.25 ng/mL for siRNA, respectively. In addition, the sequence of c-Jun siRNA used in our work was listed as follows:

Sense strand: GGCUACAGUAACCCUAAGATT

Antisense strand: UCUUAGGGUUACUGUAGCCTT

According to the reviewer's comments, we have added the necessary information in our revised manuscript:

Page 24: "*200 μg of bFGF and 2.5 μg of c-Jun siRNA were dissolved in deionized water, while 10 mg PLGA power was dissolved in dichloromethane.*"

Page 25: "*The final concentrations of bFGF and siRNA in F/R gel were calculated as 23 ng/mL and 0.25 ng/mL, respectively.*"

Page 2 in the "Supplementary Information": "*c-Jun siRNA with the sense strand of GGCUACAGUAACCCUAAGATT and the antisense strand of UCUUAGGGUUACUGUAGCCTT was purchased from GenePharma Co., Ltd. (Shanghai, China).*"

2. The resolution of images in Figure 6f should be improved for better interpretation of the data.

Our Response: We appreciate the reviewer's constructive feedback. Following the suggestion, we have improved the resolution of images in Figure 6f to enhance the clarity. The updated figure is provided as follows and also included in the revised manuscript for your review.

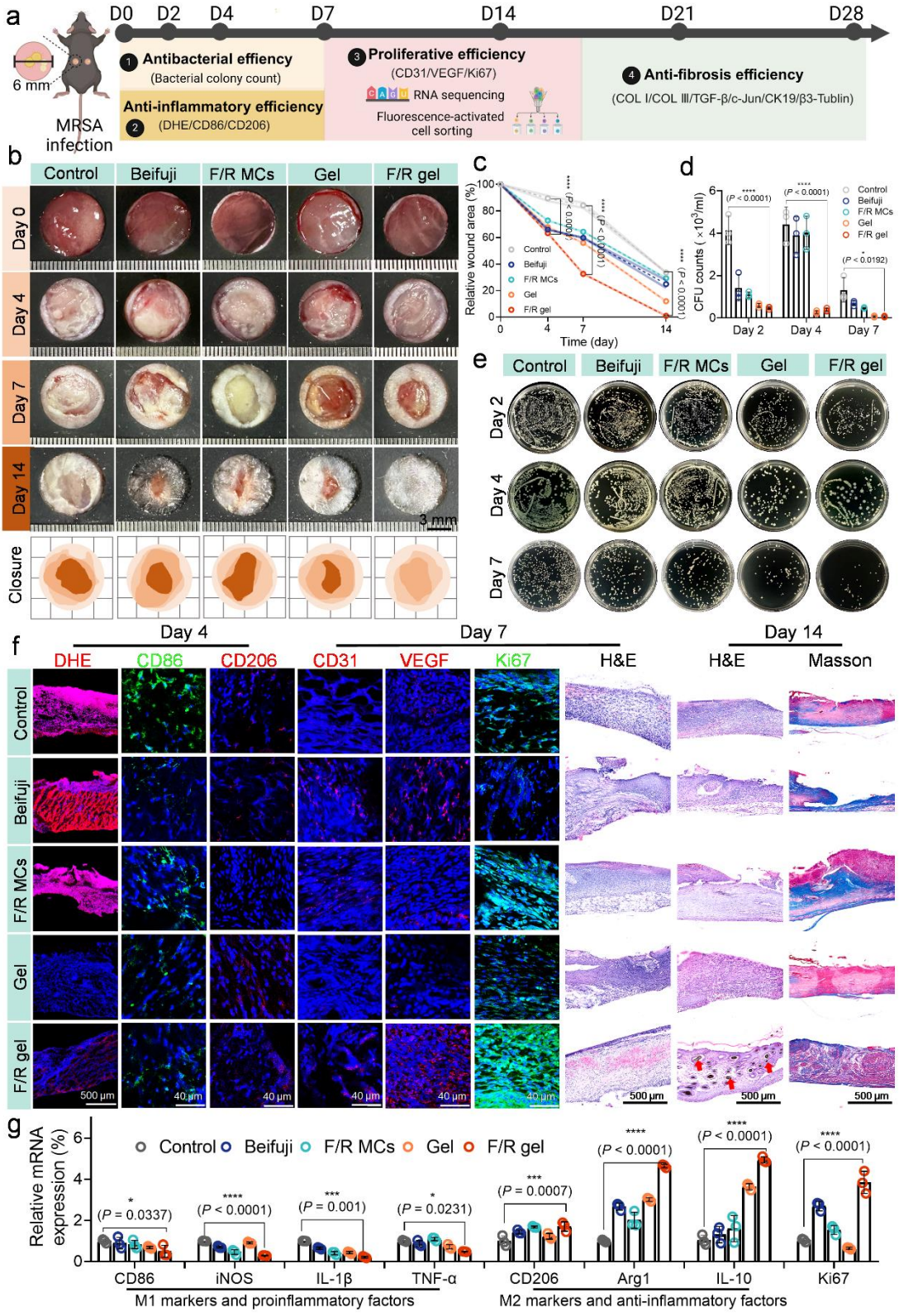
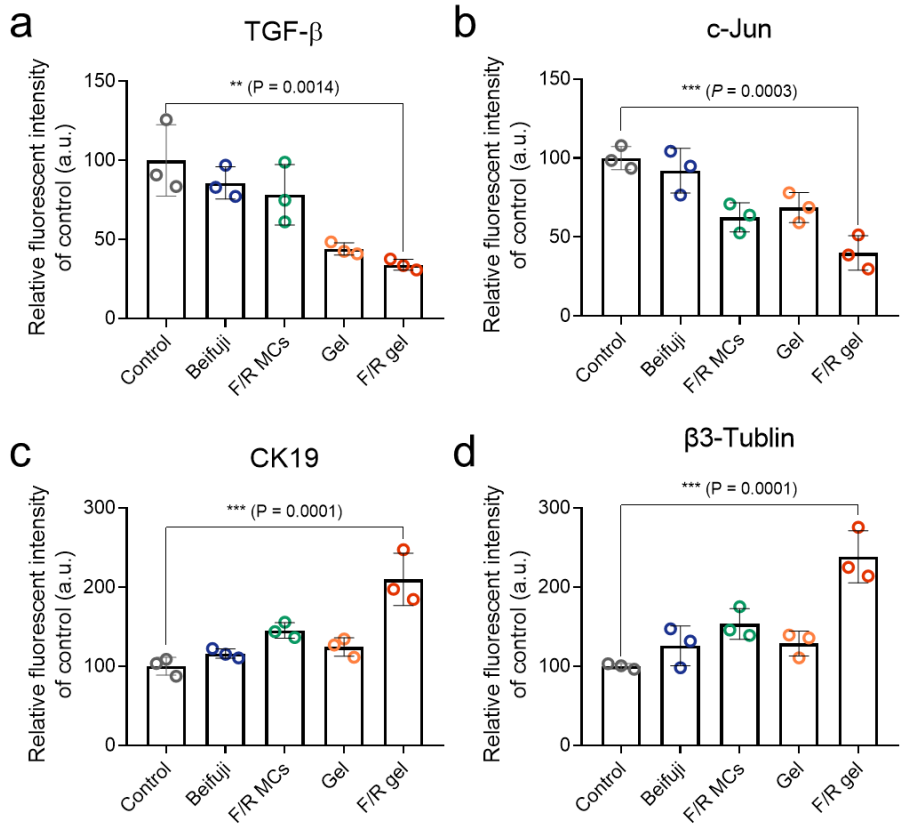


Figure 6. *In vivo* rapid healing effect of F/R gel on mouse MRSA-infected chronic wounds. (a) Experimental process of the whole animal experiments on mice (created with BioRender.com). (b) Optical photos and simulated graphs of wound closure changes from day 0 to day 14. (c) Variation curve of wound areas within 14 days (n = 3). (d) Colony counting assay using wound exudates on day 2-7 (n = 3) and (e) representative images. (f) Immunofluorescent staining, H&E staining, and Masson staining images of wound tissues during wound healing. Red arrows mark the

newborn hair follicles. (g) Relative mRNA expressions of CD86, iNOS, IL-1β, TNF-α, CD206, Arg1, IL-10, and Ki67 (n = 3).

3. In Figure 7D, showing immunofluorescence images of TGF-β, c-Jun, CK-19, and β3-tubulin, please include quantitative statistical analysis of the results across the different groups. Update the images with higher resolution for better visual presentation.

Our Response: Thanks for the reviewer's nice suggestion. We have quantitatively analyzed these immunofluorescence images and provided the data as Supplementary Fig. 51 in the resubmitted manuscript. Also, we have improved the resolution of images in Figure 7d to enhance the clarity, and updated this figure in our revised manuscript for your review.



