nature portfolio

Peer Review File

Microenvironment-feedback regulated hydrogels as living wound healing materials

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

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
In this manuscript, the authors described a microenvironment-feedback hydrogel for treating diabetic chronic wound, in which the alkaline pH of the wound bed was utilized as the cross-linking fuel, while gluconic acid was served as the anti-fuel. OSA-GEL@GC showed good wound repair efficiency both in vitro and in vivo. However, pH-sensitive hydrogels have been widely studied, and researches on the consumption of glucose in diabetic wounds have also been reported. Therefore, the novelty of this article is insufficient. Moreover, the feedback regulation proposed by the authors has not been well reflected and demonstrated in the results. Overall, I do not recommend acceptance of this manuscript in Nature Communications. It could be suitable for a more specialized journal after addressing following issues.

Major issue:

1. In Page 8, Line 145-149, the author mentioned that the diabetic mice treated with OSA-GEL@GC exhibited limited fluctuation of glucose content during day and night, showing a “homeostatic BGL during wound healing”. However, in Fig. 3B, it did not reflect the role of OSA-GEL@GC in regulating day-night fluctuation of BGL compared with the group treated with OSA-GEL.

2. In Fig.3B and Fig 3C, it is unclear why the free OSA-GEL increased the pH of the wound bed in the first 24 hours and decreases BGL after 36 hours.

3. Please further clarify the “adaptive release of Gox” and prove GOx will not be further released after BGL reaches a steady state. Is it due to the feedback as the authors mentioned or just because of the consumption of GOx that prevented further decline in BGL?

4. In Page 5, Line 188-190 in the SI, the authors mentioned that the gel dressing was changed every 2 or 3 days, but at this point, the microenvironment of the wound had relatively improved. Is the claim that the alkaline pH served as fuel still valid? Did the formation of gel and the release of GOx also be affected? Please provide corresponding data and the changes in BGL and pH after 48 hours, which may better demonstrate the characteristics of the feedback just as the authors highlighted.

Minor issues:

1. In Page 7, Line 119 in the main text, the authors mentioned that “the cell survival rate of the gel group was slightly higher than that of the control group”, but from Fig. 2B, there is no significant difference between these two groups. This statement is inaccurate.

2. Please mark the significant difference in Fig. 2B and Fig. 2C.

3. Please supplement the main role of Tegaderm TM dressing in wound repair process to better support the subsequent comparisons.

4. Please add the scale bar in Fig. 3F (line 2).

5. The method of significance analysis needs to be added to the figure legend. The type of statistical testing used for making comparisons should also be included in the methods section.

Reviewer #2

(Remarks to the Author)
The work presents the use of a pH-responsive hydrogel to regulate the release of GOx and enhance the wound healing in real-time. Such wound healing process was demonstrated by the authors in vitro and in-vivo. While the hydrogel itself is not new in its nature, the application to real-time wound healing is noteworthy and will advance the state of art on adaptive biomaterials. The methods are explained in great detail and the conclusions are well supported. For this reasons, I recommend the publication of the paper as is.

Reviewer #3

(Remarks to the Author)
The authors developed a hydrogel composed of oxidized sodium alginate and gelatin. Though the research is interesting, it is relatively routine, and the data do not fully substantiate the conclusion.

Some comments:
1. In Figure 4, the authors want to demonstrate that the hydrogels promote angiogenesis or vascularization, but either the in vitro nor in vivo results do not show any vascular structure.
2. The authors noted that the hydrogel's catalase (CAT) component decomposes ROS generated by glucose oxidase (GOx) activity. However, only in vivo experiments were conducted. Could the authors include additional in vitro experiments to further verify the hydrogel's ROS scavenging ability?
3. The manuscript mentions that catalase (CAT) reduces ROS and produces H2O and O2, alleviating hypoxia in diabetic wounds. However, no experiments were conducted to characterize O2 generation.
4. In Figure 3E, why Group 4 was not included? Can the authors add group 4 of Figure 3D to Figure 3E?
5. For Figure 3F, can the authors quantify the data?
6. As catalase (CAT) is one of the key active substances loaded into the hydrogel, the authors should include experiments to characterize its release profile over time.
7. Most diabetic patents have type 2 diabetes, it's unclear why the authors use type 1--which is not relevant- diabetic model for this study.
8. The writing should be improved.

Reviewer #4

(Remarks to the Author)
In this manuscript, the authors developed a novel microenvironment-feedback hydrogel as a living dressing biomaterial catering diabetic chronic wounds. This dynamic hydrogel was cross-linked by pH-responsive imine bonds, and leverages the initial alkaline pH of the wound bed as fuel and employs biocatalytic acid generation as the anti-fuel. By coupling this feedback loop to pH-regulated imine crosslinks, the hydrogel facilitates adaptive sol-gel cycling with programmable glucose oxidase (GOx) release in a Type-I diabetic mouse model. Homeostatic wound pH and blood glucose level were thus successfully achieved, which favored for an accelerated in vivo wound healing and tissue repairing. The concept is interesting and novel, offering a new strategy for prototype living biomaterials and future biomedical applications. The reviewer recommends minor revision with the following issues addressed.

