nature portfolio

Peer Review File

The glycerol stabilized calcium phosphate cluster for rapid remineralization of tooth enamel by a water-triggered transformation

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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)
In this manuscript, the authors develop a stable ultrasmall mineral species, glycerol stabilized calcium phosphate cluster (GCPC), for a rapid remineralization of tooth enamel. This work is of great significance from the perspective of both science and clinical practice. First, the GCPC with ultra-smalls size and stable state is obtained in glycerol solution without any additives. Given glycerol is a normal organic solvent for many inorganic salts, it is surprising that this is the first report of GCPC, and similar clusters may exist in other mineral systems, for example calcium carbonate. Second, the transformation of GCPC, which is mediated by a state of long amorphous nanowires to form HAP nanorods, will expand the theory of amorphous solids and their crystallization. So far as I know and as stated in the manuscript, amorphous inorganic minerals tend to form spherical or irregular shapes due to the anisotropy, and elongated nanowires are very rare to obtain in the absence of amphiphilic surfactants. Third, considering the real environment of mouth with dynamic aqueous fluids and moving muscles, it is realistic to use GCPC for the enamel remineralization since its transformation can be induced by water and finished in very short time, which is highly efficient for enamel repair and can significantly avoid the impacts of hash oral environments. Fourth, it is noteworthy that the repair effect is demonstrated in the human mouths (clinical trial), validating its great prospects for clinical use. This manuscript is well organized, and provides substantial data obtained from in vitro, animal experiments and clinical trial that have been carefully conducted. I would suggest accepting this manuscript after a minor revision.

1. It is mentioned in mineralization experiments in vitro that the GCPC is completely crystallized at 30 min. The author needs to explain how to judge the complete crystallization.
2. According to the in vitro study, 3X-GCPC may be better than GCPC in restoring enamel morphology. The author should explain why 3X-GCPC is not used as the main experimental group in animal and clinical studies.
3. In general, the frequency of material application is very important for the result of enamel remineralization. More detailed description regarding the frequency of using GCPC on enamel blocks should be provided in animal study, namely once or many times a day.
4. The indications of asterisks in figures are confusing. For example, on figure 5G and figure 5H, the comparison between which two groups the asterisk represents needs to be indicated.
5. Scale bars on SEM images are too thin to view, and need to be adjusted.

Reviewer #2

(Remarks to the Author)
The manuscript entitled "The glycerol stabilized calcium phosphate cluster for rapid remineralization of tooth enamel by a water-triggered transformation" by Luo et al. shows the development of a glycerol stabilized calcium phosphate cluster. The cluster can perform a fast enamel repair through a water-triggered transformation in vitro and in vivo, and in the clinical trial as well. In general, the work is well presented and the experimental evidence supports the claims. The preparation process is simple and easy to scale up, therefore the materials are promising for dental clinical applications. I recommend that this manuscript can be published in Nature Communications after minor revision.

Specific comments:
1. The diameters of GCPC and GCPC-ACP were determined by TEM, I suggest additional measurements of dynamic light scattering to confirm the diameters of these materials.
2. According to the manuscript, the Ca/P ratio is kept at 1.52 when preparing the GCPC. What is the Ca/P ratio in the resultant GCPC and GCPC-ACP? It can differ from the initial Ca/P ratio.
3. Please add the information regarding the concentration of Ca2+ and PO43- when preparing the clusters in the caption of Fig. 1A
4. In Fig.S6, the authors show the TEM image of nanowires on repaired enamel surface, please describe the sample preparation process for TEM measurement.
5. Please provide the product and company name of CPP-ACP.
6. The results of Vickers-hardness are presented in Fig.5 and Fig.6, but in the characterization part, it is written “A Knoop microhardness tester (HXD-1000 TMC, China) was used to assess the surface hardness”. Please clarify whether Vickers-hardness or Knoop-hardness was measured.

Reviewer #3

(Remarks to the Author)
Biomimetic strategies have been widely used by researchers to repair hard tissues, such as bone and teeth, and plenty of achievements have been achieved. Among these studies, the biomimetic materials of calcium phosphate, including ionic clusters, ACP, TCP, and HAP, have attracted more attention. In particular, calcium phosphate ion clusters (CPICs) prepared in ethanol and developed by the Tang lab have been used to regenerate the enamel, recovering the complex structure, and mechanical properties. This work conducted by Luo et al. prepared a calcium phosphate cluster stabilized by glycerol (GCPC) used to repair the demineralized enamel in vivo and in vitro, even carried out in human clinical trials. Although the GCPC treatment could to some extent deposit a layer of HAP mineral on the enamel surface, the thickness (one time approximately 1 um), is far less than what would be expected in a patient with caries and does not have an apparently ordered structure. Furthermore, the lack of solid evidence to support the conclusions they propose means that this work does not appear to be rigorous. For instance, the mechanism by which GCPC remineralizes the enamel is unclear. In light of this, I do not recommend that this paper be published in the high-impact journal Nature Communications. Some important comments are listed below.

1. The authors suggest that glycerol could dissolve calcium and phosphate salts and inhibit their reaction. However, they only provide a digital image of the solution and an OD value to support this conclusion. These experiments cannot exclude the formation of calcium phosphate clusters in the glycerol solvent. It is possible that the amount of clusters formed is below the detection limit of the instrument. Here, the DLS, NMR, or TEM observation could provide more solid evidence to support such conclusions. In addition, the 1-2 nm particles dispersed in the solution should appear a light blue rather than a white view in the digital image (see GCPC in mixed solvent).

2. Glycerol may stabilize the calcium phosphate cluster, but the authors don’t present any related results to prove this.

3. The authors using ACTEM observe the GCPC at the initial stage of preparation. After 2 weeks the ACTEM observation also needs to examine the GCPC to further confirm that its size has not changed and then propose that the GCPC is stable, rather than just simply providing a digital image that does not observe any visible phase separation. Figure S1E shows that the white solution could be due to the GCPC aggregating to a larger size, but it can't cause precipitation.

4. As the sample used for the TGA analysis was washed with ethanol 6 times, which could lead to the exchange of ethanol and glycerol molecules, how the authors excluded the ethanol content when analyzing the S3 stage to calculate the glycerol weight. As ethanol and glycerol have similar properties in the FTIR spectra, it is difficult to exclude the presence of ethanol.

5. During in vitro enamel repair, the authors brushed the GCPC solution onto the enamel surface. As the glycerol has a high boiling point (290 °C), the glycerol hardly evaporates and remains on the surface before being immersed in the artificial saliva, and then the GCPC could diffuse into the enamel crystal surface and may interact with it, and it is still difficult to aggregate into larger size particles due to its excellent stability. When the enamel coating with the GCPC solution is immersed in the artificial saliva, how can it be ensured that the GCPC still wanders around the enamel surface and does not diffuse into the aqueous solution? In addition, how to ensure that the GCPC crystallization process occurs on the enamel surface rather than in the bulk solution due to the water-induced transformation, resulting in a disordered structure.

6. The nanowires originated from GCPC on the enamel surface were observed at a very early stage (5 min) as shown in Figures 2 and S6. The crucial evidence to prove that the nanowires are composed of ACP, the element analysis should be included except that the SAED pattern.

7. Figure 2 shows the result of the enamel repaired with GCPC solution. The knot with an elongated shape is observed and judging whether it is perpendicular to the enamel surface should be from the cross-sectional view to obtain a clearer SEM image to support such a result. And cross-sectional view could also further confirm whether the newly formed HAP has an ordered structure. In addition, ultrasonic treatment of the repaired samples could more accurately confirm that the newly formed crystals adhere strongly to the enamel because the stress produced by the rinsing solution is too low.

8. The formation of nanowires is attributed to the interaction between GCPC and the specific substrate and is also confirmed with other substrates shown in Figure S9. The question arises as to how the nanowires at the top were formed without contact with the HAP substrate (see Fig.S9 A and B).

9. The thickness of the repaired layer is ~1 um resulting from the side view, which is less accurate than the cross-section. The authors could consider observing the cross-section of repaired specimens. The thickness could be increased by cyclic treatment. However, the authors performed experiments in which the enamel was remineralized with GCPC for 24h and 48h, rather than for 30 min and 60 min. Since the artificial saliva could remineralize the enamel over a long time, the authors should exclude the role of artificial saliva in long-term repair.

10. The authors describe that “with the incubation time prolongated to 24 h, the repaired layer has no significant changes from that of 30 min” (page 9, lines 26, 27). The authors should specify which properties in the repaired layer do not change because the change in hardness occurs between the repaired enamel for 30 min and 24h. The increase in hardness is usually accompanied by the formation of a denser compact mineral on the enamel surface.

11. In the animal study using GCPC materials, it is not clear whether the newly formed HAP crystals are simply deposited on the enamel surface or whether they form a whole. This is difficult to confirm from the SEM image.

12. In the method section, one drop of GCPC solution was used for enamel remineralization. Please clarify how much of this solution was used.

13. The HAP nanorod disk was prepared using a synthetic HAP prepared by the authors. The characterization of these nanorods, such as XRD or FTIR, is necessary.

14. To study the mechanical properties of the enamel samples, the nanoindenter could be used to obtain a variety of values, such as hardness and Young’s modulus, for a comprehensive assessment of the performance of the materials.

15. The style of the reference is not consistent. For example, references 22, 24, and 28 lack the page number; for references 2, 7, 10, 17, 18, 22, 24, 33, 34, 37, 38, and 39 the initial of the article title should be in lower case; and so on.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have provided substantial materials to make response to the Reviewers' comments. These results have further strengthened the significance of this work. I recommend the manuscript to be accepted in the present form.

Reviewer #2

(Remarks to the Author)
I have no further comments. The manuscript can be accepted.

Reviewer #3

(Remarks to the Author)
After long-term efforts, the authors have addressed some issues and improved the manuscript. However, some key issues have still not been resolved. For example, the mechanism by which the glycerol stabilizes the calcium phosphate cluster remains unclear. Despite pointing this out in my previous comment #2, the authors only presented a speculative conclusion in the revised manuscript. Illustrating this mechanism is a highlight, but the authors ignored it. In addition, the authors presented some crucial results existing contradiction, and cannot be resolved by conducting more experiments (refer to Comments #2 and #4 in this version). Moreover, the structure or morphology of the newly formed minerals on the enamel surface repaired with GCPC is similar to that of repaired with CPP-ACP, indicating that GCPC has no apparent advantage in terms of constructing an ordered structure, as demonstrated by Figure 6. This is difficult to achieve an ordered structure, even with in vitro experiments using GCPC. In summary, all these points make it difficult to capture the novelty of this work and its contribution to the advancement of materials science.

1. In my previous comment #2, the question is how glycerol stabilizes the calcium phosphate cluster, the molecular mechanism should be proposed. In response to this comment, the authors provide supplementary Figures 1E, 4, and 5 to confirm the critical role of glycerol for calcium phosphate cluster stabilization. I agree with that. However, the authors just rely on these results to give a speculated conclusion that glycerol molecules have a strong affinity to GCPC. Given the starring role of glycerol in this study, what exactly is the mechanism of glycerol stabilization? For example, what is the interaction between the glycerol molecules and the calcium phosphate clusters; could it be that the solvent effect of the glycerol molecules, due to their high viscosity, inhibits the collision of the clusters and prevents the formation of larger particles, thus stabilizing the clusters? (This point has been verified to some extent in this study. Despite the ethylene glycol having two hydroxyl groups, less one than glycerol, its stabilization for calcium phosphate clusters is far less than that of glycerol,

perhaps because of the higher viscosity of glycerol.) The authors should perform some experiments to confirm it. This will contribute to understanding how certain organic molecules stabilize the inorganic ionic cluster.

2. In my previous comment #3, after storage at room temperature for 2 weeks, the GCPC solution presents white turbidity as shown in Figure S1E in the original paper, and can exclude the interference of the blue background plate. In response, the authors use ACTEM to confirm that the solution contains mainly 1-2 nm clusters along with very few larger particles. Under this state, the solution should be present the light blue, resembling the one shown in Fig. R4. Therefore, there is a contradiction between the result shown in Figure S1E and the ACTEM result. The authors should explain these results, in particular why the white turbidity is presented, to avoid misleading the reader.

3. In FTIR (Figure 1E), the authors focus on the analysis of the GCPC-ACP rather than the GCPC which is a core material to use to repair the enamel. Can you please explain this?

4. As stated by the authors, there is no difference between the repaired layer after 30 min and after 24 h (according to the morphology of the repaired layer and GIXRD results). Thus, the authors claimed that all GCPC crystallized after 30 min. Furthermore, based on the SEM result (Figure S20), the authors concluded that the artificial saliva had a very low capacity for enamel remineralization. It is reasonable to conclude that the newly formed minerals in the repaired layer are mainly attributable to GCPC. However, the cross-section of the enamel coated with GCPC shows that the constructed repaired layer is in the range of 400 - 600 nm (Figure 2B1). After 24 h of remineralization, the authors concluded that the thickness of the repaired layer was around 1 um. HOW??? Where do the extra minerals come from?