Supplementary Figure 51. Quantitative expression levels of (a) TGF-β, (b) c-Jun, (c) CK19 and (d) β3-Tublin at the wound sites on day 28.

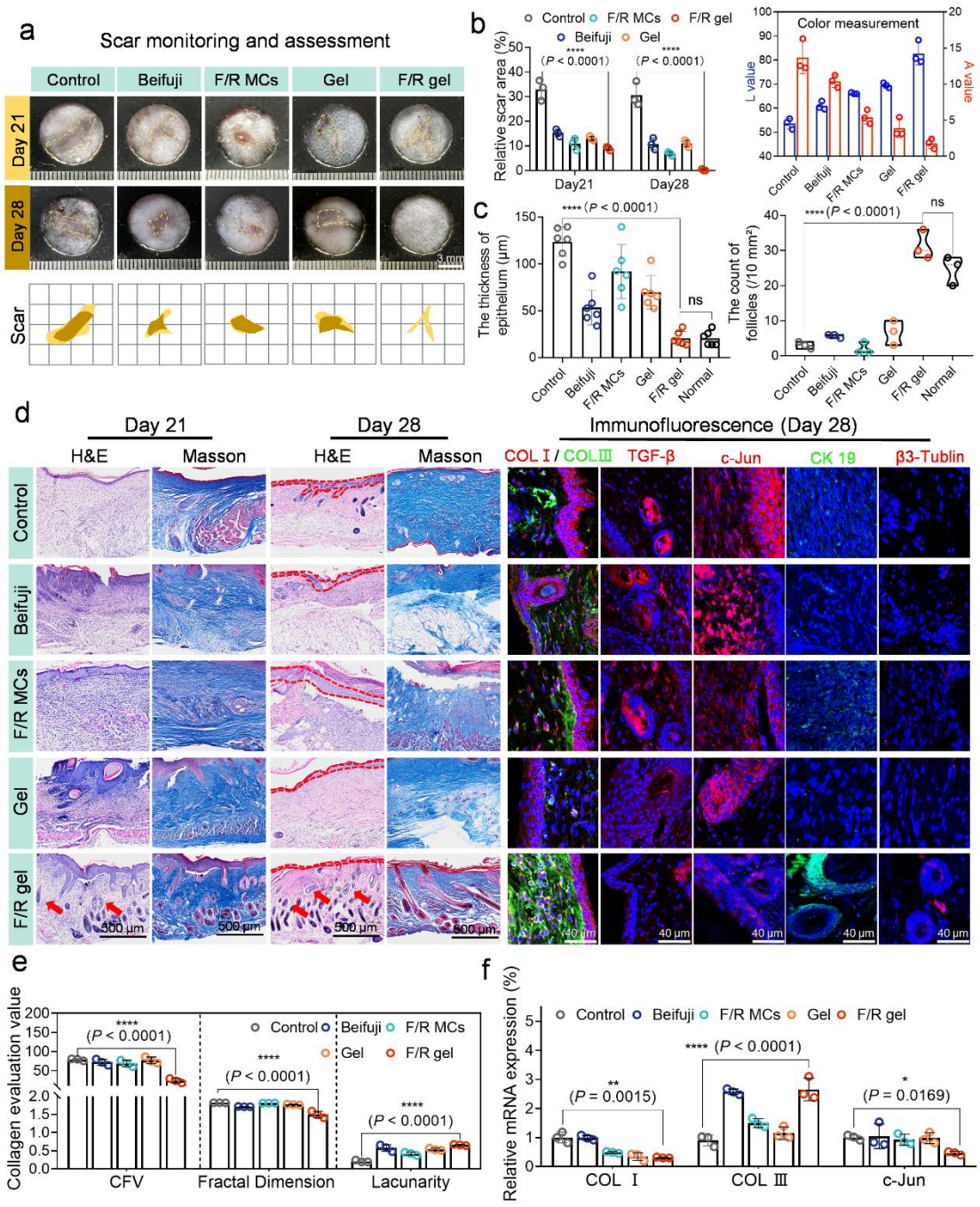


Figure 7. F/R gel alleviates fibrotic scar formation of MRSA-infected chronic wounds on mice. (a) Optical photos and simulated graphs of scar area changes from day 21 to 28. (b) Scar changing curve and color analysis of scar tissues post-treatments (a higher L value represents a whiter color, and a higher A value represents a redder color) (n = 3). (c) Epidermal thickness and number of hair follicles at the wound site of mice based on tissue section analysis. (d) H&E staining, Masson's staining, and immunofluorescent staining images of wound tissues on day 21 to 28. (e) Collagen volume ratio, fractal dimension value, and lacunarity value based on the tissue slice analysis. (f) Relative mRNA expressions of COL I, COL III, and c-Jun on day 28.

4. It appears that F/R MCs alone do not exhibit c-Jun blocking in the mouse model, even though c-Jun gene silencing was confirmed in fibroblasts (Figure 7f). Please provide an explanation for this discrepancy. It seems likely that c-Jun siRNA works synergistically with other components of the

hydrogel to achieve c-Jun blocking. Please elaborate.

Our Response: We fully agree with the reviewer on this point. Indeed, as seen in Fig. 7f, no significant blocking of c-Jun was observed in F/R MCs group on day 28 in the animal experiments, and this phenomenon was also confirmed by immunofluorescence staining results in Fig. 7d. We analyzed the possible reasons for this outcome as follows:

Unlike *in vitro* cellular experiments, free F/R MCs were applied directly to the wound tissues through dropwise addition of aqueous dispersion. This method could not ensure the F/R MCs to continuously remain in wounds during the whole healing cycle, in which fibroblasts were mainly active for ECM production in the wound proliferation stage. With the progress of wound healing, free F/R MCs quickly ran out and thus hardly accelerated a scar-free wound healing effect. In our animal experiments, we observed significant scar tissue formation in F/R MCs group (Fig. 7a). Therefore, both PCR assay and immunofluorescence staining of these scar-forming tissues on Day 28 showed a high c-Jun expression, consistent with the results of c-Jun overexpression in scar tissues reported previously (Michelle F Griffin, et al. *Sci. Transl. Med.* 13, eabb3312, 2021.).

In F/R gel group, the sustained release capacity of hydrogel network could effectively prolong the drug retention to better fit with the wound healing stages, and guaranteed MCs' activity in the critical proliferative phase dominated by fibroblasts. Therefore, effective *c-Jun* blocking effect *in vivo* could only be achieved when synergistically combined with our hydrogel system, rather than in free F/R MCs form. This also explains the necessity of our proposed F/R gel system in promoting scarless wound healing.

Following the reviewer's comments, we have added explanation for this in our revised manuscript:
Page 17: "*It should be noted that the uninfluenced c-Jun expression in F/R MCs group might be due to the rapid loss of free F/R MCs in healing process, highlighting the necessity of gel system for synergistically promoting scarless wound healing.*"

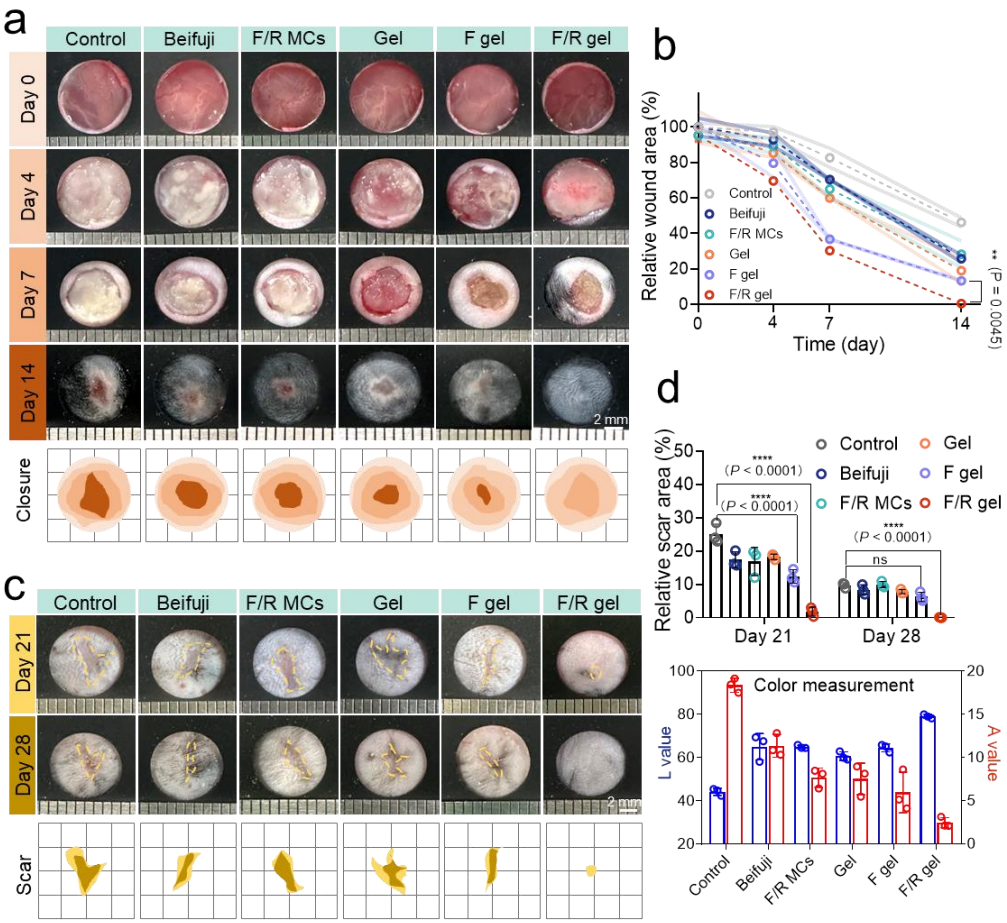
5. It's interesting to note that collagen I expression in the F/R gel group is significantly reduced, while collagen III expression shows the opposite trend. Given that c-Jun is reported to directly bind to the promoters of COL1A1 and COL3A1 and inhibit their transcription, could these results be related to c-Jun activity? How would you interpret the differential regulation of collagen types I and III in this context?