1.The authors developed a GOx-loaded hydrogel dressing with pH feedback regulation, based on oxidized sodium alginate (OSA) and gelatin (GEL). Considering the pH changes during the formation and dissociation of the hydrogels, how do these changes affect the activity of GOx?
2.What is the gelation speed of the hydrogel, as it relates to practical clinic applicability?
3.The characterization of oxidized polymer OSA should be provided, for example NMR.
4.There are many instances of "HUVEC cells" in the article, which should be corrected to "HUVECs".
5.Lines 176-177, the branching length of HUVEC tubes lacks unit.
6.In the in vivo experiments, the expression of α-SMA in group 3 is elevated on day 7, indicating more myofibroblasts. However, Masson's staining reveals a low number of myofibroblasts in group 3 on day 21. How can this be explained?

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have addressed reviewers' comments.

Reviewer #3

(Remarks to the Author)

The authors have addressed some of the reviewer's concerns, but several issues remain:
1. There is no mention of the tube formation assay in either the manuscript or the supplementary information. Were the cells encapsulated within the gel or seeded on top of it?
2. In Figure 4, both Cd31 and α-SMA staining show that Group 4 exhibits more robust angiogenesis than the gel-treated group. This raises questions about how the gel itself is being credited for promoting angiogenesis. The comparison with normal wounds is also unclear. If a comparison is necessary, the authors should consider using the gel as a control in Group 4 rather than Tegaderm. Many results in Group 4 show significant improvement, but this effect does not appear to be directly attributable to the gel.
3. In Figure 3C, there seems to be more inflammation (possibly indicated by yellow exudate?) in Groups 2 and 3 on day 7. However, Figure 5C presents different findings.
4. It appears that the gel degrades quickly that caused mechanical changes and promotes wound contraction. Further clarification on this aspect would be helpful.
5. There are still typos and grammatical errors throughout the manuscript. For example, in Figure S13, the caption states: "Serious of photographs…," which should be corrected.

Reviewer #4

(Remarks to the Author)
The author has revised this manuscript according to my suggestions. I think this revised manuscript could be suitable for publication.

Version 3:

Reviewer comments:

Reviewer #3

(Remarks to the Author)
The authors addressed the reviewer's concern.The reviewer has no more questions.

Response letter

Reviewer #1 (Remarks to the Author):

Thank you for reviewing our manuscript and giving valuable comments that would help us improve our manuscript. However, we feel that the reviewer underestimated the significance and importance of the present study.

— In fact, we present a new paradigm of biomaterials that would dynamically adapt to in vivo microenvironment changes. It is based on the kinetically asymmetric "feed-back" design principle that is inspired by the dynamic, far-from-equilibrium nature of biological systems (*Walther et al., Adv. Mater. 2020, 32, 1905111*). It opens a whole new area of possible applications of microenvironment interactive and self-regulatory hydrogels.

— Our work contrasts with the preponderance of literatures in passive pH or glucose-responsive materials that aim at degrading materials for straightforward and continuous release of therapeutic species (e.g., insulin). Instead, our strategy offers a route to provide an improved while homeostatic microenvironment for diabetic wound healing, via making full use of two key factors – pH and blood glucose as fuel and feed-back regulator that dynamically interact with our designed materials.

— Compared to most fundamental researches of fueled-feedback materials, the present work offers a rare example of dynamic hydrogel using a disease-relevant fuel to deploy a bio-medical application.

We wish to state that the concept and accomplishments of the present paper are different from most reported pH-sensitive hydrogels and researches on the consumption of glucose in diabetic wounds mentioned by the reviewer. Furthermore, we believe that the content of the present paper opens new dimensions to the field. In the near future, we will use the principles of the present paper to develop dynamic materials that operate under out-of-equilibrium conditions, performing their function (delivery of bioactive agents, eg. NO) in a transient manner in response to evolving environmental factors. Such dynamic materials hold great potential as an important self-adjustable agent to prevent overdose and to minimize undesired side-effects (e.g., tissue destruction or chronic inflammatory disorders) during therapies.

In order to better demonstrate the conceptional novelty and contribution of our work towards both fueled-feedback adaptive materials and dynamic biomaterials, we have carefully revised the paper according to the reviewer's suggestions with additional experiments and discussions to clarify the feed-back regulation effect of our hydrogel material. The followings are point-to-point responses to the reviewer's comments.