Even after 48 h of remineralization, the thickness of the repaired layer reached 3 to 4 um, indicating that each time treatment with GCPC formed a layer of at least 1.5 to 2 um at the initial stage. These results also contradict the results from Figure 2. This is incomprehensible. The authors should explain this carefully.

In addition, the authors should clearly indicate the interface between natural enamel and repaired enamel in Figure 2E1 and Figure S19 (24 h) with a magnified SEM image, because it is hard to distinguish the newly formed mineral from the natural enamel.

5. When the authors respond to my previous comment #8, they weaken the crucial role of ACP nanowires in enamel repair in the revised manuscript. In the original paper (version 1), the authors believe that ACP nanowires play a critical role. What are the reasons that lead the authors to believe that ACP nanowires do not play a decisive role? Just because the ACP nanoparticles were detected after more than 10 repetitions or for other reasons. Authors should provide a detailed scientific explanation rather than replacing the original description. And it is also better to explain how the ACP nanowires are formed. In addition, the characterization of the ACP nanowires was carried out and the results are shown in Supplementary Figures 11, 12, and 13. The authors described the diameter of the ACP nanowires as 5-10 nm, which is inconsistent with the result in Figure 12, which shows that the diameter is at least 40 nm in some parts, and which is consistent with the result shown in Supplementary Figure 13. The authors need to clearly confirm this and explain the cause of the difference between the Supplementary Figures 12 and 13.

6. In the authors' response to my previous comment #9, they stated that the artificial saliva had a very poor performance in remineralizing enamel, as demonstrated by Supplementary Fig. 20. An equally magnified SEM image as the insets in Figures 4A and D should be included for comparison with that repaired with the GCPC.

7. The method of preparing glycerol containing calcium and phosphate ions should be provided in the Experimental section.

Version 2:

Reviewer comments:

Reviewer #3

(Remarks to the Author)
In this revised manuscript, the authors have addressed almost all the issues I raised. However, there is one very important issue that is not well addressed. The issue is the confirmation of the thickness of the repaired layer, including the initial GCPC deposited onto the enamel surface and the final newly formed mineral layer. Although the author has shown the SEM results (Figs. R2, R8, R9 and R10), I don't think that the dotted line is the boundary between the natural enamel and the repaired layer. The SEM results presented here show that the newly formed mineral has a structure that is similar to that of natural enamel (Figs. R2, R9 and R10). Therefore, if this is the case, the SEM image is inaccurate for confirming the boundary. Here, the authors should provide the basis behind their determinations of the boundary the repaired layer and natural enamel. Additionally, employing alternative technologies (such as AFM) to validate the thickness measurements and further confirms SEM findings would enhance the robustness of the study. In Fig. R8, the enamel is repaired by GCPC at an early stage. From the cross-sectional SEM images provided by the authors, I cannot even distinguish the repaired layer from the natural enamel, although they are significantly different in structure. The authors should provide an SEM image to clearly show the boundary, especially for 0 min.

Response to reviewers

We appreciate the important and constructive comments provided by reviewers, based on which this manuscript has been carefully revised. We are also very grateful for the provided deadline extension to enable additional experiments. Our responses to the comments, as well as explanations of the changes in our manuscript, are explicated as follows. We hope that the revised manuscript is now acceptable for publication, and look forward to the further evaluation.

Our replies appear indented, and corresponding changes in the manuscript are highlighted in yellow.

Reviewer #1 (Remarks to the Author):

In this manuscript, the authors develop a stable ultrasmall mineral species, glycerol stabilized calcium phosphate cluster (GCPC), for a rapid remineralization of tooth enamel. This work is of great significance from the perspective of both science and clinical practice. First, the GCPC with ultra-smalls size and stable state is obtained in glycerol solution without any additives. Given glycerol is a normal organic solvent for many inorganic salts, it is surprising that this is the first report of GCPC, and similar clusters may exist in other mineral systems, for example calcium carbonate. Second, the transformation of GCPC, which is mediated by a state of long amorphous nanowires to form HAP nanorods, will expand the theory of amorphous solids and their crystallization. So far as I know and as stated in the manuscript, amorphous inorganic minerals tend to form spherical or irregular shapes due to the anisotropy, and elongated nanowires are very rare to obtain in the absence of amphiphilic surfactants. Third, considering the real environment of mouth with dynamic aqueous fluids and moving muscles, it is realistic to use GCPC for the enamel remineralization since its transformation can be induced by water and finished in very short time, which is highly efficient for enamel repair and can significantly avoid the impacts of hash oral environments. Fourth, it is noteworthy that the repair effect is demonstrated in the human mouths (clinical trial), validating its great prospects for clinical use. This manuscript is well organized, and provides substantial data obtained from in vitro, animal experiments and clinical trial that have been carefully conducted. I would suggest accepting this manuscript after a minor revision.

We appreciate the positive comments by the reviewer.

1. It is mentioned in mineralization experiments in vitro that the GCPC is completely crystallized at 30 min. The author needs to explain how to judge the complete crystallization.

We thank the reviewer for the important suggestion. We would like to explain that, in the previous manuscript, the complete crystallization of GCPC was judged by the process of morphology change. Specifically, when all of the coated GCPC transformed into nanorods and no further change was observed, it then completed the crystallization.

We agree that the morphology changes may not accurately reflect the crystallization states, thus the grazing incidence X-ray diffraction (GIXRD) of GCPC treated enamels at different time points was measured to provide further details on it.

As shown in **Fig. R1**, indicated by weak crystalline diffractions and noisy background, GCPC generates an amorphous or very weakly crystallized calcium phosphate layer on enamel by water-triggered transformation at 5 min (the weak HAP signals on the pattern may be contributed to the enamel substrate underneath the GCPC). Then over time, the intensity of characteristic reflections of HAP on GIXRD pattern increases, suggesting that the GCPC gradually evolves into HAP on the enamel, consistent with obversion by SEM. Moreover, there is no significant difference in diffraction peak intensity of the patterns between the specimens collected at 30 min and 24 h, further supporting the previous conclusion that GCPC is completely crystallized at 30 min.

Related result and discussion (**Page 8**), experimental method (**Page 21**) and supporting information (**Page 10, Supplementary Fig. 15**) are added in the revised version.

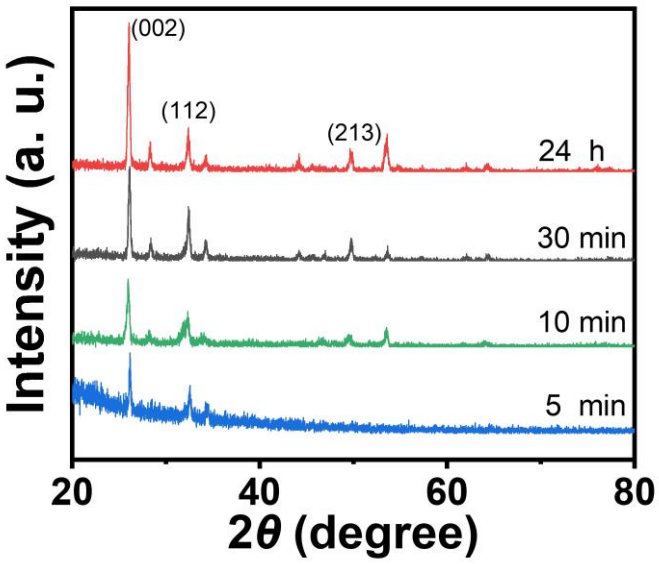


Fig. R1. Grazing incidence X-ray of enamel surface repaired by GCPC for 5 min, 10 min, 30 min and 24 h. **This graph is displayed as Supplementary Fig. 15 in the revised Supporting Information.**

2. According to the in vitro study, 3X-GCPC may be better than GCPC in restoring enamel morphology. The author should explain why 3X-GCPC is not used as the main experimental group in animal and clinical studies.

We thank the reviewer for this comment.

As displayed in the manuscript, 3X-GCPC had a more compact repair layer at 30 min compared to GCPC, suggesting that the higher concentration of clusters results in a more effective crystallization process. However, we chose GCPC rather than 3X-GCPC as the experimental group in vivo studies, because we were concerned about the potential side effects caused by the high salt concentrations in the solution. Although such concern has not been observed, we were very careful about the safety during the in vivo tests. Having said that, we will carefully investigate the performance and biosafety of 3X-GCPC, optimize its formulation, and explore its application in future studies.

3. In general, the frequency of material application is very important for the result of enamel remineralization. More detailed description regarding the frequency of using GCPC on enamel blocks should be provided in animal study, namely once or many times a day.

We thank the reviewer for this comment. Following the suggestion, we supplemented the frequency (once a day) of using GCPC on enamel blocks in animal study as follows:

"Then they were treated with GCPC, CPP-ACP paste, and deionized water (blank) once a day after enamel surface was wetted by saliva."

Related experimental details (**Page 22**) are added in the revised version.

4. The indications of asterisks in figures are confusing. For example, on figure 5G and figure 5H, the comparison between which two groups the asterisk represents needs to be indicated.

According to the reviewer's suggestion, the caption including the indications of asterisks is updated as follows (**Page 12 and 15**):

"The asterisk (*) denotes significant differences between the indicated group and the etched group."

5. Scale bars on SEM images are too thin to view, and need to be adjusted.

We thank the reviewer for raising this issue. We have adjusted the scale bars to ensure that they are clearly visible in the revised images, and further have reviewed all the SEM images in our manuscript to ensure that the scale bars are appropriately sized and positioned.

Reviewer #2 (Remarks to the Author):

The manuscript entitled "The glycerol stabilized calcium phosphate cluster for rapid remineralization of tooth enamel by a water-triggered transformation" by Luo et al. shows the development of a glycerol stabilized calcium phosphate cluster. The cluster can perform a fast enamel repair through a water-triggered transformation in vitro and in vivo, and in the clinical trial as well. In general, the work is well presented and the experimental evidence supports the claims. The preparation process is simple and easy to scale up, therefore the materials are promising for dental clinical applications. I recommend that this manuscript can be published in Nature Communications after minor revision.

Specific comments:

1. The diameters of GCPC and GCPC-ACP were determined by TEM, I suggest additional measurements of dynamic light scattering to confirm the diameters of these materials.

Following the reviewer's suggestion, we measured the dynamic light scattering (DLS) to further determine the diameters of GCPC (in the preparation solution: glycerol-dominant solvents) and GCPC-ACP (in ethanol).

The DLS (**Fig. R2**) measurements show that the average particle size of GCPC is 2.0 ± 0.2 nm, while GCPC-ACP with the size of 28.2 ± 4.9 nm (aggregated clusters), which are consistent with the TEM observations.

Related result and discussion (**Pages 5 and 6**), experimental method (**Page 20**) and supporting information (**Page 3, Supplementary Fig. 3; Page 5, Supplementary Fig. 6**) are added in the revised version.

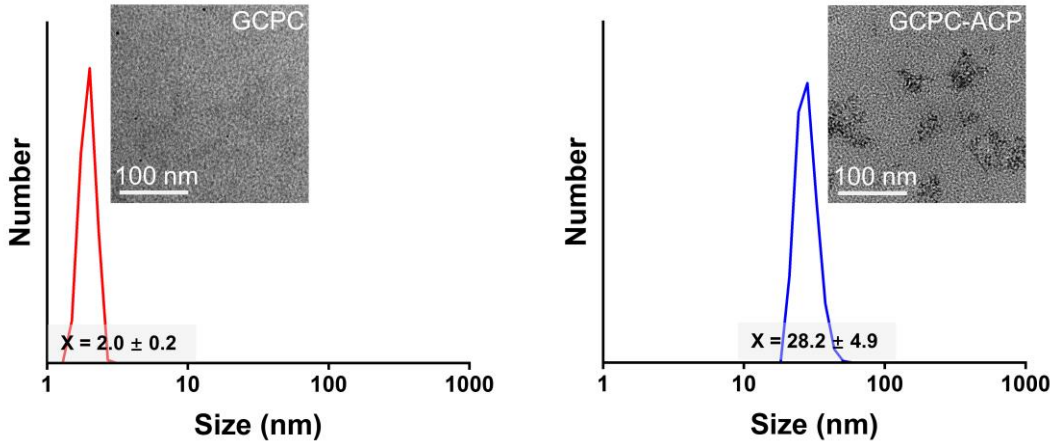


Fig. R2. DLS analysis and TEM images of GCPC and GCPC-ACP. **This graph is displayed as Supplementary Fig. 3 and 6 in the revised Supporting Information.**

2. According to the manuscript, the Ca/P ratio is kept at 1.52 when preparing the GCPC. What is the Ca/P ratio in the resultant GCPC and GCPC-ACP? It can differ from the initial Ca/P ratio.