Our Response: We thank for the reviewer's enlightening comment. As a major component of heterodimeric transcription factor AP-1, c-Jun predominantly expressed in fibroblasts has been demonstrated as a critical driver of tissue scarring in multiple organs (Gerlinde Wernig, et al. *Proc Natl. Acad. Sci.* 114, 4757-4762, 2017; Lu Cui, et al. *Nat. Commun.* 11, 2795, 2020; Tristan Lerbs, et al. *JCI. Insight.* 5, e140458, 2020; Deshka S Foster, et al. *Nat. Commun.* 11, 4061, 2020). c-Jun N-terminal kinase (JNK) was originally identified as a protein kinase that specifically phosphorylated the N-terminal transactivation domain of transcription factor c-Jun, and subsequently activated AP-1 to induce profibrotic gene expressions, such as *COL1A1*, *COL3A1*, and *ACTA2* (Junlan Zhu, et al. *Cancer. Prev. Res.* 7, 1270-81, 2014; Yonggang Sha, et al. *Biochim. Biophys. Acta.* 1820, 701-711, 2012; Miao Zhou, et al. *Acta Pharmacol. Sin.* 43, 602-612, 2022). However, regulation of these profibrotic gene transcription is highly complex and involves various enhancing or inhibitory elements (Serena Fineschi, et al. *FASEB. J.* 20, 562-564, 2006). Their promoters have different transcription factor binding sites such as Sp1, Sp3, collagen-Kruppel box, AP-1, and AP-2 (Magdalini Kypriotou, et al. *J. Biol. Chem.* 282, 32000-32014, 2007; P Rossi, et al.

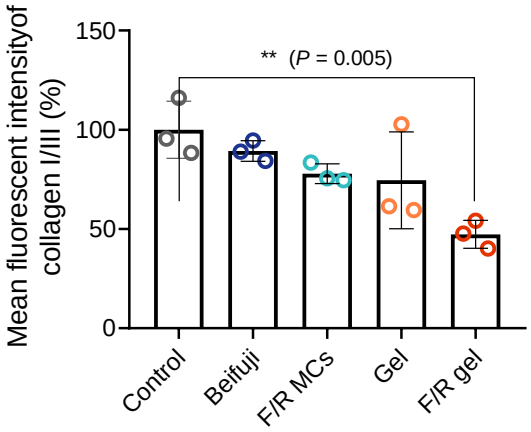
Cell 52, 405-414, 1988; Keun Jae Ahn, et al. *Open. Med. (Wars).* 17, 1473-1482, 2022)

In our work, the *in vivo* data of both immunofluorescent staining and PCR experiments indicated that collagen I expression in the F/R gel group was significantly reduced, while the trend was reversed for collagen III in wound tissues collected on day 28 (Fig. 7d-f). These results were consistent with previous reports regarding to the ratio changes of collagen I and III in scarless tissues after wound closure (Meilang Xue, et al. Adv. Wound. Care. (New Rochelle). 4, 119–136, 2015; Tong Wu, et al. *ACS Nano* 15, 20087-20104, 2021). To further figure out the specific role of c-Jun siRNA in F/R gel for scar prevention, we have re-assessed wound repair effect of different treatments on MRSA-infected mouse wounds, in which the F gel (gel containing only bFGF-loaded microspheres) group was added to the original groups. Consistent with the previous experimental data in Fig. 6b-c, the F/R gel group exhibited the fastest wound healing effect with a healing rate of approximately 69.66% on day 7, which was significantly higher than that of the Control group (approximately 17.33%) (Supplementary Fig. 42a-b). The healing efficiency of F gel group was similar to that of the F/R gel group during the first 7 days; however, by day 14, the healing efficiency reached approximately 86.60%, whereas the wounds in F/R gel group were almost completely healed (approximately 99.45%). Importantly, we observed no visible scars in the F/R gel group, while the F gel group also generated significant scarring at wound areas on day 28 (Supplementary Fig. 42c-d). Besides, serious scarring with a higher collagen I/III ratio was also found in the free F/R MCs group (Supplementary Fig. 52), revealing that direct administration of F/R MCs to wounds was very difficult to properly regulate collagen-rated gene expressions and thus inhibit scar formation. Therefore, we speculated that the differential expression of collagen I and III following F/R gel treatment was likely due to the coordinated regulatory effects of gel's bioactive components.

We acknowledge very much for the reviewer's attention on this important and heuristic point. A more comprehensive understanding of how c-Jun gene specifically regulates the expressions of collagen I and III during wound healing is undoubtedly enlightening for us to better manipulate collagen deposition by our hydrogel for scarless wound repair in practical applications. Our next research plan will focus more on such specific regulatory mechanisms, which we believe to be of great benefit to our work.



Supplementary Figure 42. (a) Optical photos and simulated graphs of wound closure changes from day 0 to day 14. (b) Variation curve of wound areas within 14 days (n = 3). (c) Optical photos and simulated graphs of scar area changes from day 21 to 28. (d) Scar changing curve and color analysis of scar tissues post-treatments (a higher L value represents a whiter color, and a higher A value represents a redder color) (n = 3).



Supplementary Figure 52. Quantitative expression levels of collagen I/III ratios analyzed at the wound site.

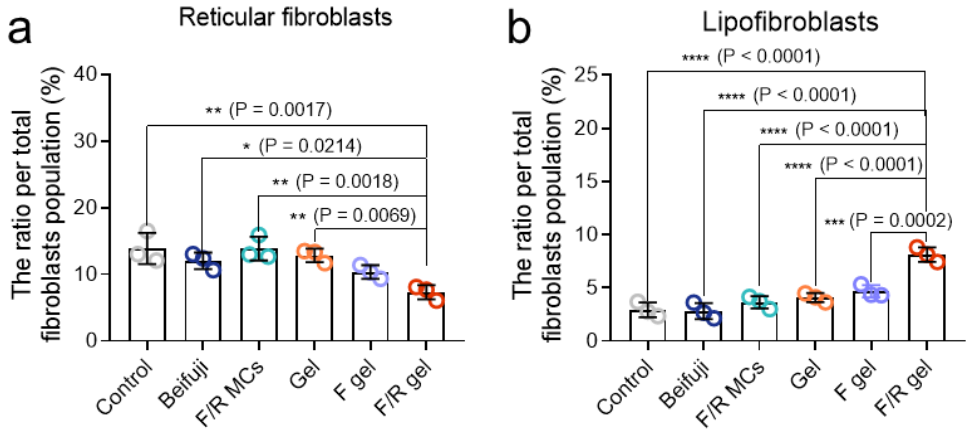
Base on the reviewer's comment, we have added these data and more discussions in our revised manuscript as follows:

Page 17-18: "*To further elaborate the specific role of c-Jun siRNA in F/R gel for scar reduction, we*

supplemented another batch of animal experiments in which the F gel (gel containing only bFGF-loaded microspheres) group was added (Supplementary Fig. 42 and 49). The results clearly revealed that F gel treatment only accelerated wound closure rate but hardly inhibited scarring tissues, hence highlighting the importance of F/R gel's components for anti-scar wound healing."