Major issue:

1. In Page 8, Line 145-149, the author mentioned that the diabetic mice treated with OSA-GEL@GC exhibited limited fluctuation of glucose content during day and night, showing a "homeostatic BGL during wound healing". However, in Fig. 3B, it did not reflect the role of OSA-GEL@GC in regulating day-night fluctuation of BGL compared with the group treated with OSA-GEL.

Answer: Thank you for pointing it out. It is reported that either basic pH (~8.0) or

highly acidic pH (<6.0) is detrimental towards wound healing (*Wound Repair Regen*. 2014, 22, 174-186; *Adv. Wound Care*. 2024, 13, 446-462). Similarly, neither hyperglycemia nor hypoglycemia is desired during diabetic wound treatment. Hence, the ultimate goal of our work is to downgrade BGL and pH in diabetic wound bed, and remain in a steady and optimized condition that facilitates the wound healing.

To reflect the effect of fuel-feedback design on homeostasis of wound bed microenvironment, we prolonged the wound treatment experiments to 5 days and examined the pH and BGL values on the wound (see revised Figure 3A and 3B). It was found that the wound BGL treated with OSA-GEL@GC first decreased from original 1.19 mmol/g to 0.60 mmol/g in the first 12 hours, and then gradually fell to *ca*. 0.25 mmol/g in the following 36 hours and maintained almost unchanged in the next 3 days without continuous descend, showing a good BGL downgrading and homeostatic effects (Figure 3A). The wound pH showed a similar trend and was eventually maintained around 6.5±0.2 till Day 5 (Figure 3B). While in the control group (Group 2 treated with OSA-GEL), both wound BGL and pH values remained in high values even after 5 days, showing a relatively inefficient wound healing effect.

We have to apologize for the misunderstanding on the claim of "regulating day-night fluctuation". In our original design, we would like to examine whether the fuel-feedback hydrogel could counter the possible variation in diabetic wound BGL. In order not to mislead our readers, we have revised the manuscript and re-written the relevant contexts (Please see page 8-9 and Figure 3A-B in the revised manuscript).

2. In Fig.3B and Fig 3C, it is unclear why the free OSA-GEL increased the pH of the wound bed in the first 24 hours and decreases BGL after 36 hours.

Answer: Thanks for the comment. Actually, OSA-GEL as a control group did not interfere with the diabetic wound microenvironments and facilitate the healing process (see revised Figure 3A and 3B). In the initial stage of OSA-GEL dressing group, the basic microenvironment of diabetic wound might result in an increased pH (*ca.* 7.8) of the tested wound bed, which is a typical character of diabetic wound microenvironment (*Adv. Mater*. 2016, 28, 5732-5737). Compared to OSA-GEL treated group, OSA-GEL@GC obviously downregulated pH value (*ca*. 6.6) and BGL level (0.60 mmol/g) of wound microenvironment after 12 h. However, both BGL (0.99 mmol/g) and pH value (*ca.* 7.5) exhibited a slight decline after 36 h, and maintained almost steady in the following days, indicating a limited improvement in diabetic wound microenvironment (see revised Figure 3A and 3B). These results confirm that OSA-GEL would not have significant effect on optimizing the diabetic wound microenvironment and facilitating the healing process (Please see page 9, second paragraph in the revised manuscript).

3. Please further clarify the "adaptive release of Gox" and prove GOx will not be further released after BGL reaches a steady state. Is it due to the feedback as the authors mentioned or just because of the consumption of GOx that prevented further decline in

BGL?

Answer: Thanks for the reviewer's suggestion. The term "adaptive release of GOx" refers to an automatically controlled release of GOx associated with the microenvironment variation (pH, BGL). The design and working mechanism are as follows: The encapsulated GOx in OSA-GEL@GC consumes local blood glucose, produces gluconic acid and gradually dissociates the formed hydrogel via cleaving Schiff base crosslinks, thereby releasing GOx to the wound bed. The released GOx further consumes glucose in the wound bed, resulting in decreased BGL in wound microenvironment. This sequentially halts a series processes of gel acidification/degradation and GOx release, countering a continuous descend of BGL and pH. Hence, the GOx release in our system is not a routinely continuous release process but an intermittent release that adapts to the variation of surrounding microenvironment.

According to the reviewer's comments, we further performed a GOx release test under pH 6.5 (correlated with BGL in steady state). It was found that GOx release was more or less halted after 8 hours and a ca. 25% of entrapped GOx was released or consumed (Figure S20). Moreover, it could be found if not the feedback mechanism, the surrounding pH of OSA-GEL@GC under diabetic BGL could reach to a pH region as low as 4.5-5.5 (Figure 1C and 1D) instead of a homeostatic pH of 6.5 that facilitates the wound healing. Therefore, we consider the steady BGL was mainly resulted from the feedback mechanism rather than just GOx consumption (Please see page 9, second paragraph, Figure S20 and Figure 1C-D in the revised manuscript).