Following the reviewer's suggestion, the Ca /P molar ratios of GCPC and GCPC-ACP were determined using ICP-OES. The Ca /P ratio of GCPC is found to be 1.42 ± 0.01, and that of GCPC-ACP is 1.39 ± 0.02.

3. Please add the information regarding the concentration of Ca^{2+} and PO_4^{3-} when preparing the clusters in the caption of Fig. 1A.

Following the reviewer's suggestion, the concentrations of PO_4^{3-} and Ca^{2+} have been included in the caption of Fig.1 A (**Page 5**) as follows:

"(A) Schematic and digital images of the prepared solutions by adding the same concentrations of PO_4^{3-}/ Ca^{2+} (0.033 and 0.050 mol/L, respectively) in different solvents (glycerol, mixed (glycerol + water, water content 16.7 v/v%) and water solvents)."

4. In Fig.S6, the authors show the TEM image of nanowires on repaired enamel surface, please describe the sample preparation process for TEM measurement.

We thank the reviewer for this comment. Following the suggestions, we added experimental details (**Page 21**) as follows:

"GCPC-ACP and nanowires on repaired enamel surface at 5 min were characterized by transmission electron microscopy (FEI Talos F200X) … Nanowires were collected by ultrasound irrigation of the enamel immersed in ethanol for 30 s."

5. Please provide the product and company name of CPP-ACP.

Thank the reviewer for this comment. We apologize for not including this information in the manuscript, as we were concerned about potential conflicts of interest. We here clarify that the CPP-ACP paste (full name: GC Tooth Mousse®) was from GC Corporation in Japan, but respectfully request not to add this information to manuscript due to same reason as mentioned above.

6. The results of Vickers-hardness are presented in Fig.5 and Fig.6, but in the characterization part, it is written "A Knoop microhardness tester (HXD-1000 TMC, China) was used to assess the surface hardness". Please clarify whether Vickers-hardness or Knoop-hardness was measured.

After carefully reviewing our records, we confirm that Vickers-hardness tests were applied in this work, and realize that there was an error regarding the description of hardness test. We thank the reviewer for raising this error and apologize for it. While this error does not affect the interpretations and conclusions of this study, it should have been avoided.

In the revised manuscript, we have revised the corresponding text to correct this error as follows:

"Fig. 5G and H illustrate the microhardness of the sound, acid etched (caries model) and GCPC repaired enamels characterized by a Vickers hardness tester" (**Page 11**) and "Nanoindentation (Bruker Hysitron TI980, Germany) and a Vickers hardness tester (0.490 N, 15 s; HXD-1000 TMC, China) were used to assess the surface hardness of the samples." (**Page 21**).

Reviewer #3 (Remarks to the Author):

Biomimetic strategies have been widely used by researchers to repair hard tissues, such as bone and teeth, and plenty of achievements have been achieved. Among these studies, the biomimetic materials of calcium phosphate, including ionic clusters, ACP, TCP, and HAP, have attracted more attention. In particular, calcium phosphate ion clusters (CPICs) prepared in ethanol and developed by the Tang lab have been used to regenerate the enamel, recovering the complex structure, and mechanical properties. This work conducted by Luo et al. prepared a calcium phosphate cluster stabilized by glycerol (GCPC) used to repair the demineralized enamel in vivo and in vitro, even carried out in human clinical trials. Although the GCPC treatment could to some extent deposit a layer of HAP mineral on the enamel surface, the thickness (one time approximately 1 um), is far less than what would be expected in a patient with caries and does not have an apparently ordered structure. Furthermore, the lack of solid evidence to support the conclusions they propose means that this work does not appear to be rigorous. For instance, the mechanism by which GCPC remineralizes the enamel is unclear. In light of this, I do not recommend that this paper be published in the high-impact journal Nature Communications. Some important comments are listed below.

We appreciate the comments and suggestions.

Regarding the previous studies of enamel remineralization, we agree that there have been various noteworthy studies over the past few years demonstrating significant insights into the development of the enamel repair strategies. Particularly, the calcium phosphate species-based remineralization materials which were developed by the Tang lab (Sci. Adv. 2019, 5, eaaw9569; Aust. Endod. J. 2022, 00, 1; Adv. Mater. 2011, 23, 4695) can result in enamel-like structures. But, as we have noted in the manuscript, the remineralization materials including the aforementioned ones still have limitations that hinder the translation from bench to bedside. For example, the potential cytotoxicity and biocompatibility issues from toxic stabilizers and solvents (e.g., triethylamine and ethanol) required for preparation of remineralization materials impair their clinical use. Also, experimental procedures of tooth treatments by these materials in laboratory are often too idealized, which require long time for material attachment, dried enamel surface and static artificial saliva, neglecting the real oral environment clinical practice, leading to limited operability and poor patient compliance in clinical practice. More importantly, the real oral environments, in contrast, are much harsher since the sustained flow of the liquid in the mouth and friction on the tooth surface may lead to the detachment of materials from enamels before finishing the repairs. Therefore, a rapid repair rate in harsh dynamic environments is desired to avoid or diminish these influences for more effective enamel remineralization.

This study aims to address the challenges above. First, we have developed a stable ultrasmall (1-2 nm) mineral species (GCPC) in the glycerol-dominant mixed solvents (major glycerol and minor water) via a simple mixture process, which is the first time to report calcium phosphate clusters in such biocompatible organic solution. As an indispensable stabilizer, glycerol effectively stabilizes GCPC but endows it with the character of water-responsive transformation. Moreover, we have demonstrated that GCPC, with remarkable biocompatibility, can perform a rapid remineralization of tooth enamel by such rapid water-triggered transformation and significantly avoid the impacts of dynamic oral environments on tooth enamel remineralization and improve its repair efficacy. We again would like to emphasize that "rapid" is highly desired for remineralization materials to avoid the impact of dynamic environment in mouth, especially from the perspective of dental care and restoration. Besides, we report a challenging clinical trial to confirm the recovery of microstructure and mechanical properties of enamel. We press that clinical trial is not only an important way to validate the remineralization capacity of GCPC in the real oral environment, but also reflects the high biocompatibility of GCPC and its promising prospects for practical applications, which are rare in the related previous studies. Hence, GCPC indeed exhibits promising remineralization performance as we confirmed in multiple ways, and we believe that the work reported in this manuscript made a significant advance in the related fields and deserves the publication on this journal.

We understand the reviewer's concern about the thickness of repair layer, and agree that enamel repair by minerals with substantial thickness is important in dental remineralization treatment, which remains a persistent pursuit in the field of biomimetic mineralization strategies for the reconstruction of dental hard tissue. However, as shown in **Table 1 (Page 12-14)**, the thickness of the repaired layers reported in various works, even with longer mineralization time, is comparable to that in our study. We also would like to explain that remineralization method generally focuses on the treatments of early caries although it should be prevented before developing into deep caries. In such cases, remineralization methods, especially the one reported in this manuscript, offer distinct advantages by rebuilding and restoring enamel original composition and structure to the greatest extent possible comparing to the commonly used resin-based fillings.

In addition, we thank reviewer for the comments regarding "the lack of solid evidence to support the conclusions" we proposed. We agree that it is crucial to provide robust evidence to support any scientific claims made in our work. To address this concern, we have revised our manuscript to include additional evidence according to the reviewer's suggestions, strengthening the validity of our conclusions, which are also specified in the responses below.

1. The authors suggest that glycerol could dissolve calcium and phosphate salts and inhibit their reaction. However, they only provide a digital image of the solution and an OD value to support this conclusion. These experiments cannot exclude the formation of calcium phosphate clusters in the

glycerol solvent. It is possible that the amount of clusters formed is below the detection limit of the instrument. Here, the DLS, NMR, or TEM observation could provide more solid evidence to support such conclusions. In addition, the 1-2 nm particles dispersed in the solution should appear a light blue rather than a white view in the digital image (see GCPC in mixed solvent).

We appreciate the reviewer's comment on the species in the pure glycerol solution of PO_4^{3-}/ Ca^{2+}. Following the suggestions, we measured the liquid-cell ACTEM of this solution mentioned above, but can hardly find clusters or other solid species. Then we turned to DLS (Malvern Zetasizer Nano ZS90), which shows that the scattering intensity is below the measurable range of the instrument (**Fig. R3**). As a comparison, the same DLS instrument detects significant light scattering in the glycerol-dominant solution (major glycerol and minor water, same condition for GCPC preparation in the manuscript) of PO_4^{3-}/ Ca^{2+}. These all indicate that PO_4^{3-} and Ca^{2+} mainly keep in the form of ions in pure glycerol although the formation of clusters cannot be totally ruled out.

It also should be explained that, from the perspective of physical chemistry, they are not totally the free ions, but would be partially in the form of ion complexes, pre-nucleation clusters or other dynamic species (Small, 2022, 18, 2107735). We agree that, after a longer time, the more stable post-nucleation species (e.g., clusters) would increase in the solution driven by thermodynamic effects.

On **Page 4** of the revised manuscript, we added the above discussion to further clarify the PO_4^{3-} /Ca^{2+} ion states in pure glycerol as follows:

"In addition, dynamic light scattering (DLS) measurement of the glycerol solution (**Supplementary Fig. 2**) shows that the light scattering intensity of the species in it is beyond the measurable range of instrument, indicating that $PO_4^{3-}$and Ca^{2+} mainly keep in the state of ions and other prenucleation species [28], but should be much less in the form of post-nucleation precipitates"

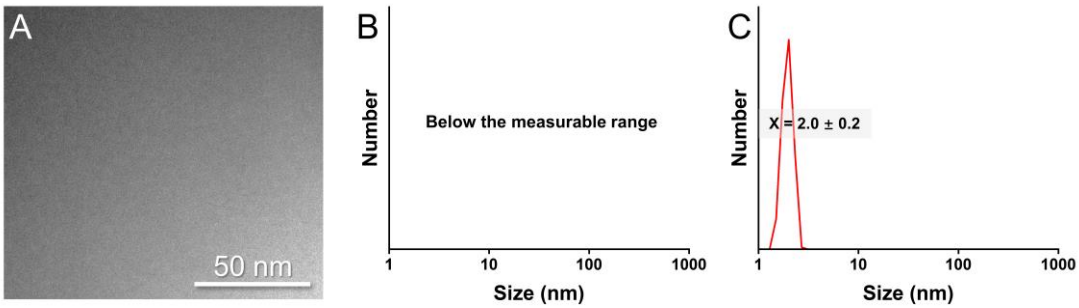


Fig. R3. (A, B) liquid-cell ACTEM image (A) and DLS analysis of the solution containing 0.033 mol/L PO_4^{3-} and 0.050 mol/L Ca^{2+} ions in pure glycerol solvent. (C) DLS analysis of the solution containing same ions as A and B in glycerol-dominant solvent (glycerol+water; water content 16.7 v/v%). **The graph B is displayed as Supplementary Fig. 2 and Supplementary Fig. 3 in the revised Supporting Information.**

We also thank reviewer for the comment regarding the optical properties of 1-2 nm calcium phosphate clusters in the solvent. Actually, the GCPC solution does show a weak blue appearance in normal conditions, but the digital photos in the former version failed to display it due to the use of a blue background plate. Therefore, we here took the digital image of light scattering (with white light source) of the fresh GCPC solution (**Fig. R4**). In this case, the GCPC solution indeed exhibits a bluish appearance (in comparison, the pure glycerol shows a transparent state).

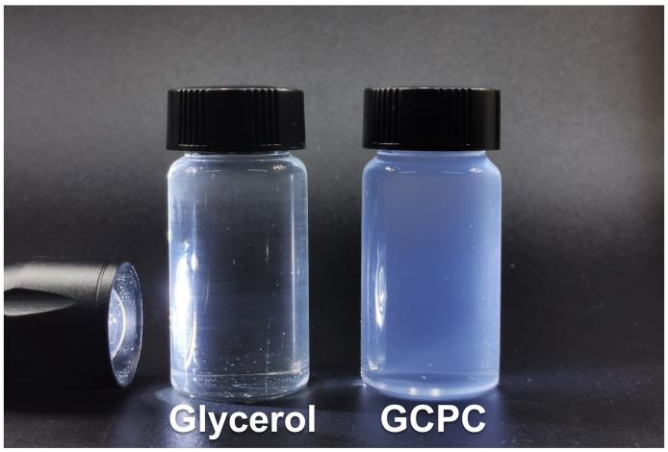


Fig. R4. The digital image of light scattering (white light source) of the pure glycerol and fresh GCPC solution (mixed solvent).