6. In Figure 8a, to further support your hypothesis that bFGF and c-Jun siRNA stimulate fibroblast proliferation while modulating distinct fibroblast subpopulations for anti-fibrotic wound healing, you should perform FACS experiments to analyze the proportions of fibroblast subtypes across all control groups. Especially the differences in fibroblast subpopulations between scarred wound healing (Beifuji/ blank Gel) and scarless wound healing (F/R gel) groups.
Once addressed, the manuscript would be well-suited for publication.

Our Response: We thank the reviewer's valuable comments. In response to this suggestion, we have conducted additional FACS experiments for the Beifuji group, the F/R MCs group, the Gel group, and the F gel group to further investigate the changes of distinct fibroblast subpopulations in these control groups. As shown in Supplementary Fig. 54, the proportion of reticular fibroblasts in F/R gel group was consistently the lowest among all the groups, and the differences were statistically significant when compared to all the control groups. In contrast, the proportion of lipofibroblasts exhibited the opposite trends. Therefore, these findings further validated the effect of F/R gel on modulating fibroblast subtypes to promote scar-free wound repair.



Supplementary Figure 54. Fluorescence-activated cell sorting results on fibroblast subtypes of wound tissues in different groups (conducted in additional independent experiments).

Following the reviewer's comments, we have added these new data and more description on this part in our revised manuscript:

Page 18: "*To verify this speculation, we performed FACS experiments to analyze the subtype proportions of fibroblasts in traumatic tissues on day 7 (Fig. 8a). The proportion of reticular fibroblasts in F/R gel group was significantly lower than all the control groups, while the variation tendency for lipofibroblasts was quite the other way (Fig. 8b, Supplementary Fig. 53-54).*"

Reviewer #3 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Our Response: We also appreciate the co-reviewer's attention on our work. Now we have carefully addressed each of the reviewers' comments and thoroughly revised our manuscript.

Reviewer #4 (Remarks to the Author):

The manuscript introduces an innovative phase-adaptive hydrogel with dynamic properties to promote rapid and scar-free healing of chronic wounds. It effectively addresses challenges such as multidrug-resistant infections, oxidative stress, and excessive fibrosis in wound healing by leveraging a hierarchical drug release mechanism. The study presents a robust combination of experiments, from material synthesis and in vitro assessments to in vivo testing on wound models, showing substantial promise for clinical translation. However, the manuscript requires revisions to enhance clarity, address technical gaps, and improve experimental rigor. Below are detailed comments for revisions.

Our Response: We really appreciate the very positive remarks and valuable suggestions from the reviewer. In response to these comments, we have carefully revised the manuscript, improved the clarity of images, and added necessary experiments to strengthen the validity of conclusions.

1. The introduction, although comprehensive, should place more emphasis on how this hydrogel design compares to existing wound healing technologies. A clearer statement of novelty should be added.

Our Response: Thanks for the reviewer's constructive suggestion. Following the comments, we have summarized a table for "Comparison of recent key publications related to wound dressings in regenerative medicine and our work" as Supplementary Table 5, highlighting the innovative applications of our F/R gel system. In addition, we have added more discussions on the related literatures of existing wound healing technologies in our revised manuscript as follows:

Page 3-4: "*Up to now, various wound dressings have been developed for chronic wound managements, among which promoting transition from inflammation to proliferation phase is the most-proposed and studied therapeutic target for healing*[16-19]*. Notwithstanding these advanced wound dressings meet the needs of antimicrobial activity, maintaining redox balance, and promoting proliferation to some extent, most of them may ignore how to rationally orchestrate essential facets of the different healing process to achieve scarless wound repair.*"

Page 4-5: "*Currently, as a classical regulatory pathway for fibrosis, transforming growth factor beta (TGF-β) has been adopted as a promising therapeutic target for anti-scarring wound repair29. Recently, Wu et al. developed a photo-crosslinking hydrogel dressing that facilitated scarless wound repair through pulsatile release of TGF-β inhibitors*[30]*. This innovative dressing significantly enhanced skin wound closure and effectively suppressed scar formation in both murine skin wound model and preclinical large animal wound model. In another recent pioneering study, Gu et al. designed a core-shell structured microneedle patch loaded with verteporfin drug as photo-controlled programmed platform to facilitate scarless skin repair by modulating immune microenvironment and blocking Engrailed-1 (En1) activation*[28]*. However, studies on achieving scar-free repair of chronic wounds are very scarce at present, and the known reports are still limited to traditional targets. TGF-β, for example, has pleiotropic effects for wound healing, in which it promotes wound closure by regulating cell migration, proliferation, and extracellular matrix (ECM) deposition in early stages, whereas it causes fibrosis and scar formation in later stages*[30,31]*. Therefore, wound dressings targeting TGF-β should be carefully administered, otherwise normal healing process may be interfered.*"

Page 21: "*However, there are few suitable therapeutic approaches that simultaneously accelerate wound closure rate and inhibit fibrotic scarring by dynamically modulating regeneration cascades*

and influencing proportion of fibroblast subtypes during wound management (Supplementary Table 5)."

2. The abstract and introduction provide an ambitious overview of the hydrogel's design and functions, but certain key terms, such as "phase-adaptive" and "hierarchical drug release" are not fully explained in their initial introduction. It is essential to clarify what is meant by "phase-adaptive regulating functions" and how they specifically contribute to the wound healing process at a molecular level.

Our Response: We thank the reviewer for these constructive suggestions. Now we have supplemented more clarifications for the terms (including "phase-adaptive", "hierarchical drug release") and the crucial roles of different components (εPL, εPL-CeO_v, bFGF and *c-Jun* siRNA) for wound healing process in our revised manuscript:

Page 5-6 for the term of "phase-adaptive": "*Drug-resistant bacteria are highly prone to forming weakly acidic biofilms on wound surfaces, which pose a significant obstacle to wound healing. The Schiff base bond in hydrogel matrix is very sensitive to cleavage under acidic condition, leading to fast hydrogel degradation. During the early stages of wound healing, low-molecular εPL and ultrasmall εPL-CeO_v rapidly released, exerting their anti-biofilm and ROS-scavenging activities. The large specific surface area and abundant oxygen vacancies on εPL-CeO_v surface allowed cerium ions to interconvert between Ce^{3+} and Ce^{4+}, which laid the foundational molecular mechanism for its enzymatic activity such as superoxide dismutase and catalase. Combined with the inherent antimicrobial property of εPL, the εPL-CeO_v could further modulate local inflammation via alleviating oxidative stress of wound tissues, which was critical for promoting smooth transition of inflammation to proliferation. The F/R MCs, which were uniformly distributed in the hydrogel and relatively larger in size, gradually released as the hydrogel degraded, and this process was accompanied by the release of bFGF as well as c-Jun siRNA. Specifically, in the proliferative phase, bFGF accelerated wound healing by promoting cell proliferation and angiogenesis, while c-Jun siRNA inhibited c-Jun overexpression and regulate the ratio of fibroblast subtypes, leading to scar-free skin regeneration. Therefore, the immunomodulatory and fibroblast subtypes regulation by F/R gel's phase-adaptive functions promoted anti-bacterial infection, angiogenesis, opportune ECM deposition, and dermal fibrosis suppression.*"

Page 8 for the term of "hierarchical drug release": "*Therefore, the gel system had the property of hierarchical drug delivery, in which 1) antibacterial εPL-containing components (free εPL, εPL-CeO_v) released first to fight against bacterial infections, 2) closely followed by ROS and inflammation clearance regulated through CeO_v nanozyme, and then 3) pro-proliferative bFGF and c-Jun-inhibiting siRNA started to facilitate neoangiogenesis, cell proliferation, and simultaneously modulate fibroblast subtypes. This strategy offers the possibility of precise drug delivery on demand according to different wound healing stages, and holds great potentials for achieving rapid anti-fibrotic wound healing (Fig. 2l).*"

3. While the manuscript provides a comprehensive analysis of the hydrogel's components (e.g., εPL, CeO_v nanozyme, siRNA, and bFGF), the mechanistic elucidation of their interactions within the dynamic wound microenvironment can be further refined. The transition from bacterial clearance to immunomodulation and scar inhibition through c-Jun suppression necessitates a more robust mechanistic linkage between various phases of healing.