4. In Page 5, Line 188-190 in the SI, the authors mentioned that the gel dressing was changed every 2 or 3 days, but at this point, the microenvironment of the wound had relatively improved. Is the claim that the alkaline pH served as fuel still valid? Did the formation of gel and the release of GOx also be affected? Please provide corresponding data and the changes in BGL and pH after 48 hours, which may better demonstrate the characteristics of the feedback just as the authors highlighted.

Answer: Thanks for the reviewer's suggestion. As mentioned above, after treating with the OSA-GEL@GC dressing for 2 days, the microenvironment of the wound was relatively improved with a decreased pH and a decline of BGL. Moreover, a prolonged experiment on wound bed treatment was conducted to 5 days and the pH and BGL values on the wound was examined, respectively (see revised Figure 3A-B). It was found that wound BGL decreased from original 1.19 mmol/g to ca. 0.25 mmol/g in the first 48 hours and maintained almost unchanged in the next 3 days without continuous descend, showing a homeostatic state. Similarly, wound pH also decreased in the first 48 hours and almost unchanged in the following days. The homeostatic state of pH could be attributed to the fact that the alkaline pH and physiological buffer serve as fuels to adjust pH, otherwise the surrounding pH of OSA-GEL@GC would reach to the range of 4.5-5.5 under diabetic glucose level (as indicated in Figure 1C and 1D).

In addition, we investigated the formation of gel at the homeostatic pH. Expectedly, OSA-GEL@GC could be generated under relatively acidic pH of 6.5, although with

attenuated stiffness compared to those formed under alkaline conditions (Figure S19). GOx release experiment was further carried out. The result demonstrated a slow yet gradual release of GOx from OSA-GEL@GC under pH of 6.5, which was later halted with ca. 25% of release (Figure S20). Therefore, these results indicate that the fuel-feedback cycle would still work, which might facilitate a steady process (Please see page 8-9, Figure S19-20, Figure 1C-D and Figure 3A-B in the revised manuscript).

Minor issues:

1. In Page 7, Line 119 in the main text, the authors mentioned that "the cell survival rate of the gel group was slightly higher than that of the control group", but from Fig. 2B, there is no significant difference between these two groups. This statement is inaccurate.

Answer: Sorry for the misleading description. In order not to mislead our readers, the sentence was corrected as "the cell survival rate of the gel group was similar to the control group within 5 days, which proved the good biocompatibility of OSA-GEL@GC" (Please see page 6-7 in the revised manuscript).

2. Please mark the significant difference in Fig. 2B and Fig. 2C.

Answer: Following the reviewer's suggestion, we added significant difference in Fig. 2B and Fig. 2C (Please see updated Fig. 2B and 2C in the revised manuscript).

3. Please supplement the main role of Tegaderm TM dressing in wound repair process to better support the subsequent comparisons.

Answer: Tegaderm™ dressing is made of semi-permeable polymeric films with good conformability. It is one of the most widely used commercial dressings in wound healing research. In general, it serves as a barrier to protect wound from external contaminants such as bacteria, viruses, blood and body fluids, while water vapor, oxygen and carbon dioxide can easily be exchanged through the dressing. Tegaderm™ dressing seals in natural wound fluid to maintain a moist environment, which promotes the healing process. In our work, Tegaderm™ dressing is used in control groups, and its healing efficacy was compared to that of our designed hydrogel dressing. Please see page 8, second paragraph.

4. Please add the scale bar in Fig. 3F (line 2).

Answer: Following the reviewer's suggestion, the scale bars in images of H&E staining were added (please see revised Figure 3H, line 2).

5. The method of significance analysis needs to be added to the figure legend. The type of statistical testing used for making comparisons should also be included in the methods section.

Answer: Following the reviewer's suggestion, the method of significance analysis and

the type of statistical testing used were provided. The unpaired t-test is employed for comparisons between two groups, while one-way ANOVA is utilized for comparing three or more groups.

Reviewer #2 (Remarks to the Author):

The work presents the use of a pH-responsive hydrogel to regulate the release of GOx and enhance the wound healing in real-time. Such wound healing process was demonstrated by the authors in vitro and in-vivo. While the hydrogel itself is not new in its nature, the application to real-time wound healing is noteworthy and will advance the state of art on adaptive biomaterials. The methods are explained in great detail and the conclusions are well supported. For this reasons, I recommend the publication of the paper as is.

Answer: We appreciate very much the positive comments of the reviewer. We have further improved our manuscript and made it more qualified for publication. Thanks again!

Reviewer #3 (Remarks to the Author):
Thanks the reviewer's comment and the followings are point-to-point responses to the reviewer's comments.

1. In Figure 4, the authors want to demonstrate that the hydrogels promote angiogenesis or vascularization, but either the in vitro nor in vivo results do not show any vascular structure.