2. Glycerol may stabilize the calcium phosphate cluster, but the authors don't present any related results to prove this.

We are sorry for the reviewer's confusion, thus would like to summarize the corresponding evidence regarding the role of glycerol in stabilizing the clusters here. Basically, when the manuscript first introduced GCPC in the introduction section, the stabilization effect by glycerol was claimed that "glycerol can effectively retard the transformation and aggregation of the substances formed in it;" then, in the discussion section, we further proved it as follows:

First, the GCPC solution (water content 16.7 v/v%) can keep a homogeneous state without visible phase separation for at least 2 weeks (**Supplementary Fig. 1E**). In comparison, when introducing more water in the GCPC solution, precipitation is immediately formed, which turns out to be ACP nanoparticles according to TEM and SEAD characterizations (**Supplementary Fig. 4**). Besides, mixing GCPC with methanol, ethanol or ethylene glycol at a volume ratio of 1:10 to exchange glycerol with each of them, solid precipitation occurs immediately (**Supplementary Fig. 5**). This suggests that glycerol functions to stabilize GCPC in the solution, and comparatively, other organic alcohols cannot stabilize it. Moreover, as a high content of glycerol is still detected in GCPC-ACP washed by ethanol for many times and dried in vacuum thoroughly (please also see our response to comment 4), it is reasonable to speculate that glycerol molecules have strong affinity to GCPC, which may cause their high stability as discussed above (however, glycerol also endows GCPC with the character of water-responsive transformation).

3. The authors using ACTEM observe the GCPC at the initial stage of preparation. After 2 weeks the ACTEM observation also needs to examine the GCPC to further confirm that its size has not changed and then propose that the GCPC is stable, rather than just simply providing a digital image that does not observe any visible phase separation. Figure S1E shows that the white solution could be due to the GCPC aggregating to a larger size, but it can't cause precipitation.

We appreciate the very important suggestion. Thus, we further measured liquid cell-ACTEM of GCPC after storage at room temperature for 2 weeks. As shown in **Fig. R5**, we still mainly observe 1-2 nm sized clusters in the solution, and very fewer bigger particles can be found as well. We acknowledge that the GCPC clusters will further aggregate in the solutions over time, increasing the quantity of the bigger particles.

Further details regarding the data have been added to the Result and discussion section (**Page 6**) and Supporting Information (**Page 6; Supplementary Fig. 7**).

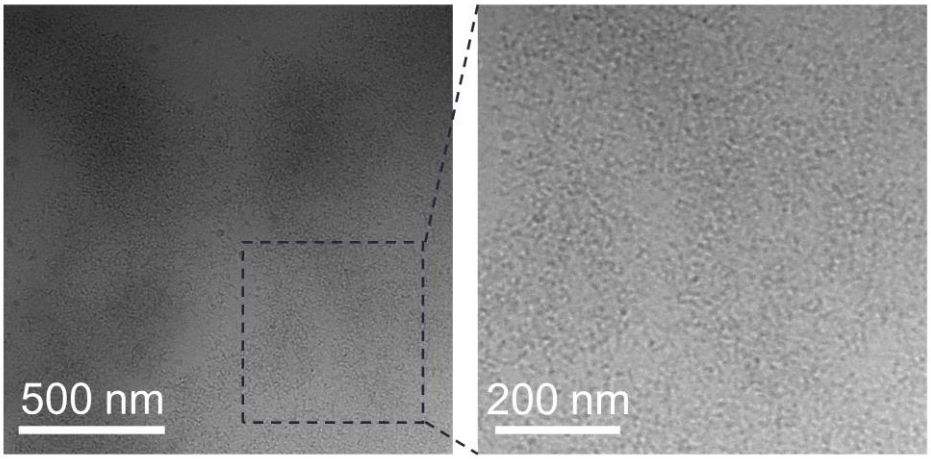


Fig. R5. Liquid cell ACTEM image of GCPC solution after storage at room temperature for 2 weeks. **This graph is displayed as Supplementary Fig. 7 in the revised Supporting Information.**

4. As the sample used for the TGA analysis was washed with ethanol 6 times, which could lead to the exchange of ethanol and glycerol molecules, how the authors excluded the ethanol content when analyzing the S3 stage to calculate the glycerol weight. As ethanol and glycerol have similar properties in the FTIR spectra, it is difficult to exclude the presence of ethanol.

We thank the reviewer for raising this important query.

In order to address this concern, gas chromatography-mass spectrometry (GC-MS, GCMS-QP2020 NX, China) and Gas chromatograph (GC, Agilent 8890, USA) system coupled with headspace sampling and flame ionization detector were used to determine the content of glycerol and potential ethanol in GCPC-ACP, respectively.

Briefly, GCPC-ACP was cultured in water at 37 ºC for 24 h to crystallize and release ethanol and glycerol in water, then the supernatant was collected after centrifugation for tests. A reported derivatization protocol (Anal. Bioanal. Chem. 2011. 400, 1405) was used to test the content of glycerol, whereby the glycerol was trimethylsilylated, then detected by GC-MS. For ethanol, GC was directly used for the determination without transformation. Detailed protocol is included in the experimental section of the revised manuscript (**Page 20 and 21**).

As shown in **Fig. R6**, the content of glycerol and ethanol in GCPC-ACP sample was determined to be 21.2 wt% and 0.000176 wt%, respectively.

Hence, we confirm that almost all of the ethanol in GCPC-ACP has been removed during the drying process, which is consistent with the content of glycerol assessed by TGA-FTIR analysis.

Further details regarding the data have been added to the Result and Discussion section (**Page 6**) and Supporting Information (**Page 6, Supplementary Fig. 8**).

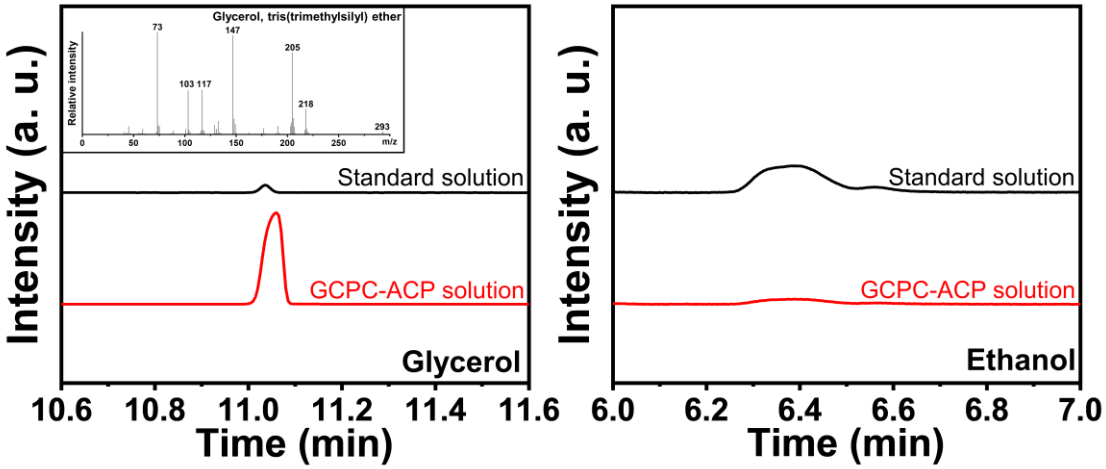


Fig. R6. The GC-MS (left) and GC (right) analyses of GCPC-ACP extracted solutions for determining the content of glycerol (left) and ethanol (right) in GCPC-ACP. Inset: mass spectrums of trimethylsilyl ether of glycerol derived from glycerol in GCPC-ACP extracted solution. **This graph is displayed as Supplementary Fig. 8 in revised Supporting Information.**

5. During in vitro enamel repair, the authors brushed the GCPC solution onto the enamel surface. As the glycerol has a high boiling point (290 ºC), the glycerol hardly evaporates and remains on the surface before being immersed in the artificial saliva, and then the GCPC could diffuse into the enamel crystal surface and may interact with it, and it is still difficult to aggregate into larger size particles due to its excellent stability. When the enamel coating with the GCPC solution is immersed in the artificial saliva, how can it be ensured that the GCPC still wanders around the enamel surface and does not diffuse into the aqueous solution? In addition, how to ensure that the GCPC crystallization process occurs on the enamel surface rather than in the bulk solution due to the water-induced transformation, resulting in a disordered structure.

We thank the reviewer for raising the concerns, and would like to address them as follows.

First, we would like to clarify that GCPC can be significantly retained on the enamel during its transformation in artificial saliva. Actually, when GCPC is immersed in the artificial saliva, the diffusion rate of glycerol is much slower than water since the former has much higher molecular mass and viscosity, so the diffusion behavior of water-into-glycerol, rather than glycerol-into-water, prevails in the two diffusion processes. Besides, the diffusion of clusters into water can be significantly retarded due to the high viscosity of glycerol and their rapid transformation/ aggregation when contacting the water (that diffuses into the glycerol or at the interface of the glycerol and water phases). This can be demonstrated by an experiment as follows (**Fig. R7**). Briefly, an aluminum plate with the size of 2 x 2 cm was used as the substrate where we smeared 100 mg of GCPC to the center of its surface. Then, we submerged it at an angle of 45º (with respect to the bottom of the beaker) in artificial saliva at 37 ºC for 30 min (the time for completing crystallization of GCPC). Then we took out the plate for observation. After sufficient drying, we weighed and calculated the mass of the remaining CaP on the plate (**Fig. R7, middle**). For comparison (control), 100 mg of GCPC was added to the same volume of artificial saliva at 37 ºC and incubated for the same duration, followed by centrifugation, drying, and weighing the mass of the collected CaP. The results show that a uniform CaP layer is formed and retained on the plate. By comparing the mass of CaP on the aluminum plate with that formed in the control, it is calculated that 60.9 wt% of CaP remains on the substrate.

Second, crystallization of GCPC can occur on the enamel surface. As described in the manuscript, the surface of enamel must keep wet (not dry) prior to the application of GCPC on enamels. So, the water on/in the enamel initially destabilizes GCPC during adsorbing it on the surface of enamel. Also importantly, the inductive effect by the HAP nanorods of enamel should be noted, which favors the primary crystallization sites on the top ends of nanorods and the preferential crystal growth to keep the order of the formed new HAP nanorods (Sci. Adv. 2019, 5,

eaaw9569; Adv. Mater. 2011, 23, 4695). Moreover, as a reference, in some previous reports, the repair materials are also coated on the enamel to form an even more dense coating, then immersed in aqueous solution to induce the transformation (ACS Nano 2022, 16, 3119; Adv. Mater. 2020, 32, 2002080; Adv. Sci. 2021, 2103829). Typically, in one study (Sci. Adv. 2019, 5, eaaw9569), a dried ACP layer is densely formed on enamel using a CaP precursor, then the crystallization is induced in artificial saliva. In that case, the mobility of ACP is very low, but the repair layer of HAP is still well formed. Having said that, like in other reports, we cannot avoid that a fraction of GCPC crystallizes in the bulk solution, resulting in disordered HAP nanorods. In this case, it requires a water flushing and brushing after each treatment, as we stated in the manuscript, to remove these weakly attached HAP nanorods.

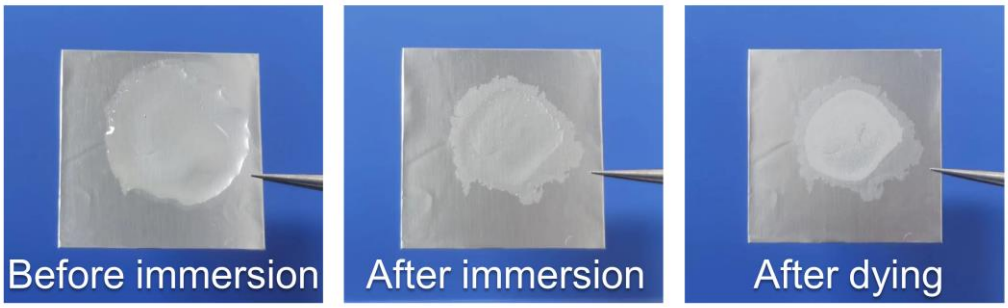


Fig. R7. Digital images of an aluminum plate after being coated with GCPC (left), subsequently immersed in artificial saliva for 30 min (middle) and dying (right).

6. The nanowires originated from GCPC on the enamel surface were observed at a very early stage (5 min) as shown in Figures 2 and S6. The crucial evidence to prove that the nanowires are composed of ACP, the element analysis should be included except that the SAED pattern.

We thank the reviewer's comment. According to the suggestion, the element distribution of nanowire was characterized by energy dispersive spectroscopy (EDS) mapping. As shown in **Fig. R8**, the distribution of calcium and phosphorus elements is consistent with the morphology of nanowire, further supporting that it is indeed an ACP nanowire.