Our Response: We are grateful to the reviewer for this good suggestion, which we believe is a very constructive point to improve the clarify of our hydrogel system's superiority. Based on the comment, we have added more elucidations of the important interactions between F/R gel's components (εPL, CeO_v, siRNA, bFGF) and dynamic wound microenvironment during various healing phases, which are now highlighted in the "Discussion" section of our revised manuscript:

Page 21: "*Effective clinical treatment options for chronic wounds are very scarce at present, especially for those caused by severe drug-resistant bacterial infections, and preventing scar formation in the healing skin is even more difficult. The mechanisms of scarring are complex, involving multiple cell types, signaling molecules, and biochemical pathways. While some common wounds can heal spontaneously with a normal skin appearance, chronic wounds are difficult to restore fully functional skin. Moreover, scar treatment after wound healing is usually limited in therapeutic effectiveness and proneness to recurrence. Therefore, to achieve rapid wound healing without scarring, two key issues should be addressed: 1) accelerate wound healing process to ensure repair speed, and 2) inhibit profibrotic pathway and prevent scar formation to ensure repair quality. If these two challenges are tackled separately, the treatment process would require the elaborated use of different drugs at various wound healing stages, which inevitably complicates the approach and hardly achieves ideal curative effect.*"

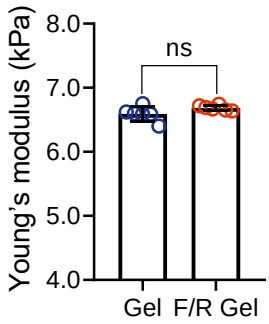
Page 21-22: "*The systematic in vivo and ex vivo experiments revealed that in early stages of wound healing, the wound microenvironment influenced by bacterial biofilms accelerated the degradation of hydrogel matrix. The controlled release of low-molecular εPL and ultrasmall εPL-CeO_v performed antimicrobial effect by inhibiting bacteria survival and biofilm formation. Suppressed biofilm growth and dissociated mature biofilms of Gram-positive as well as Gram-negative bacteria with higher efficiency were observed when compared to conventional antibiotics. Meanwhile, the released εPL-CeO_v nanozyme exerted its ROS scavenging activity to regulate local immune microenvironment by neutralizing excess inflammatory cytokines and inhibiting macrophage polarization toward a pro-inflammatory phenotype, thereby helping the wound rapidly pass through inflammatory phase. Previous studies have highlighted the critical importance of smooth transition from the inflammatory to the proliferative phase for successful would healing, and a failure in this transition can lead to prolonged inflammation as well as impaired re-epithelialization, ultimately resulting in intractable nonhealing wounds*[62,63]*. As shown in our results, the untreated mice in control group exhibited much increased inflammation and delayed epithelialization. Taking advantage of the larger size and slower degradation characteristic of F/R MCs, the loaded bFGF and siRNA drugs gradually released from the disintegration of MCs. bFGF accelerated wound healing rate by promoting cell proliferation and angiogenesis in the proliferative phase. Meanwhile, c-Jun siRNA regulated fibroblast subsets (reduced the number of reticular fibroblasts while increased lipofibroblasts) by inhibiting c-Jun overexpression, thereby preventing excessive fibrogenesis and reducing scar formation.*"

4. The characterization of the mechanical properties of the hydrogel, including its stiffness and adhesiveness, should be more comprehensively linked to its in vivo performance. Were any assessments performed to determine the hydrogel's adherence to the wound surface under physiological conditions? Could this influence its efficacy in promoting wound healing?

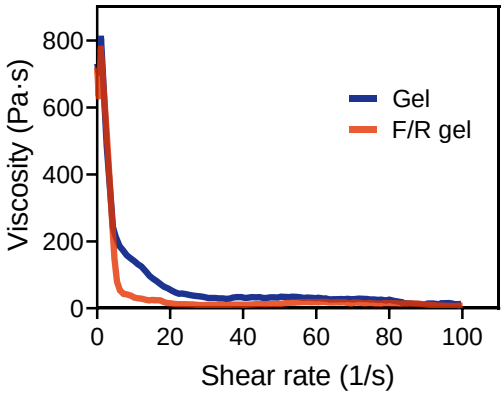
Our Response: Thanks for the reviewer's instructive advice on the characterization of hydrogel. Following this suggestion, we have added the corresponding hydrogel characterization (including

stiffness, viscosity) and *in vivo* adhesiveness of F/R gel for wound healing application.

Firstly, we evaluated the stiffness and viscosity of F/R gel by rheometer. Young's modulus is one of the most essential mechanical properties for hydrogels, which directly reflects their stiffness or flexibility, and is crucial for their adaptability in wound repair. A hydrogel that is too soft may not provide enough mechanical support, while a hydrogel too stiff may cause additional irritation or compression to the wound and interfere with the healing process. The Young's modulus of normal human skin ranges from 4.5 to 8 kPa (C Pailler-Mattei, et al. *Med. Eng. Phys.* 30, 599-606, 2008). As shown in Supplementary Fig. 12, the F/R gel's Young's modulus was ~ 6.69 kPa, which was in line with this range and not significantly different from that of the blank Gel. In addition, as shown in Supplementary Fig. 13, the viscosity of both Gel and F/R gel decreased gradually with the increase of shear rate, and the addition of F/R MCs did not change the original fluidity and viscosity of hydrogel matrix.



Supplementary Figure 12. Young's modulus of the blank Gel and F/R gel.



Supplementary Figure 13. Viscosity of the blank Gel and F/R gel.

For adhesion performance test, we demonstrated that F/R gel could steady adhered to the experimenter's skin whether finger joint was cyclically straight or bent (Fig. 2g). Besides, we also found that the F/R gel could adhere to different types of fresh wounds created on mice (Supplementary Fig. 16), revealing its excellent adhesion capacity. After applied on the wound sites, the hydrogel directly contacted with wound tissues, and immediately formed multiple interfacial bonds between the abundant reactive groups (e.g., amino, carboxyl) of hydrogel and the amine or thiol groups of warm tissue surface (Yanan Jiang, et al. *ACS Nano*. 16, 8662-8676,2022.). The excellent adhesion property ensured positive effects on wound protection and rapid healing efficiency during F/R gel treatment.

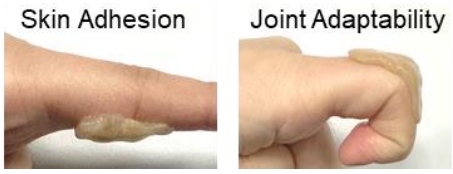


Figure 2. (g) Representative photographs of F/R gel's adhesion on experimenter's fingers

Supplementary Figure 16. Representative photographs of F/R gel's adhesion to different types of mouse wounds.

We have added these new data and more descriptions on this issue in our revised manuscript as follows:

Page 7: "*Rheological results revealed that stable three-dimensional gel was formed in about 3.0 seconds (Fig. 2c), with the Young's modulus at ~ 6.69 kPa comparable to that of the blank Gel (Supplementary Fig. 12). By adjusting shear strain values at 0%-1000% (1 Hz), both F/R gel's storage modulus (G') and loss modulus (G") kept decreasing with increase of shear stress, while an inflection point for G" > G appeared at about 770%, indicating shear thinning behavior (Fig. 2d, Supplementary Fig. 13).*"

"*Besides, excellent adhesion performance was also demonstrated by steady adhering to both the experimenter's skin whether finger joint was cyclically straight or bent (Fig. 2g), and the various types of fresh wounds created on mice (Supplementary Fig. 16).*"

5. The drug release profile (Fig. 2k) needs more clarification. The current graph suggests a step-wise release, but the dynamics of drug diffusion from the gel need to be explained more comprehensively. The manuscript would benefit from including data on the stability and degradation of the hydrogel matrix in vitro.

Our Response: We sincerely thank for the reviewer's constructive comment. Following this suggestion, we have performed more detailed drug releasing experiments, and also added new data on the stability/degradation of F/R gel *in vitro* as follows:

(1) Drug releasing property of the F/R gel

To elucidate the drug release profiles, we conducted the drug releasing experiments of F/R gel by

using two different pH conditions: neutral PBS (pH = 7.4) and acidic PBS (pH = 4.5). As shown in Fig. 2k, the results clearly verified the nature of sequential releasing properties in both neutral and acidic conditions, in which εPL-CeO_v released first, then followed by bFGF/siRNA drugs to the external solutions. Moreover, acidic microenvironment (pH = 4.5) triggered faster drug release rates especially for εPL-CeO_v nanoparticles, which was consistent with the acid sensitivity of Schiff base bond. Furthermore, we compared the drug release rates of free F/R MCs and F/R gel, and found that the encapsulation of MCs in hydrogel matrix significantly delayed the release of bFGF and siRNA drugs, regardless of the solution's pH values (Supplementary Fig. 20). Therefore, these results suggested that F/R gel system had a hierarchically delivering property, in which εPL-CeO_v and the loaded drugs released in a certain sequence, rather than simultaneously from the gel.