Answer: Thanks for the reviewer's comment. To further demonstrate that the hydrogels promote angiogenesis or vascularization, we re-performed tube formation experiments at different time intervals. It was observed that the HUVECs treated with the **OSA-GEL@GC** group exhibited significant tube formation compared to the control group at the time interval of 6 hours (Figure 4A-C). In **OSA-GEL@GC** hydrogel-treated group, a decent number of junctions (550) and longer branching length (>60000 px) were obviously observed, while negligible tube formation was observed in the control group (high-glucose DMEM). To obtain a more intuitive understanding of angiogenesis, we conducted immunofluorescence staining to assess the vascularization of diabetic wounds. It has been reported that CD31 serves as a marker for vascular endothelial cells (*Theranostics* 2020, 10, 426-436; *Nat. Commun.* 2017, 8, 1600), and its expression is positively correlated with the extent of angiogenesis (*Adv. Mater*. 2021, 33, e2102624; *Front. Bioeng. Biotechnol.* 2023, 11, 1129187). The *in vivo* experiment showed that the positive area of CD31 in Group 3 is significantly higher than that in other diabetic control Groups 1 and 2 at Day 7 (Figure 4D-E), indicating a probable promoting effect of **OSA-GEL@GC** on neovascularization (Please see page 11, second paragraph and revised Figure 4A-C).

2. The authors noted that the hydrogel's catalase (CAT) component decomposes ROS

generated by glucose oxidase (GOx) activity. However, only in vivo experiments were conducted. Could the authors include additional in vitro experiments to further verify the hydrogel's ROS scavenging ability?

Answer: Following the reviewer's suggestion, supplementary *in vitro* experiments were performed to prove the hydrogel's ROS scavenging ability. In the **OSA-GEL@GC** hydrogel system, GOx can effectively catalyze the oxidization of glucose into gluconic acid and H_2O_2 in the presence of O_2, and the subsequently generated H_2O_2 was converted by catalase into H_2O and O_2. Intracellular ROS level was evaluated using 2',7'-dichloro-dihydro-fluorescein diacetate (DCFH-DA) as a fluorescent probe. As shown in Figure 2H and 2I, the intracellular fluorescence of **OSA-GEL@GC** hydrogel-treated Group 3 was significantly decreased compared to Group 2 that without catalase, indicating the effective ROS scavenging of the catalase component (Please see page 7, third paragraph and Figure 2H-I).

3. The manuscript mentions that catalase (CAT) reduces ROS and produces H_2O and O_2, alleviating hypoxia in diabetic wounds. However, no experiments were conducted to characterize O_2 generation.

Answer: Following the reviewer's suggestion, the O_2 content at the cellular level was characterized by using a O_2 probe $Ru(dpp)_3Cl_2$. A relatively weaker intracellular fluorescence of **OSA-GEL@GC** hydrogel-treated Group 3 was observed compared to Group 2 that without catalase (Figure 2J-K), indicating effective O_2 production induced by catalase component. Meanwhile, the levels of O_2 in both groups were checked by dissolved oxygen meter (Figure S15). In the control group, O_2 was consumed due to GOx-catalyzed oxidation of glucose, while O_2 rapidly increased in the **OSA-GEL@GC** hydrogel-treated Group 3. These results reveal that catalase (CAT) is able to convert ROS into H_2O and O_2, which would alleviate hypoxia in diabetic wounds (Please see related discussion from page 7, third paragraph to page-8, first paragraph, Figure 2J-K and Figure S15).

4. In Figure 3E, why Group 4 was not included? Can the authors add group 4 of Figure 3D to Figure 3E?

Answer: Sorry for the unclear demonstration of the data. Following the reviewer's suggestion, the data of group 4 was added in the revised manuscript (Please see revised Figure 3D).

5. For Figure 3F, can the authors quantify the data?

Answer: Following the reviewer's suggestion, we quantified the data and the results were provided in Figure 3E-3G. The wounds treated with **OSA-GEL@GC** (Group 3) was basically healed with the continuous epithelium, which was similar to that of normal mouse control group (Group 4; Figure 3E and 3H, line 1-2). The average wound diameter of Group 3 was 1.03 nm. However, the re-epithelialization level of control Group 1 and 2 remained slow, as indicated by the average wound diameters of 3.15 mm and 2.51 mm, respectively (Figure 3E). The collagen deposition and arrangement were then thoroughly investigated to gain a further understanding of the wound healing effect.

Both Masson's trichrome and Sirius red staining exhibited similar results that the collagen deposition in the dermis was denser and exhibited a more regular pattern in **OSA-GEL@GC** (Group 3) and normal mice (Group 4) compared to the other two diabetic control groups (Group 1&2; Figure 3H, line 3-4). The average tissue collagen content of Group 3 was 15.3% (Figure 3F), which was significantly higher than that of Group 1 (7.5%) and Group 2 (12.4%). During the maturation phase of wound healing, the ratio of Type I to Type III collagen increases. The results of Sirius red staining indicate that the Type I/III collagen ratio in Group 3 (1.46) was higher than that in Group 1 (1.17) and Group 2 (1.29), respectively, Figure 3G. Please see Figure 3E-G and the related discussion in the revised manuscript, from page 10, second paragraph to page 11, first paragraph.