Further details regarding the data have been added to the Result and Discussion section (**Page 8**), Experimental Method (**Page 21**) and Supporting Information (**Page 9; Supplementary Fig. 13**).

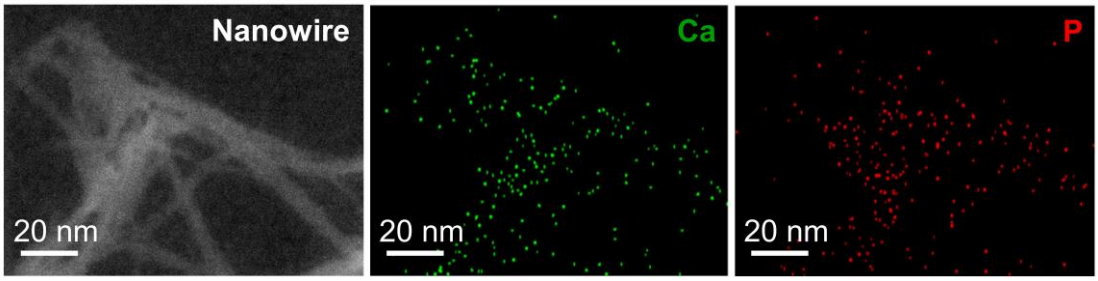


Fig. R8. STEM (left) image and EDS (middle and right) elemental mapping of ACP nanowires. **This graph is displayed as Supplementary Fig. 13 in revised Supporting Information.**

7. Figure 2 shows the result of the enamel repaired with GCPC solution. The knot with an elongated shape is observed and judging whether it is perpendicular to the enamel surface should be from the cross-sectional view to obtain a clearer SEM image to support such a result. And cross-sectional view could also further confirm whether the newly formed HAP has an ordered structure. In addition,

ultrasonic treatment of the repaired samples could more accurately confirm that the newly formed crystals adhere strongly to the enamel because the stress produced by the rinsing solution is too low.

We thank the reviewer's important comments.

Following the suggestion of the reviewer, we have undertaken a renewed effort to capture SEM images of the enamel samples repaired by GCPC at early stage from the surface and cross-sectional view.

According to the previous and new observations, we re-conclude that the evolution of GCPC on enamel surface as follows. First, after acid etching, the enamel samples expose the tightly arranged hydroxyapatite nanorods (**Fig. R9A and A1**). Upon GCPC treatment, attributed to ultra-small size and high fluidity, GCPC enables rapid penetration into the exposed interstices on the acid-etched enamel surface (**Fig. R9B and B1**). When further being incubated in artificial saliva for 5 min, GCPC on enamel evolves into ACP nanoparticles and ACP nanowires adhered along enamel crystals by water-triggered transformation (**Fig. R9C and C1**). At 10 min, a complete and uniform ACP layer can be observed on enamel surface, which is derived from the fusion of the previous species (**Fig. R9D and D1**), and finally transforms into nanorods (30 min, **Fig. R9E and E1**).

Based on the above observations, we would like to clarify that, by numerously repeating the observations (more than 10 times of the characterizations), it is found that nanowires are not the only intermediate species towards HAP formation, in contrast, ACP nanoparticles derived from GCPC are involved in the pathway as well. Besides, the majority of resulting HAP nanorods are aligned perpendicular to the enamel substrate (**Fig. R9E and E1)**. Also, the nanowires directly transform into the mineralization layer without forming other shapes (e.g., the knots) (**Fig. R10**). We apologize for the inappropriate statement on the role of intermediate nanowires in the previous version. In light of these findings, we have carefully revised the manuscript to more accurately reflect the observed transformation pathways. Further details regarding the data have been added to the Result and Discussion section (**Page 7, 8 and 9, Fig. 2**). Moreover, the schematic diagram (**Page 9, Fig. 2A2, B2, C2 and D2; Page 10, Fig. 3**) illustrating the process of enamel repaired by GCPC has been appropriately modified to align with experimental results observed.

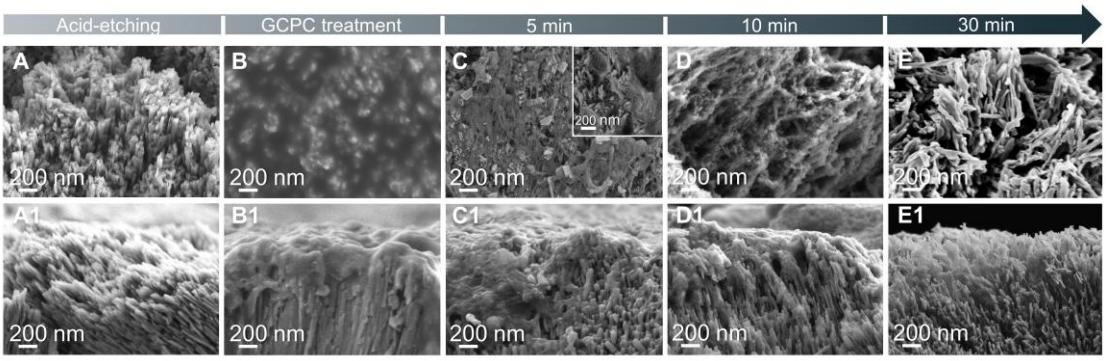


Fig. R9. Rapid repair of demineralized enamel using GCPC. (A-A1, B-B1, C-C2, D-D1) SEM images from the top surface and cross-sectional view of etched enamel before (A-A1) and after treatment for 0 min (B-B1), 5 min (C-C1), 10 min (D-D1) and 30 min (E-E1). **This graph is displayed as Fig. 2 in revised manuscript.**

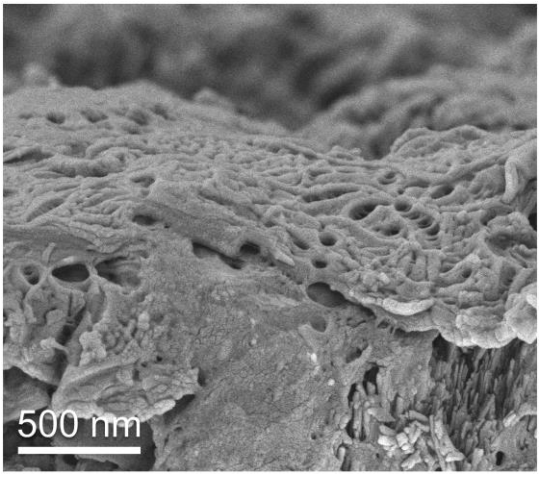


Fig. R10. The cross-sectional view of the ACP nanowires formed on the acid etched enamel at 5 min. **This graph is displayed as Supplementary Fig. 11 in revised Supporting Information.**

Regarding the concern about the attachment of the newly formed hydroxyapatite crystals to the enamel, we performed ultrasonic treatment (1500 W, 40 KHz, 30 s) on enamel formed by GCPC treatment (30 min) in artificial saliva. The SEM images (see below **Fig. R11**) show that the HAP crystals derived from GCPC remain tightly attached on the etched enamel substrate, indicating that they were indeed growing closely to the enamel rather than simply adhering on the surface, which is consistent with the results presented in the manuscript. Having said that, we acknowledge that a fraction of the GCPC distant from the enamel surface may form crystals that are weakly attached to it, but they can be removed by water flushing or brushing. Further details regarding the data have been added to the Result and Discussion section (**Page 8**), Experimental Method (**Page 21**) and Supporting Information (**Page 11**).

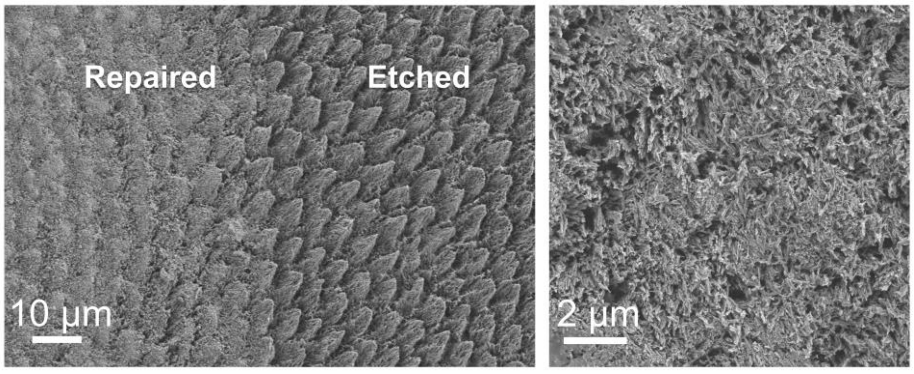


Fig. R11. SEM images of enamel repaired by GCPC at 30 min after ultrasonic treatment. The right picture is a high magnification image of the repaired area on the left picture. **This graph is displayed as Supplementary Fig. 16 in Supporting Information.**

8. The formation of nanowires is attributed to the interaction between GCPC and the specific substrate and is also confirmed with other substrates shown in Figure S9. The question arises as to how the nanowires at the top were formed without contact with the HAP substrate (see Fig.S9 A and B).

We thank the reviewer for the important comment.

As introduced above, we have numerously repeated the observations (more than 10 times of the characterizations) on the evolution of GCPC on the enamel, we find that nanowires are not the only intermediate species towards HAP formation. In contrast, ACP nanoparticles derived from GCPC are involved in the pathway as well.

Again, with further numerous characterizations, we find that the nanowire formation is not dependent on the use of specific substrates, and different substrates can also induce short/long nanowires. This indicates that the interaction between GCPC and the incubation solutions may cause the formation of nanowires. We would like to apologize for inappropriate descriptions before in the manuscript, thus have made the necessary revisions according to the previous and new observations to accurately reflect our findings.

9. The thickness of the repaired layer is ~1 um resulting from the side view, which is less accurate than the cross-section. The authors could consider observing the cross-section of repaired specimens. The thickness could be increased by cyclic treatment. However, the authors performed experiments in which the enamel was remineralized with GCPC for 24h and 48h, rather than for 30 min and 60 min. Since the artificial saliva could remineralize the enamel over a long time, the authors should exclude the role of artificial saliva in long-term repair.

Thank the reviewer for the comments on the side view of repaired specimens.

Firstly, we would like to clarify that the previous "side view" samples are actually a cross-sectioned one (collected by breaking the enamel in perpendicular), and observed at a tilted angle of 60°.

Here, following the reviewer's suggestion, we have supplemented the cross-sectional image (tilted angle of ~90°) of enamel repaired by GCPC at 24 h and 48 h. The results of SEM (**Fig. R12**) show that the repaired layer is dense (fully filling the interstices) and composed of HAP nanorods, and has a thickness of up to 1 μm at 24 h. The thicker repaired layer with a thickness of 3~4 μm is formed by adding one more cycle of GCPC treatment, in line with those presented in the manuscript.

Besides, we would like to explain that soaking enamel samples in artificial saliva for 24 h or repeatedly for 48 h is to observe the repair effect of GCPC at a frequency of at least once a day, which follows our daily oral care routine. Moreover, we did display the remineralization effects of sole artificial saliva in **Supplementary Fig. 20**, which, with very weak performance, excluded the potential long-term remineralization contributed by artificial saliva itself, and confirmed that the observed mineralization is mainly contributed by GCPC.

Further details regarding the data have been added to the Result and Discussion section (**Page 10**) and Supporting Information (**Page 12; Supplementary Fig. 19**).

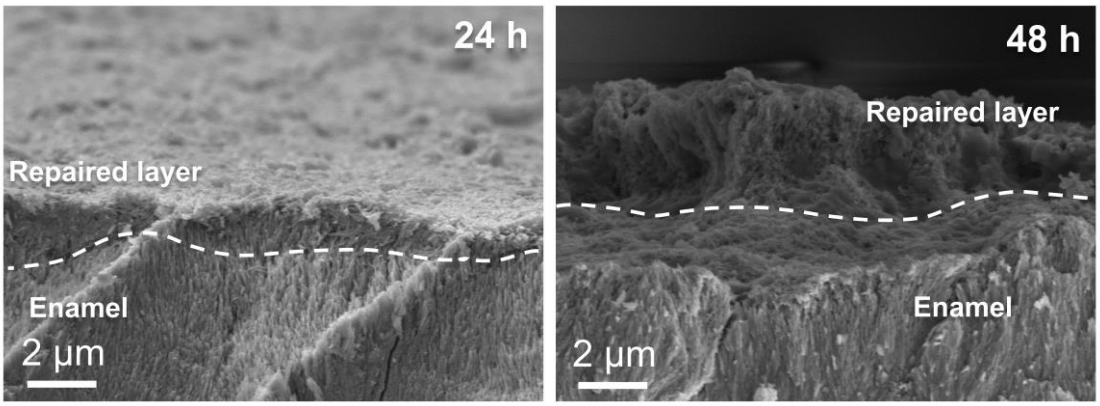


Fig. R12. SEM images of cross-section of etched enamel repaired by GCPC for 24 h and 48 h. **This graph is displayed as Supplementary Fig. 19 in Supporting Information.**

10. The authors describe that "with the incubation time prolongated to 24 h, the repaired layer has no significant changes from that of 30 min" (page 9, lines 26, 27). The authors should specify which properties in the repaired layer do not change because the change in hardness occurs between the repaired enamel for 30 min and 24h. The increase in hardness is usually accompanied by the formation of a denser compact mineral on the enamel surface.