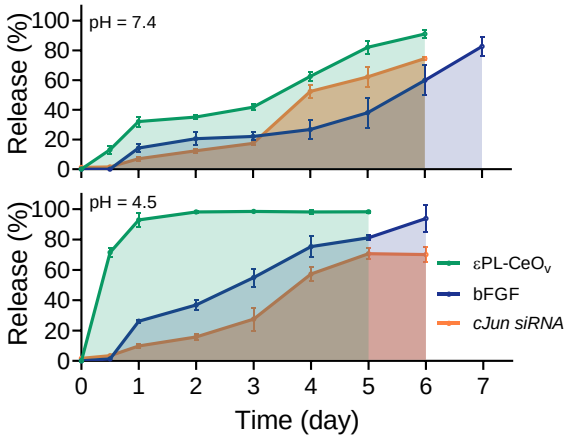
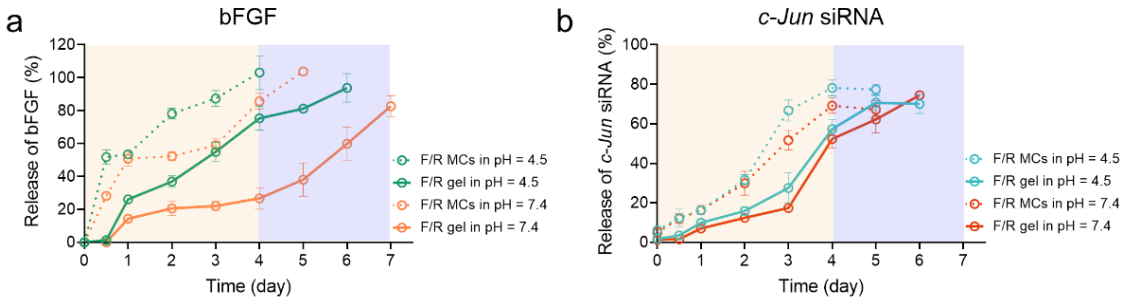


Figure 2. (k) *In vitro* release properties of F/R gel's components in neutral PBS (pH = 7.4) and acidic PBS (pH = 4.5).



Supplementary Figure 20. *In vitro* release properties of (a) bFGF and (b) c-Jun siRNA from F/R MCs and F/R gel.

We have added these new data and more descriptions in our revised manuscript as follows:

Page 8: "*As shown in Fig. 2k, when incubated in neutral PBS, εPL-CeO_v released first from the gel, in which ~ 91.13% was detected to release into external solutions at day 6. With gel degradation, release of bFGF and siRNA drugs became gradually detectable. By day 7, the cumulative release amount of bFGF was close to 85%, while the siRNA drug reached ~ 74.48%. Moreover, acidic microenvironment (pH = 4.5) triggered faster drug release rates especially for εPL-CeO_v, which was consistent with the acid sensitivity of Schiff base bond. In addition, the accelerated breaking of Schiff-base hydrogel network in weakly acidic environment was further demonstrated by the in vitro hydrogel degradation experiments (Supplementary Fig. 19), which was more favorable for the release of εPL-CeO_v and F/R MCs from F/R gel. We also compared the drug release rates of free*

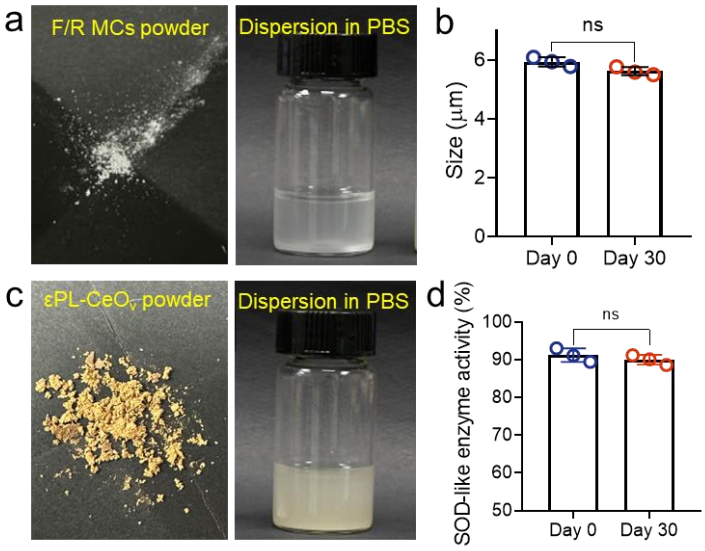
F/R MCs and F/R gel, and found that the encapsulation of MCs in hydrogel matrix significantly delayed the release of bFGF and siRNA drugs, regardless of the solution's pH values (Supplementary Fig. 20)."

(2) Stability and degradation of the F/R gel *in vitro*

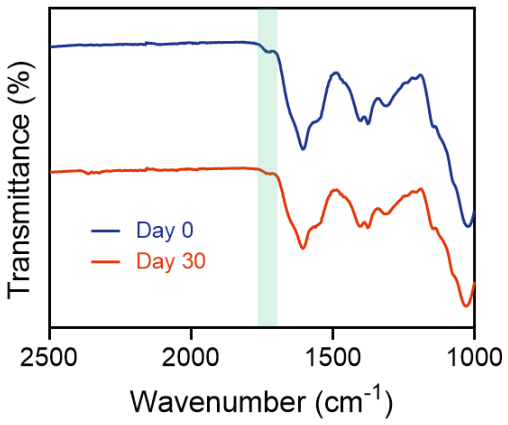
The F/R gel system in our work is a kind of ready-to-use hydrogels, in which each component for gel can be stored separately and mixed for instant use when needed. Therefore, we first evaluated the storage stability of each component separately to ensure the overall gel stability for actual applications.

We stored F/R MCs at 4°C and εPL-CeO_v at room temperature after preparation for up to 30 days, and carefully checked their properties including powder appearances, aqueous dispersions, size changes, and enzyme activity. As shown in Supplementary Fig. 14-15, no significant changes were found in these samples after long-term storage. In addition, HA-CHO, as another hydrogel's main component, was also tested after storage. The similar infrared spectra of HA-CHO on day 0 and day 30 clearly indicated its robust stability.

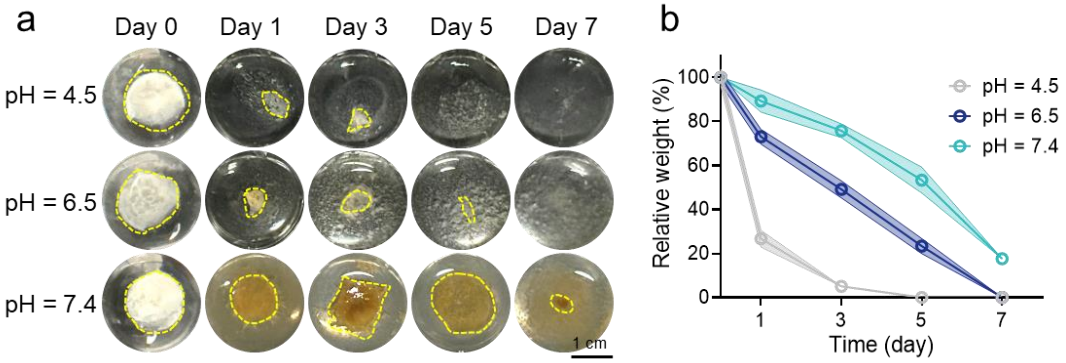
The degradation of F/R gel *in vitro* was then studied by continuous incubation in three PBS solutions with different pH values for a duration. As shown in Supplementary Fig. 19, F/R gel degraded faster in an acidic environment. At pH = 4.5, the gel matrix was almost completely degraded after 5 days. In a weakly acidic environment of pH = 6.5, the hydrogel degraded almost completely after 7 days. The slowest rate of degradation was observed in neutral PBS solution (pH = 7.4), in which ~ 17% of the hydrogel remained by day 7. These findings are consistent with the original design intentions, as chronic wound infections typically create a weakly acidic microenvironment (Lei Chen, et al. *Aggregate* 6, e640, 2024.). In such an environment, Schiff-base F/R gel can respond rapidly and begin to degrade, thereby releasing εPL-CeO_v and the loaded drugs for subsequent wound repair.



Supplementary Fig. 14. The stability of F/R MCs and εPL-CeO_v. (a) Appearance and (b) Particle size of F/R MCs after 30 days. (c) Appearance and (d) SOD enzyme activity of εPL-CeO_v after 30 days.



Supplementary Fig. 15 Infrared spectroscopy of the HA-CHO after 30 days.



Supplementary Figure 19. (a) Representative photographs showing F/R gel morphology changes after incubated with pH = 4.5, pH = 6.5, or pH = 7.4 PBS. (b) The corresponding weight changes of F/R gel during the 7-day degradation process.