6. As catalase (CAT) is one of the key active substances loaded into the hydrogel, the authors should include experiments to characterize its release profile over time.

Answer: Following the reviewer's suggestion, we performed experiments to monitor the release of catalase over time. The loaded catalase was labelled with Fluorescein isothiocyanate (FITC) and its release from the hydrogel matrix was monitored by the fluorescence signal of FITC (please see the following Figure R1).

To investigate the catalase release profile in gel matrices, fluorescein isothiocyanate (FITC) conjugation was performed through the following procedure: First, 3 mL of catalase solution (3.3 mg/mL) was prepared in pH 9.0 buffer. Subsequently, 50 μL of FITC solution (10 mg/mL in dimethyl sulfoxide) was added dropwise under continuous stirring. The reaction mixture was then continuously stirred for 4 hours at room temperature in light-protected conditions. The resulting solution was purified using a PD-10 desalting column to remove unreacted FITC molecules. Finally, the eluted FITC-labeled catalase reconstituted to a final volume of 3 mL with phosphate-buffered saline.

In the presence of high concentration of glucose (4 mg/mL), no obvious fluorescence changes were observed at different time intervals. The results indicate a limited release of CAT through the hydrogel matrix. This is probably attributed to its molecular weight and isoelectric point that hamper its free diffusion (*ACS Appl. Mater. Interfaces* 2022, 14, 57408). Similar phenomenon of CAT release from alginate hydrogels were observed in previous studies (*ACS Appl. Mater. Interfaces* 2024, 16, 68816).

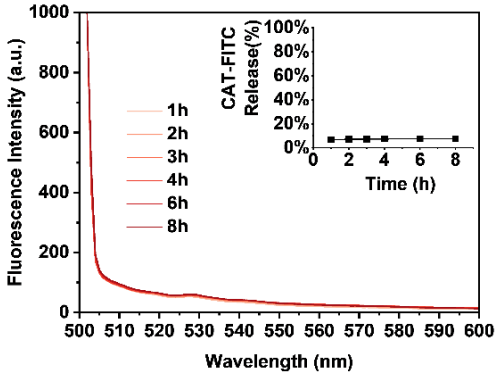

Figure R1. Release of CAT-FITC from the OSA-GEL@GC hydrogel at different time intervals.

7. Most diabetic patents have type 2 diabetes, it's unclear why the authors use type 1--which is not relevant- diabetic model for this study.

Answer: Type 1 diabetes develop due to either spontaneous autoimmune destruction or the chemical ablation of pancreatic beta cells. Type 2 diabetes display insulin resistance and dysfunctional pancreatic beta cells. Although the etiologies of the two types of diabetes are different, the reasons for retarded wound healing are the same, which is excessively high blood glucose and tissue glucose levels (*Diabetes Care,* 2008, 31, 1288-92). Both types of diabetes have high-glucose environment, which increase tissue inflammation, increase ROS, decrease fibroblast proliferation and migration, weaken the migration of keratinocytes and endothelial cells, reduce neovasculogenesis, and diminish macrophage phagocytosis (*Sci. Adv.* 2023, 9, eadj1019; *Bioact. Mater.* 2025, 44, 131-151). Given that the type 1 diabetes model reliably achieves a stable hyperglycemic state (*Br. J. Pharmacol.* 2012, 166, 877-894), it was chosen as the in vivo model for demonstration.

8. The writing should be improved.

Answer: Following the reviewer's suggestion, we carefully polished the writing in the paper.

Reviewer #4 (Remarks to the Author):

1.The authors developed a GOx-loaded hydrogel dressing with pH feedback regulation, based on oxidized sodium alginate (OSA) and gelatin (GEL). Considering the pH changes during the formation and dissociation of the hydrogels, how do these changes affect the activity of GOx?

Answer: Thanks the reviewer's comment. We carefully checked the activities of GOx under different pH. As shown in Figure S6, the activity of GOx almost unchanged in the range of pH 5-7.5 and showed a slight decrease at pH 8. The results were provided in the revised manuscript (Please see page 5 and Figure S6).

2.What is the gelation speed of the hydrogel, as it relates to practical clinic applicability?

Answer: Thanks the reviewer's comment. In our work, the gelation occurs within several minutes and their storage modulus increase with the gelation time (Figure S11-12 and Figure 1E). As rapid gelation benefits for bio-medical applications, particularly in areas of drug delivery, tissue engineering and wound healing, we will investigate hydrogels with even faster gelation speed in future study.

3.The characterization of oxidized polymer OSA should be provided, for example NMR.