We thank the reviewer for the important suggestion and are sorry for any confusion caused by the lack of explicit description in the previous version of the manuscript.

We would like to explain that the claim "the repaired layer has no significant changes from that of 30 min" in manuscript was mainly based on the morphology change of the GCPC coating layer which also reveals its crystallization process. As the response to **Reviewer #1** above, all of the coated GCPC transformed into short HAP nanorods at 30 min and no further morphology change was observed at 24 h.

Besides, following the suggestion by **Reviewer #1**, the Grazing Incidence X-ray Diffraction (GIXRD) is additionally measured, and the data are displayed in the revised manuscript, which further confirm the complete crystallization of GCPC in 30 min. (see **Fig. R1**). That means the short time of 30 min is sufficient for GCPC to complete the full or majority of its repair process.

We agree that the increased hardness after 24 h may be resulted from the formation of a denser compact repair layer. We presume that the ripening process after GCPC crystallization contributes to the denseness of the repair layer, which can hardly be observed but indeed leads to the improvement of enamel hardness.

Thus, we have revised the corresponding texts as follows to clarify our findings in the revised version (**Page 10**).

"With the incubation time prolongated to 24 h, the morphology of repaired layer (i.e., HAP short rods) has no significant changes from that of 30 min… Moreover, the results of GIXRD (**Supplementary Fig. 15**) show that there is no significant difference in crystallinity between the specimens collected at 30 min and 24 h, supporting that nearly all of the GCPC is crystallized at 30 min."

11. In the animal study using GCPC materials, it is not clear whether the newly formed HAP crystals are simply deposited on the enamel surface or whether they form a whole. This is difficult to confirm from the SEM image.

We understand the concern raised by the reviewer, and agree that it is difficult to confirm integrity between enamel and the formed crystals via SEM. It is reported that ultrasonic treatment could be used to confirm whether new crystals grow or adhere tightly on the enamel surface. Hence, following the strategy in other reports (ACS Nano 2022, 16, 3119; Adv. Healthcare Mater. 2022, 11, 2200872), we subjected the enamels repaired by GCPC at 30 min and 24 h in animal study to ultrasonic treatment (1500 W, 40 KHz, 30 s), then observed the repair effect using SEM. As shown in **Fig. R13**, there is still a dense repair layer on the enamel surface despite treatment by ultrasound, suggesting that newly formed HAP crystals form an integrated structure on the enamel substrates.

Further details regarding the data have been added to the Result and discussion section (**Page 14**) and Supporting Information (**Page 15, Supplementary Fig. 23**).

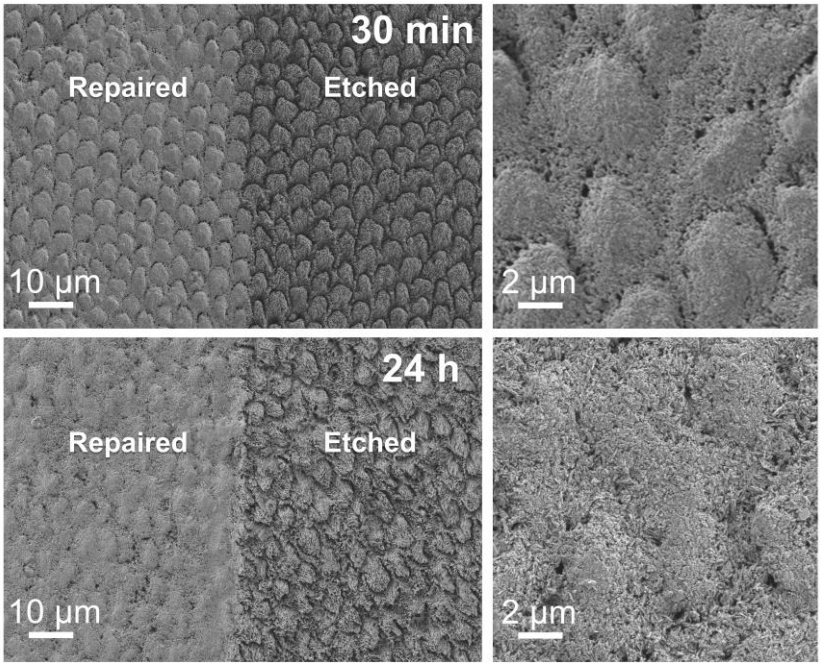


Fig. R13. SEM images of enamel repaired by GCPC in animal study at 30 min and 24 h after ultrasonic treatment. The right one is a high magnification image of the repaired area on the left one. **This graph is displayed as Supplementary Fig. 23.**

12. In the method section, one drop of GCPC solution was used for enamel remineralization. Please clarify how much of this solution was used.

We thank the reviewer for this comment. The volume of the applied GCPC solution for enamel remineralization is 30 μL. In the revised manuscript, the corresponding text in experimental method is updated as follows (**Page 19**):

"a drop of GCPC solution (a volume of 30 μL; water content 16.7 v/v%) was applied onto it using a micro brush…"

13. The HAP nanorod disk was prepared using a synthetic HAP prepared by the authors. The characterization of these nanorods, such as XRD or FTIR, is necessary.

Thank the reviewer's suggestion. As stated above (response to **Reviewer #3, comment 7**), we have removed the result and discussion section of manuscript about the induction effect of substrates (including HAP nanorod disk). But we still characterized the synthetic HAP nanorod by XRD and FTIR.

The crystal composition and structure of the HAP nanorods have been confirmed by FTIR and XRD (**Fig. R14**), which both reveal the characteristic peaks/bands of HAP, indicating that the synthesized product is indeed HAP.

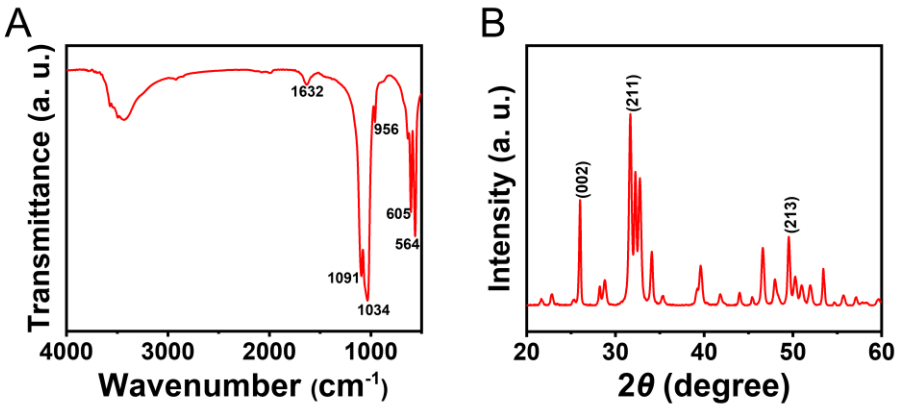


Fig. R14. (A, B) FTIR spectrum (A) and XRD pattern (B) of the synthesized HAP nanorods.

14. To study the mechanical properties of the enamel samples, the nanoindenter could be used to obtain a variety of values, such as hardness and Young's modulus, for a comprehensive assessment of the performance of the materials.

We thank the reviewer for this suggestion.

Herein, we applied the nanoindentation to further comprehensively assess mechanical properties of enamel samples at 30 min and 24 h (**Fig. R15**).

Compared with sound enamel with a hardness of 4.29 ± 0.07 GPa and Young's modulus of 125.18 ± 1.92 GPa, acid etching treatment results in significantly reduced hardness (0.851 ± 0.08 GPa) and Young's modulus (25.80 ± 2.20 GPa). After GCPC treatment for 30 min, the newly formed HAP crystals exhibit noticeably enhanced hardness 2.30 ± 0.38 GPa (Young's modulus:113.11 ± 15.86 GPa), and that of the repaired enamel by 3X-GCPC and GCPC in oscillator (Harsh GCPC) increases to 1.94 ± 0.14 GPa (Young's modulus:112.83 ± 5.79 GPa) and 2.20 ± 0.13 GPa (Young's modulus:117.35 ± 4.51 GPa), respectively. The hardness of the repaired enamel is further increased after incubation for 24 h, reaching the values of 2.79 ± 0.46 GPa (GCPC), 3.59 ± 0.29 GPa (3X GCPC) and 2.99 ± 0.64 GPa (Harsh GCPC), possibly due to ripening process of crystals. However, no significant difference of hardness is found in both 2 wt% NaF (1.20 ± 0.11 GPa) and CPP-ACP paste (1.32 ± 0.19 GPa) groups compared with that of acid etched enamel at either 30 min or 24 h, although the Young' s modulus in these group are improved.

The above results are consistent with the conclusions by Vickers-hardness test in manuscript, i.e., no matter in the static and oscillated environments, GCPC treatment recovers the mechanical properties to the values close to that of natural enamel, and shows better performance than the conventional remineralization materials/commodities (2 wt% NaF and CPP-ACP).

Further details regarding the data have been added to the Result and Discussion section (**Page 12**), Experimental Method (**Page 21**) and Supporting Information (**Page 14, Supplementary Fig. 21**).

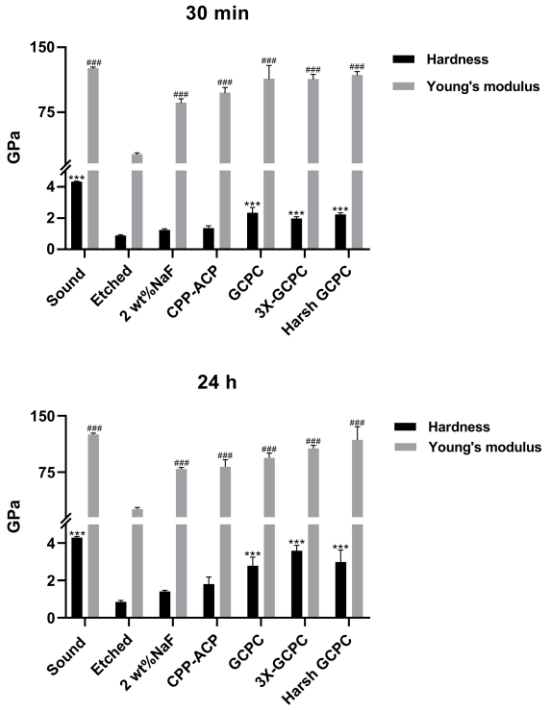


Fig. R15. Hardness and Young's modulus of different enamel samples after repair for 30 min and 24 h determined by nanoindentation. Sound, etched, 2 wt% NaF, CPP-ACP, GCPC, and 3X-GCPC correspond to the samples of sound enamel, etched enamel (blank group), enamels repaired by 2 wt% NaF, CPP-ACP paste, GCPC and 3X-GCPC in static artificial saliva respectively; Harsh GCPC represents the enamel repaired by GCPC under oscillation condition. The asterisk (*) and hashtag ($^{\#}$) denote significant differences of hardness and Young's modulus respectively between the indicated group and the etched group. The error bars represent the mean ± SD for n = 3, * or $^{\#}p < 0.05$, ** or $^{\#\#}p < 0.01$, *** or $^{\#\#\#}p < 0.001$. **This graph is displayed as Supplementary Fig. 21 in Supporting Information.**

15. The style of the reference is not consistent. For example, references 22, 24, and 28 lack the page number; for references 2, 7, 10, 17, 18, 22, 24, 33, 34, 37, 38, and 39 the initial of the article title should be in lower case; and so on.

We apologize for these issues. In the revised version of our manuscript, we have corrected the formatting of our references according to the guidelines of the journal. Additionally, we carefully reviewed all references to ensure their accuracy and completeness.

Response to reviews

We appreciate the important and constructive comments provided by reviewers, based on which this manuscript has been carefully revised. Our responses to the comments, as well as explanations of the changes in our manuscript, are explicated as follows. We hope that the revised manuscript is now acceptable for publication, and look forward to the further evaluation.

Our replies appear indented, and corresponding changes in the manuscript are highlighted in yellow.