Following the reviewer's comment, we have added the new data and more discussions on this part in our revised manuscript:

Page 7: "*As a kind of ready-to-use hydrogels, F/R gel could be conveniently applied via mixing for instant use when needed. All the free components of F/R gel possessed excellent stability after long-term storage (Supplementary Fig. 14-15).*"

Page 8: "*In addition, the accelerated breaking of Schiff-base hydrogel network in weakly acidic environment was further demonstrated by the in vitro hydrogel degradation experiments (Supplementary Fig. 19), which was more favorable for the release of εPL-CeO$_v$ and F/R MCs from F/R gel.*"

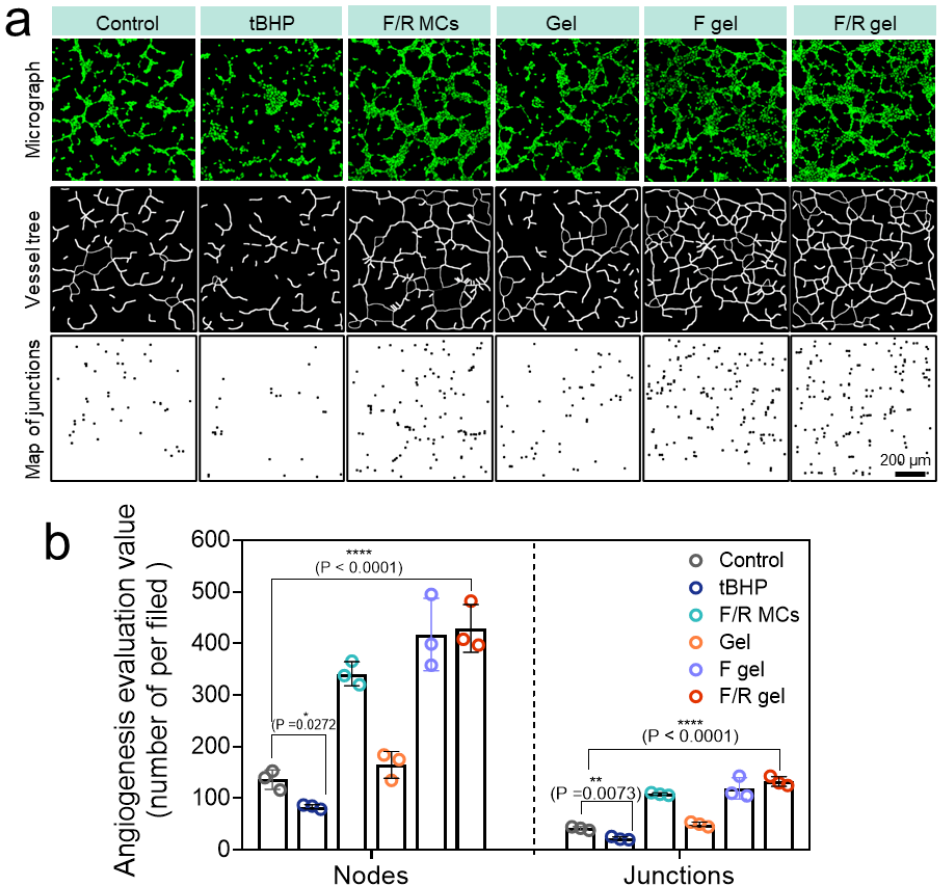
6. Angiogenesis is briefly discussed but not thoroughly evaluated. The manuscript mentions the promotion of neoangiogenesis; however, the angiogenic effects of bFGF should be explored in greater depth. Were there any specific assays done to quantify the degree of vascularization in the wound sites?

Our Response: We thank the reviewer for this valuable comment. In the original manuscript, we performed immunofluorescent staining of CD31 and VEGF markers to evaluate *in vivo*

angiogenesis effect. Higher expression levels of both CD31 and VEGF were observed in the F/R gel group on day 7, clearly indicating an increase in neovascularization following F/R gel's treatment (Fig. 6f, Supplementary Fig. 46).

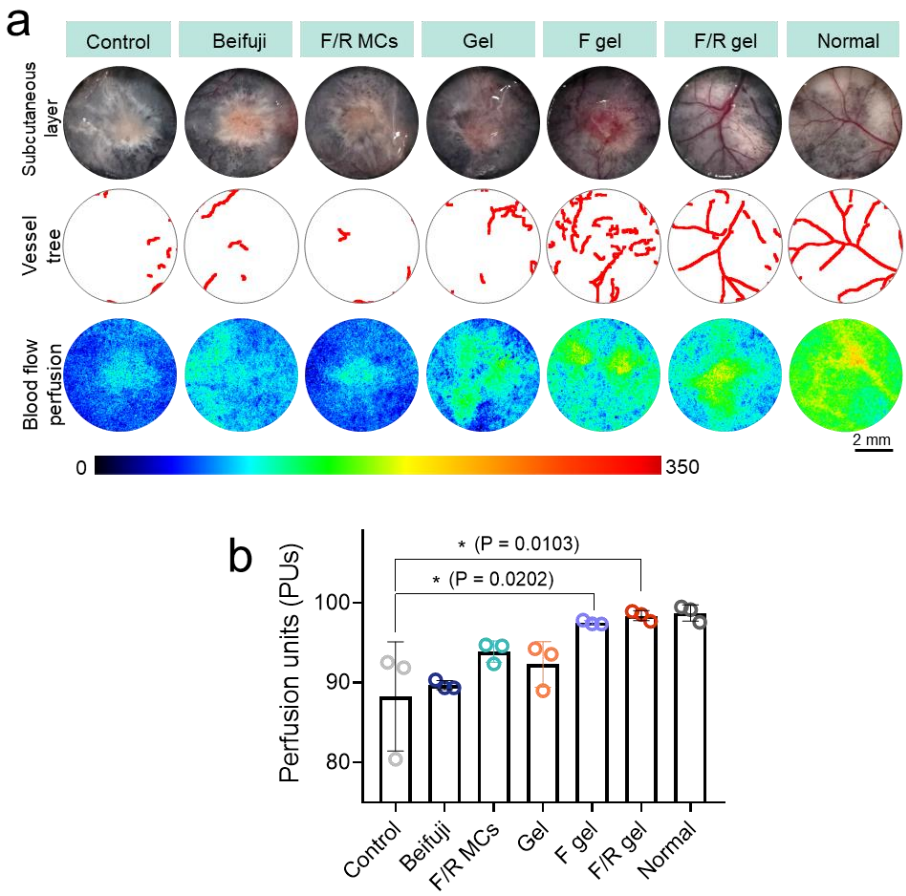
To make our results more convincing, we have supplemented the cellular tube-forming assays, and also re-conducted a new batch of animal experiments to verify vascularization *in vivo*.

(1) Strikingly, the F/R gel induced remarkable tubulogenesis of HUVEC under oxidative stress environment, in which the formation of tubules assembled by cell elongation and joining was much more than that in tBHP group, Gel group, or Control group (unstimulated by tBHP) (Supplementary Fig. 34). The Gel group scavenged ROS and protected HUVECs from oxidative stress, thus restoring tube formation to the levels similar to Control group. The increased tube formation in F/R MCs and F gel groups was also due to the presence of bFGF.



Supplementary Figure 34. Tube formation of HUVEC after tBHP stimulation followed by different treatments. (a) Representative images of the cellular tubes, vessel trees, and junction maps analyzed by image J. (b) Statistical data for tube nodes and junctions (n = 3).

(2) As shown in Supplementary Fig. 49, obvious microvascular networks at the wound site were observed in the F/R gel group on Day 14, which was the closest to the Normal group. At the same time, we further assessed the blood flow perfusion in wound tissues by using a laser speckle contrast imaging (LSCI) system, and the results also confirmed the presence of abundant blood flow in the wounds of F/R gel group. Collectively, these data suggested that F/R gel treatment could activate endothelial cells and significantly enhance neovascularization for accelerated wound healing.



Supplementary Figure 49. (a) The new blood vessels at wound sites, the corresponding vessel trees analyzed by image J, and the blood flow perfusion of wound tissues on day 14. (b) Statistical results of blood flow perfusion tests (n = 3).