Answer: Following the reviewer's suggestion, the oxidized sodium alginate was characterized by ^{1}H NMR (Figure S4). After oxidization, two new signals at 5.69 and 5.46 ppm were observed, corresponding to the protons in hemiacetals. The results confirm the formation of OSA and similar results have been reported in literatures (*Carbohydr. Polym.*, 2022, 295, 119843). Please see page 4, first paragraph and Figure S4.

4.There are many instances of "HUVEC cells" in the article, which should be corrected to "HUVECs".

Answer: We have corrected “HUVECs” in the revised manuscript.

5.Lines 176-177, the branching length of HUVEC tubes lacks unit.

Answer: The unis has been added in the revised manuscript.

6.In the in vivo experiments, the expression of α-SMA in group 3 is elevated on day 7, indicating more myofibroblasts. However, Masson's staining reveals a low number of myofibroblasts in group 3 on day 21. How can this be explained?

Answer: The myofibroblasts show different contributions in early and late stages of wound repair. During the early stage of wound repair, myofibroblasts transformed from fibroblasts synthesis collagen and exert increased contractile forces, leading to a more rigid ECM that can resist external pressures and is beneficial for wound contraction and healing *(Nat. Rev. Mol. Cell Biol.* 2024, 25, 617-638; *Int. Wound J.* 2008, 5, 477-482). While in the late stage, prolonged existing of myofibroblasts leads to an excessive collagen synthesis and ends with hypertrophic scarring. The apoptosis of myofibroblasts prevents excessive fibrosis and leads to a mature remodeled scar (*Br J. Dermatol.* 2020, 182, 974-986). In our study, Group 3 exhibits a higher count of α-SMA-positive myofibroblasts compared to Groups 1/2 on Day 7 and then a lower count on Day 21. Both observations indicate that Group 3 treated with **OSA-GEL@GC** progresses more swiftly in the wound healing process compared to Groups 1 and 2. Please see page 11, third paragraph and page 12, first paragraph in the revise manuscript.

Response letter

Reviewer #1 (Remarks to the Author):

The authors have addressed reviewers' comments.

Answer: Thank you for reviewing our manuscript and giving valuable comments that help us improve our manuscript.

Reviewer #3

1. There is no mention of the tube formation assay in either the manuscript or the supplementary information. Were the cells encapsulated within the gel or seeded on top of it?

Thank you for pointing it out, which helps us improve our manuscript. Following the reviewer's suggestion, a detailed tube formation assay was complemented in the supporting information. 50 μL of Matrigel (#356234, Corning Life Sciences, United States) was evenly spread on each well of a 96-well plate and kept at 37℃ for 30 minutes to allow polymerization. Human umbilical vein endothelial cells, with a density of 1.5×10^4 cells per well, were seeded onto the Matrigel and incubated with high-glucose Dulbecco's modified eagle medium (DMEM, Gibco) + 1% Endothelial cell growth supplement (ECGS, 1052, ScienCell). The cell culture medium containing the hydrogel was added to the experimental group, and only cell culture medium was added to the control group. Tube formation was observed and photographed at 6 h post-treatment. Please see the related information in experimental section in supporting information, page S4-S5.

2. In Figure 4, both Cd31 and α-SMA staining show that Group 4 exhibits more robust angiogenesis than the gel-treated group. This raises questions about how the gel itself is being credited for promoting angiogenesis. The comparison with normal wounds is also unclear. If a comparison is necessary, the authors should consider using the gel as a control in Group 4 rather than Tegaderm. Many results in Group 4 show significant improvement, but this effect does not appear to be directly attributable to the gel.

Thanks for pointing it out. In our design, Group 4 (normal-blood-glucose mice treated with Tegaderm dressing) was intended to demonstrate normal healing levels after treatment and serve as a reference for evaluating the healing effect of the experimental group. We agree with the reviewer that comparing Group 4 with other groups may not be necessary and cause confusion, as wound types and wound microenvironment differ between these groups.

Despite this, *in vivo* experiments of Groups 1, 2 and 3 showed that the positive area of CD31 in Group 3 is significantly higher than that in the diabetic control groups (Groups 1 and 2) on Day 7 (Figure 4D-E). This suggests a potential promoting effect of OSA-GEL@GC on neovascularization.

In addition, we previously performed several control experiments in which normal-blood-glucose mice were treated with either Tegaderm (Group 4) or OSA-GEL@GC gel dressing (Group 5), see following Figure R1 for details. On day 14, the wound in Group 5 was almost healed, showing similar healing effects as Group 4. Both groups showed complete wound closure on day 21. H&E, Masson trichrome and Sirius Red

staining showed the similar re-epithelialization and collagen deposition in both Group 4 and 5, respectively. No statistically significant differences in collagen content or the ratio of type I to type III collagen were observed between the two groups. These results demonstrate that OSA-GEL@GC dressing has a good healing effect comparable to that of Tegaderm.