Reviewer: After long-term efforts, the authors have addressed some issues and improved the manuscript. However, some key issues have still not been resolved. For example, the mechanism by which the glycerol stabilizes the calcium phosphate cluster remains unclear. Despite pointing this out in my previous comment #2, the authors only presented a speculative conclusion in the revised manuscript. Illustrating this mechanism is a highlight, but the authors ignored it. In addition, the authors presented some crucial results existing contradiction, and cannot be resolved by conducting more experiments (refer to Comments #2 and #4 in this version). Moreover, the structure or morphology of the newly formed minerals on the enamel surface repaired with GCPC is similar to that of repaired with CPP-ACP, indicating that GCPC has no apparent advantage in terms of constructing an ordered structure, as demonstrated by Figure 6. This is difficult to achieve an ordered structure, even with in vitro experiments using GCPC. In summary, all these points make it difficult to capture the novelty of this work and its contribution to the advancement of materials science.

We appreciate the comments and suggestions.

First, we agree that it is crucial to elucidate the mechanism of glycerol stabilizing the calcium phosphate cluster, thus have employed molecular dynamics (MD) simulations and relevant experimental validation to get further insights into the complex interaction between calcium phosphate clusters and glycerol, which is shown in responses of the **comment #1** below. Regarding the comment “some crucial results existing contradiction, and cannot be resolved by conducting more experiments”, we have provided additional explanations based on extra evidence, please also see our responses below **(comments #2 and #4**). We hope our responses have significantly resolved the reviewer’s concerns.

Second, we would like to stress the novelty of this work here. As shown in **Table 1 (Page 15-17)** and corresponding discussions of the manuscript, we have previously highlighted the distinctive features and advancements developed by GCPC in comparison to existing repair materials in recent representative literatures. In order to be more explicit, we would like to further specify such novelty and significance as follows.

(1) Fundamental

Finding and preparation of ultrasmall stable clusters in a biocompatible organic solvent: We developed a stable ultrasmall (1-2 nm) mineral species

(GCPC) in glycerol and glycerol-dominant solvents via a simple mixture process, which is the first time to report calcium phosphate clusters in such biocompatible organic solution. In addition, glycerol has resolved a dilemma: stabilizers are required to capture the intermediate state during synthesis and storage of efficient mineralization materials, but their transformations are unexpectedly inhibited by stabilizers when treating enamel defects. In this work, glycerol stabilizes GCPC with its affinity to the clusters and high viscosity (see further discussion in the response to the following comments), but endows it with the character of water-responsive transformation due to its noticeable solubility in water.

(2) Mineralization performances

Water-triggered response and rapid remineralization: In wet conditions, GCPC forms a compact HAP repair layer on enamel within a short time (30 min), which is much faster than the conventional materials (hours or days) and can avoid the impact of dynamic environment in mouth. The rapid enamel remineralization has been demonstrated in multiple ways, including in vitro experiments, animal studies and **double-blind clinical trials**, while most other studies still remain at the animal experimental stage.

Effective remineralization under harsh condition: The repair of enamel by GCPC does not require drying prior to treatment, a long time treatment/incubation, or a static environment.

(3) Clinical feasibility

Low cost and health-friendly: The preparation process is simple, requiring only the mixing of solutions. The used chemicals are readily available, inexpensive, and all can reach food or pharmaceutical grade.

Positive patient compliance: There's no need for patients to dry the tooth surface, spend additional time for material adhering, or limit too much mouth motion, thus these merits are beneficial for clinical promotion.

For the comments regarding the structural order of the repair layer, we agree that GCPC did not form a very high order of the HAP nanocrystals when achieving the goal of this work: a novel, rapid and clinically-feasible enamel repair. Indeed, this poses a significant challenge due to the conflicting requirements of both objectives, that is, while the former necessitates a mild control over the growth of the HAP minerals, the latter needs a swift transformation of the repair materials.

Having said that, according to our characterization on the GCPC-treated HAP nanorods in artificial saliva with cryo-TEM (mixing HAP nanorods with GCPC solution, then incubating them in artificial saliva, **Fig. R1;** also see the details in the section of **Other Changes** below), the new minerals originated from GCPC transformation prefer to grow on the ends (not the sides) of HAP nanorods. This preference essentially favors the restoration of the demineralized enamel (constructed by aligned HAP nanorods) by GCPC, and more importantly, avoids a total disordered arrangement of the formed HAP crystals during enamel repair as confirmed in our SEM data, although they are not as ordered as those of the original enamels.

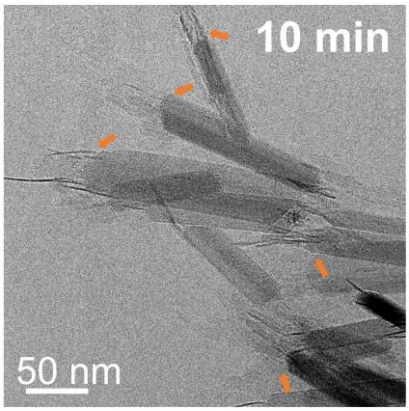


Fig. R1. Cryo-TEM image of the GCPC-treated HAP nanorods at 10 min. Newly formed structures at the ends of HAP nanorods are marked by orange arrows.

Furthermore, we noticed that many studies (Sci. Adv. 2019, 5, eaaw9569; Adv. Mater. 2024, 2311659; Adv. Funct. Mater. 2024, 2306900) have highlighted the presence of trace amounts of fluoride ions in artificial saliva or simulated body fluids, since fluoride has been shown to modulate crystal orderliness by inducing the formation of a preferential needle-like crystal structure (CrystEngComm, 2019, 21, 4684; Caries research, 43, 2, 132). Hence, we tried two ways to improve the order of the formed crystals on enamel with the aid of fluoride. First, we introduced a small quantity of NaF into artificial saliva (15 ppm, F-AS) to replace normal artificial saliva during the in vitro remineralization experiment (i.e., enamels were treated by the materials but then incubated in F-AS instead of normal artificial saliva). As displayed in the SEM images of **Fig. R2**, comparing to the blank group (without treatment by any materials but also incubated in F-AS for 24 h), the GCPC group can form a dense repair layer composed of highly ordered HAP crystals at 30 min and 24 h, while the crystals in CPP-ACP group still show disordered arrangement. Second, we introduced a small quantity of NaF into GCPC (0.5 ppm, F-GCPC) and subsequently conducted an in vitro remineralization experiment (i.e., enamels were treated by F-GCPC then incubated in normal artificial saliva). Similarly, enamel samples treated by F-GCPC exhibit a flat surface with the growth of dense and oriented crystals (shown in cross-sectional SEM images, **Fig. R2**) as well. These findings demonstrate that, at an optimized condition (e.g., adding fluorine), **GCPC is also able to form the highly ordered crystals in the repair layers**.

Further details regarding the data have been added to the Result and discussion section (**Page 13**) and Supporting Information (**Supplementary Fig. 23**).

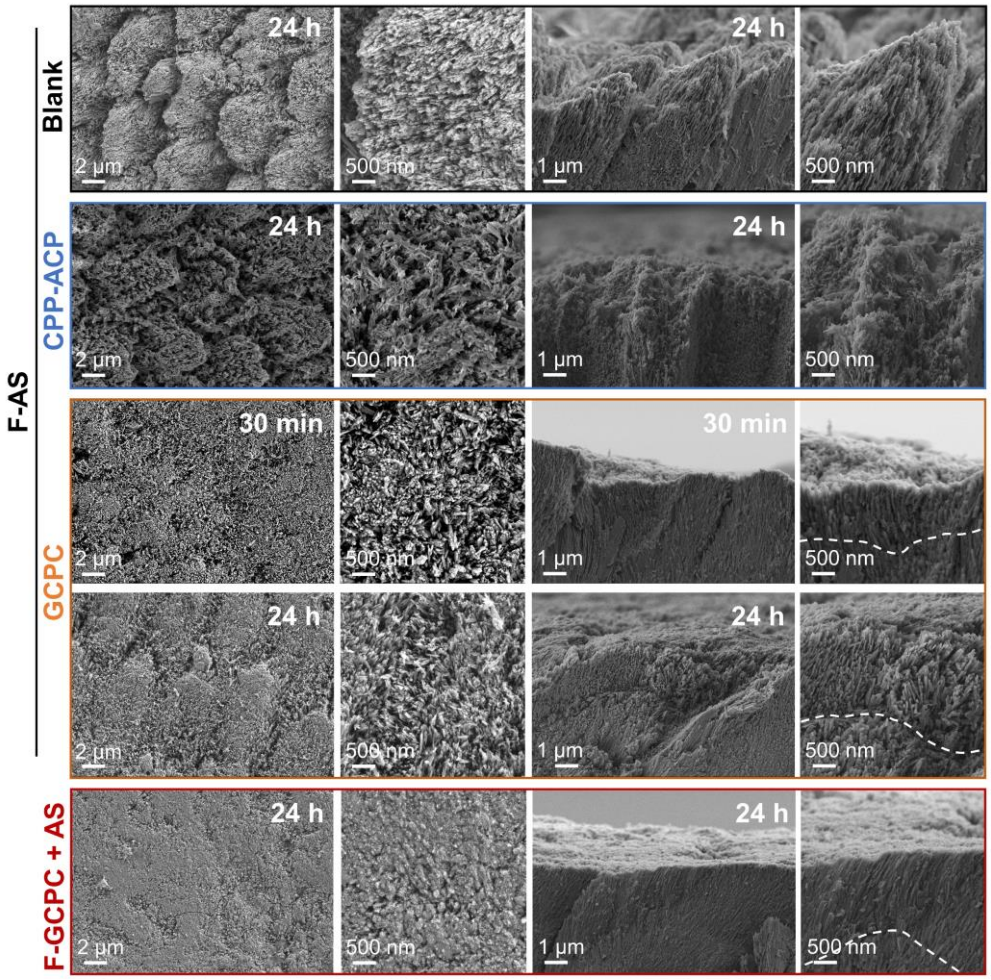


Fig. R2. SEM images from the top surface and cross-sectional view of etched enamel without any treatment (Blank, 1st row) or with a treatment (2nd to 4th rows) as indicated. The incubation of the samples treated with CPP-ACP and GCPC are in fluorine-containing artificial saliva (F-AS), while those with fluorine-containing GCPC (F-GCPC) are in normal artificial saliva (AS) for the durations as indicated on the figure. The dotted line indicates the boundary between the repair layer and the natural enamel.

1. In my previous comment #2, the question is how glycerol stabilizes the calcium phosphate cluster, the molecular mechanism should be proposed. In response to this comment, the authors provide supplementary Figures 1E, 4, and 5 to confirm the critical role of glycerol for calcium phosphate cluster stabilization. I agree with that. However, the authors just rely on these results to give a speculated conclusion that glycerol molecules have a strong affinity to GCPC. Given the starring role of glycerol in this study, what exactly is the mechanism of glycerol stabilization? For example, what is the interaction between the glycerol molecules and the calcium phosphate clusters; could it be that the solvent effect of the glycerol molecules, due to their high viscosity, inhibits the collision of the clusters and prevents the formation of larger particles, thus stabilizing the clusters? (This point has been verified to some extent in this study. Despite the ethylene glycol having two hydroxyl groups, less one than glycerol, its stabilization for calcium phosphate

clusters is far less than that of glycerol, perhaps because of the higher viscosity of glycerol.) The authors should perform some experiments to confirm it. This will contribute to understanding how certain organic molecules stabilize the inorganic ionic cluster.

We appreciate the very important suggestions on the exact mechanism of cluster stabilization contributed by glycerol. We also agree that the high viscosity of glycerol should play a role, which is reasonable since the high viscosity reduces the mobility of the clusters, and further resists the collision/aggregations/fusions between the clusters.

To explore the interplay between the cluster stability and solvent viscosity, following the experiments in the previous manuscript (mixing GCPC with different solvents), we mixed various alcohols (with different viscosity, also varied numbers of molecular hydroxyl groups and molecular chain length) with GCPC in a volume ratio of 10:1 to assess the stability at 30 min and 1 week by observing whether the white precipitates form. The images of **Fig. R3** show that monohydric alcohols (methanol, ethanol and isopropanol), with low viscosity (0.554 ~ 2.038 mPa·s at 25 °C), produce a noticeable white precipitate (indicated by red arrows) within 30 min; dihydric alcohols (ethylene glycol, 1,2-propandiol and 1,3-butanediol), with slightly higher viscosity (16.1 ~ 96 mPa·s at 25 °C) than that of monohydric alcohol, generate white precipitations after 1 week. However, no obvious precipitation occurs in ternary alcohols (glycerol and 1,2,4-butanetriol), with the highest viscosity (954 ~ 1385 mPa·s at 25 °C), after 1 week. The results above corroborate that high viscosity of solvents could indeed improve the stability of clusters. However, we find that, it is very difficult to design an experiment to strictly investigate only the influence of viscosity on the stability of clusters without changing other conditions. For example, the viscosity of those solvents above is positively related with the number of the hydroxy groups on the molecules, thus, we cannot exclude that the solvent molecules may also stabilize the clusters in other ways, e.g. stronger adsorption on the clusters via the hydroxy groups; on the other hand, if comparing the cluster stability in the solvents with the same hydroxy group levels, e.g. methanol, ethanol, propanol, butanol, etc (all are monohydric alcohols), the influence of different hydrophobicity/hydrophilicity cannot be ruled out either.