Following the reviewer's comment, we have added the new data and more discussions on this part in our revised manuscript as follows:

Page 12: "*Additionally, F/R gel could activate endothelial cells and subsequently promote endothelial tube formation under oxidative stress environment (Supplementary Fig. 34).*"

Page 15: "*On day 7, higher CD31 and VEGF expressions in F/R gel group were observed at wound site, suggesting more neovascularization after treatment (Fig. 6f, Supplementary Fig. 46).*"

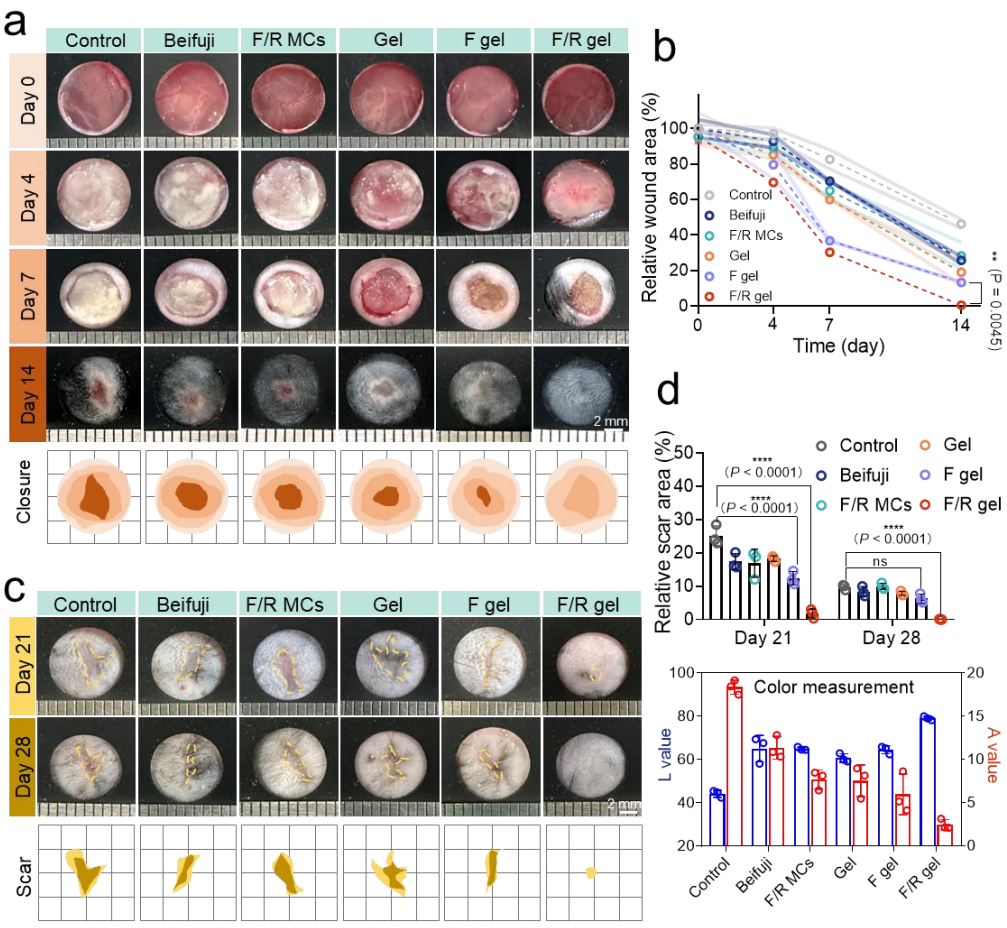
Page 15: "*On day 14, we conducted a comprehensive evaluation of neovascularization in the wound tissues. The newly formed blood vessels beneath the mouse skins were first monitored (Supplementary Fig. 49a). Similar to normal skin, the F/R gel-treated wounds exhibited denser and more regular vascular network. This observation was consistent with the results from a laser speckle contrast imaging (LSCI) system, which assessed the blood perfusion levels in the wounds. Collectively, these data clearly demonstrated a pronounced pro-angiogenic effect with higher local perfusion in the wounds after F/R gel treatment (Supplementary Fig. 49b).*"

7. The mouse wound healing model illustrates the efficacy of the hydrogel in comparison to a commercial gel (Beifuji). However, additional control groups are necessary to isolate the contributions of each component of the hydrogel. For example, assessing the effects of F/R gel without c-Jun siRNA could elucidate its specific role in scar reduction. Furthermore, it is

recommended to enhance the presentation of wound healing data, particularly through quantification of angiogenesis markers such as CD31 and VEGF.

Our Response: Thanks for the reviewer's very constructive comment. We have re-established the MRSA-infected wound model of mice and re-assessed wound repair effect after treatments, in which the F gel (gel containing only bFGF-loaded microspheres) group was added.

Consistent with the data in Fig. 6b-c, the F/R gel group exhibited the fastest healing effect with a healing rate of ~ 69.66% on day 7, which was significantly higher than that of the Control group (~ 17.33%) (Supplementary Fig. 42a-b). The healing efficiency of F gel group was similar to F/R gel group during the first 7 days; however, by day 14, the efficiency reached ~ 86.60%, whereas the F/R gel group were almost completely healed (~ 99.45%). Based on the wound appearance, we observed that from day 4 to 7, the wound surfaces in Control, Beifuji, and F/R MCs groups were covered with light yellow biofilm, which delayed wound repair. In contrast, the Gel, F gel, and F/R gel groups no longer showed any biofilms after day 4. Meanwhile, neovascularization results (see Supplementary Fig. 49a, provided in "Our Response" to the 6th comment as above) confirmed the pro-angiogenic effect of both F gel and F/R gel, however the former group displayed too dense and chaotic vascular structures, and such imperfect vessel formation might interfere with normal healing process. Importantly, we observed no visible scars in F/R gel group, while other groups (including F gel group) all generated significant scarring at wound areas on day 28 (Supplementary Fig. 42c-d). Based on the repair differences between F gel and F/R gel groups, we could speculate that c-Jun siRNA delivery played a key role in scar reduction during wound healing stages.



Supplementary Figure 42. (a) Optical photos and simulated graphs of wound closure changes from day 0 to day

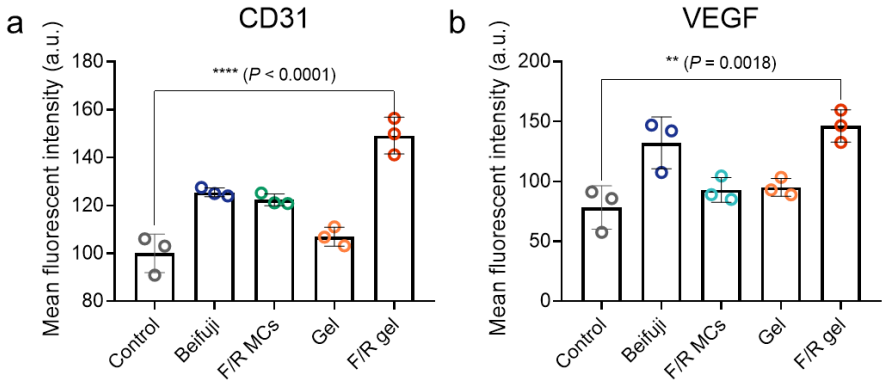
14. (b) Variation curve of wound areas within 14 days (n = 3). (c) Optical photos and simulated graphs of scar area changes from day 21 to 28. (d) Scar changing curve and color analysis of scar tissues post-treatments (a higher L value represents a whiter color, and a higher A value represents a redder color) (n = 3).

Now we have added these new data and more discussions on this part in our revised manuscript as follows:

Page 17-18: "*To further elaborate the specific role of c-Jun siRNA in F/R gel for scar reduction, we supplemented another batch of animal experiments in which the F gel (gel containing only bFGF-loaded microspheres) group was added (Supplementary Fig. 42). The results clearly revealed that F gel treatment only accelerated wound closure rate but hardly inhibited scarring tissues, hence highlighting the importance of F/R gel's components for anti-scar wound healing.*"

Besides, we have enhanced the presentation of wound healing data through quantitative analysis on angiogenesis markers (including CD31, VEGF) (Supplementary Fig. 46), and more descriptions on this part were added in our revised manuscript.

Page 15: "*On day 7, higher CD31 and VEGF expressions in F/R gel group were observed at wound site, suggesting more neovascularization after treatment (Fig. 6f, Supplementary Fig. 46).*"



Supplementary Figure 46. Mean fluorescence intensity (a) CD31 and (b) VEGF analyzed in immunofluorescence staining images (n = 3).

8. There are some grammatical issues, particularly with sentence structure in the introduction and methods sections. A thorough proofreading is necessary to ensure the text is polished for submission.

Our Response: We appreciate your valuable comments. Following the suggestion, we have carefully checked the manuscript and made the necessary changes which are highlighted in red in our revised manuscript. We have also scrutinized the revised version to ensure that the text is concise and ready for submission.

9. The supplementary information includes crucial experimental details (e.g., synthesis, characterization). However, some key results, such as the antioxidant assays, would be more impactful if moved to the main manuscript.

Our Response: Thanks to the reviewer's good suggestion. Now we have moved these important experimental methods including "Synthesis & characterization of εPL-CeO_v and F/R MCs", "Synthesis and characterization of the F/R gel", and "Measurements of ROS scavenging activity" to the main manuscript in the "Methods" part.