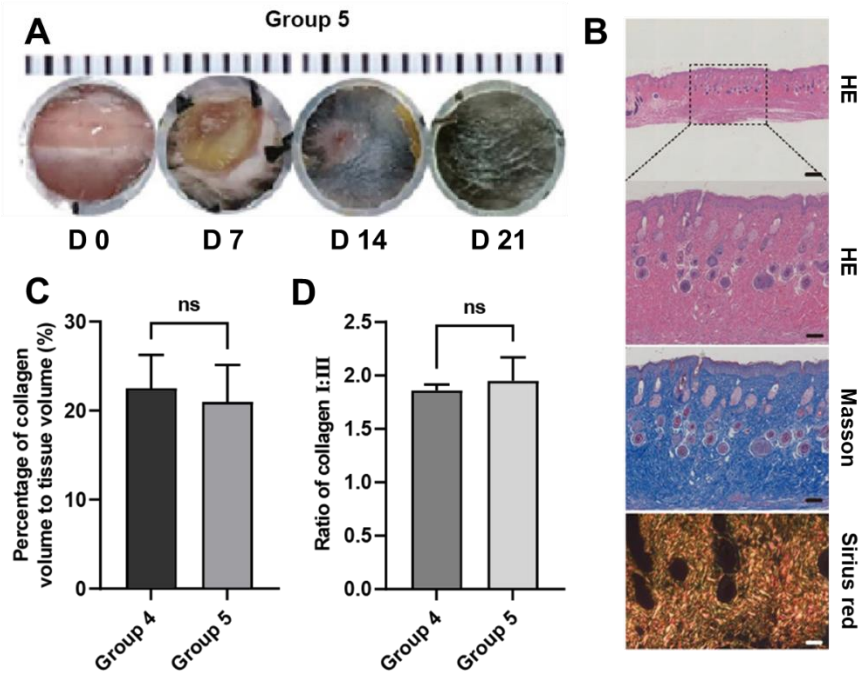

Figure R1. (A) Representative images of back wound recovery in Group 5 on days 0, 7, 14 and 21. (B) Images of H&E staining (line 1-2), Masson's trichrome (line 3) and Sirius red staining (line 4) on mouse wound tissue on day 21. Scale bar = 200 μm, 100 μm, 100 μm, and 50 μm, respectively. Percentage of collagen volume (C) and ratio of collagen I:III on wound tissue (D), n = 3.

3. In Figure 3C, there seems to be more inflammation (possibly indicated by yellow exudate?) in Groups 2 and 3 on day 7. However, Figure 5C presents different findings.
Thanks for the comment. Actually, the yellow coloration observed on day 7 in Groups 2 and 3 results from the color of the gel dressing (attributed to the imine Schiff base crosslinking, see also Figure S13) adhering to the tissue rather than yellow exudate. Wound inflammation levels were evaluated based on the M1/M2 macrophage ratio and the expression of inflammatory factors. Groups 2 and 3 exhibit lower levels of inflammation compared to Group 1 (Figure 5C). To avoid possible misunderstanding to readers, we have revised the manuscript with detailed description of our hydrogel dressing in related parts, page 4 in main text.

4. It appears that the gel degrades quickly that caused mechanical changes and promotes wound contraction. Further clarification on this aspect would be helpful.
Thanks for the reviewer's suggestion. Mechanical properties and matrix degradability at an appropriate rate are two important design consideration when engineering synthetic hydrogels (*Chem. Rev.* **2021**, *121*, 11149). The stiffness of hydrogels has an

impact on wound healing mainly by the regulation of cell activities in the proliferation phase (*Colloids and Surfaces B: Biointerfaces*, **2016**, *140*, 574). In particular, hydrogels with degradable cross-linkers promote robust cell spreading, outgrowth, and secretion of proangiogenic cytokines that are critical in wound healing (*Biomaterials*, **2023**, *301*, 122256). In our system, the encapsulated GOx in OSA-GEL@GC consumes local blood glucose, produces gluconic acid and gradually dissociates the formed hydrogel via cleaving Schiff base crosslinks, leading to dynamic mechanical changes. It probably contributes to wound healing, however, the extent to which matrix stiffness or degradability regulates cell behavior still needs further investigation. In future study, we will systematically investigate the essential role of stiffness and gel degradation on wound healing. Please see the related discussion in the Conclusion part, page 15. Related references were also added as Ref. 68-70.

5. There are still typos and grammatical errors throughout the manuscript. For example, in Figure S13, the caption states: "Serious of photographs…," which should be corrected.

Thanks for the reviewer's comment and sorry for the mistake. We have corrected the typo errors and carefully checked the manuscript.

Reviewer #4 (Remarks to the Author):

The author has revised this manuscript according to my suggestions. I think this revised manuscript could be suitable for publication.

Answer: We appreciate very much the positive comments of the reviewer.