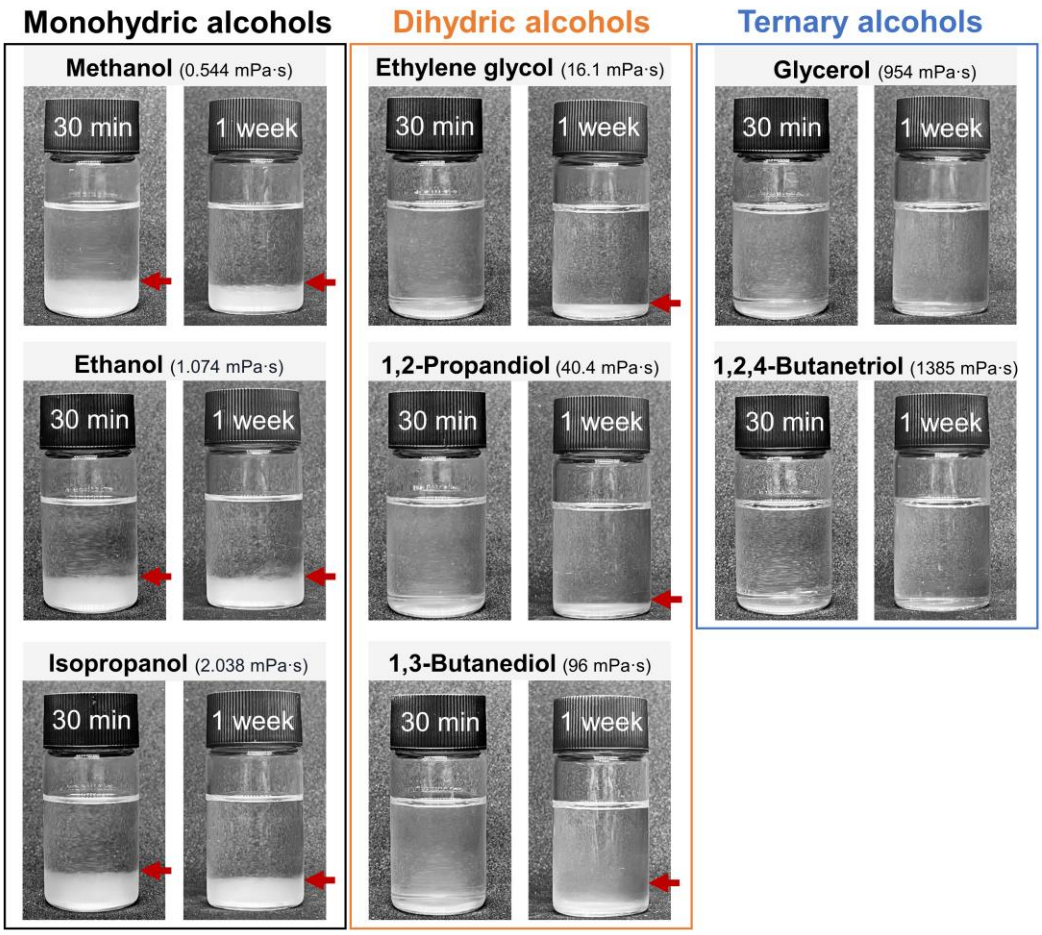


Fig. R3. Photographs of different alcohols with varied viscosity mixed with GCPC in a volume ratio of 10:1 after 30 min and 1 week. Viscosity of each solvent at room temperature (25 °C) is displayed beside its name. The red arrows mark the boundary between the visible precipitate and the transparent liquid.

Therefore, **a molecular dynamic simulation** (**Fig. R4**) was conducted to further analyze role of glycerol on both the formation and stabilization of calcium phosphate clusters. Such simulation is based on the simulated evolution of calcium phosphate cluster in the glycerol-dominated solvent (glycerol + water, water content 16.7 v/v%, same as GCPC preparation) with those in pure water and pure glycerol solvents as the controls. It should be explained that, due to the limited computation time, the clusters displayed here should correspond to the early state of the clusters instead of the mature ones. However, we can still observe the differences of the species and interactions in the different solvent systems, which already reveals the influence of the glycerol. According to the results, glycerol affects the state of cluster in the following ways comparing to water.

Retarding the formation of clusters in the solvents: Fig. R4A shows the spatial conformation of Ca^{2+} and PO_4^{3-} ions in three solution systems at start (0 ns) and end (20 ns) of simulation. At the initial stage of simulation, Ca^{2+} and PO_4^{3-} ions in all solvents are randomly distributed in the solvent. Over time, their

reactions differ in different solvent systems. In water solvent, all Ca^{2+} and PO_4^{3-} ions are coordinated together to form clusters with various numbers of constituent ions (from 2 to 4) after 20 ns. In the glycerol-dominated solvent, most of the ions form the primary clusters (constituted by one Ca^{2+} and one PO_4^{3-}) without further aggregation into larger ones. However, only one primary cluster is obtained in glycerol solvent at the end, and the other ions are still distributed in the solvent box in an uncoordinated state.

Weakening the interaction between Ca^{2+} and PO_4^{3-} in the solvents: The total interaction energy between Ca^{2+} and PO_4^{3-} in these systems were further assessed throughout the simulation process. It can be found in **Fig. R4B** that the interaction energy (absolute value) between Ca^{2+} and PO_4^{3-} ions in water solvent consistently rises within 15 ns, along with the continuous formation and aggregation of calcium phosphate clusters. In the glycerol-dominant solvent, the interaction energy has an increase before 6 ns when clusters forms, followed by gradual stabilization with minor fluctuations. The interaction energy in glycerol solvent does not exhibit significant change before 13 ns, and only an increase occurs between 13-15 ns upon the cluster formation, which then comes to stable again. Besides, comparing the final interaction energy between Ca^{2+} and PO_4^{3-} in these three solvent systems, the highest value is in water solvent (~-21000 kJ/mol), followed by glycerol-dominant solvent (~-1200 kJ/mol), and lowest in glycerol solvent (~-900 kJ/mol). This result indicates that glycerol can significantly reduce the interaction between the Ca^{2+} and PO_4^{3-}, which is also consistent with the retarded formation and aggregation of the clusters demonstrated above. Such reduction could be attributed to the varied interaction between ions and solvent molecules. As shown in **Fig. R4C**, at the beginning of 0-2 ns when the ions are mostly free in the solvents, either Ca^{2+} or PO_4^{3-} has a stronger interaction with glycerol than that with water.

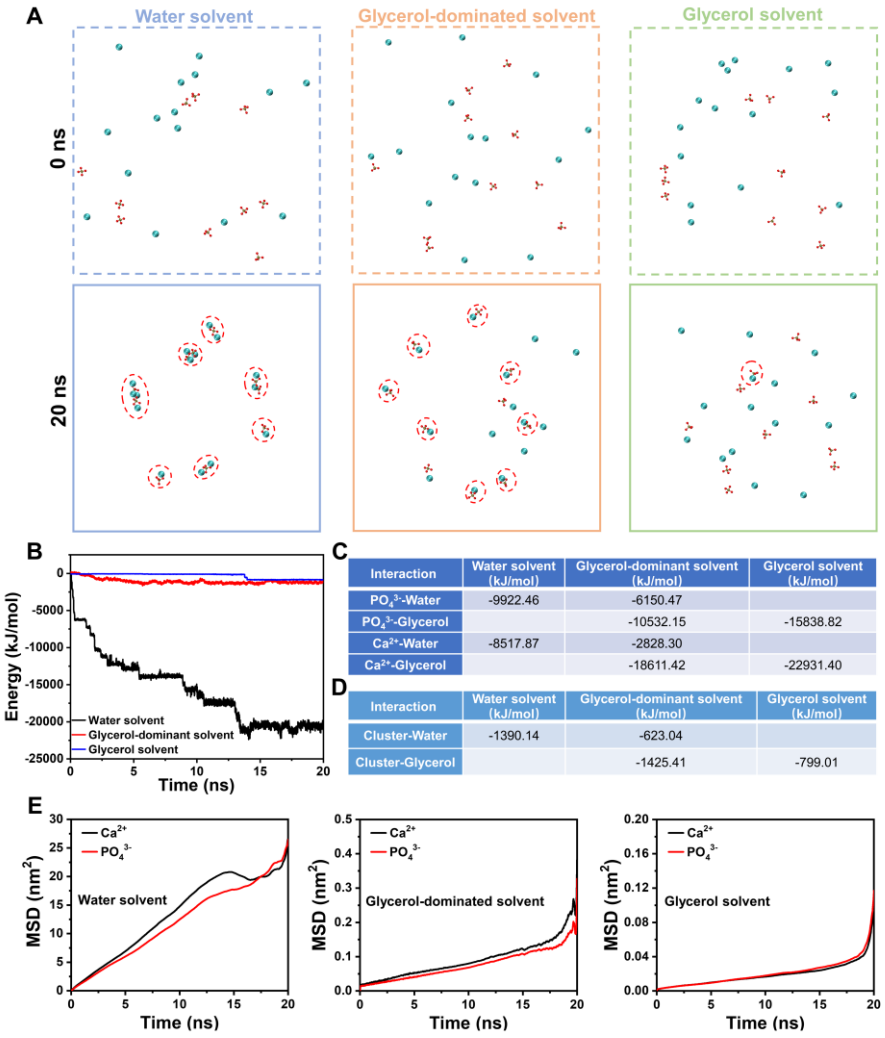
Enhancing the interaction between clusters and solvents: We chose a primary cluster formed before 15 ns in each solvent system with the same constituents (constituted by one Ca^{2+} and one PO_4^{3-}) and without aggregate before 20 ns, then comparing its interactions with different solvent molecules by averaging the energy in the time range of 15-20 s. As shown in **Fig. R4D**, the interaction energy between cluster and water is -1390.14 kJ/mol in water solvent, while that between cluster and glycerol in glycerol solvent is -799.01 kJ/mol, indicating that glycerol has a stronger interaction with the primary clusters than water, which thus have impacted their further aggregation.

Reducing the mobility of the ions in the solvents: The behaviors of mean square displacement (MSD) as a function of time reveal dynamics of the ions in solutions systems and reflect the influence of solvent properties (e.g., **viscosity**) on it. As shown in **Fig. R4E**, Ca^{2+} and PO_4^{3-} ions in water solution have the highest MSD values, followed by those in glycerol-dominant solvents and then pure glycerol. Accordingly, Ca^{2+} and PO_4^{3-} ions have the highest diffusion coefficient 0.205 and 0.192 in water solvent, respectively, and those in glycerol-dominant solvent are 0.0012 and 0.0011. The lowest diffusion coefficient of Ca^{2+} (0.00025)

and PO_4^{3-} (0.00030) are found in glycerol solvent. Hence, glycerol does not favor the movement of ions in it, which in turn reduce their reaction towards the formation, growth and aggregation of clusters.

In brief, the present findings reveal the crucial role of glycerol in forming and stabilizing clusters. Firstly, glycerol exhibits stronger interactions with both Ca^{2+} and PO_4^{3-} compared to water, thereby resisting the formation and growth of clusters. Secondly, the early clusters themselves demonstrate stronger affinity to glycerol than to water, preventing their aggregation. Furthermore, although both water and glycerol possess hydroxyl groups, they differ significantly in viscosity, consequently, the mobility of ions and clusters, which is a prerequisite for the formation, growth, or aggregation of clusters, displays less activity in glycerol compared to that in water.

Further details regarding the data have been added to the Result and discussion section (**Page 7-9, Fig. 2**) and Supporting Information (**Supplementary Fig. 6**).



C

Interaction	Water solvent (kJ/mol)	Glycerol-dominant solvent (kJ/mol)	Glycerol solvent (kJ/mol)
PO_4^{3-}-Water	-9922.46	-6150.47	
PO_4^{3-}-Glycerol		-10532.15	-15838.82
Ca^{2+}-Water	-8517.87	-2828.30	
Ca^{2+}-Glycerol		-18611.42	-22931.40

D

Interaction	Water solvent (kJ/mol)	Glycerol-dominant solvent (kJ/mol)	Glycerol solvent (kJ/mol)
Cluster-Water	-1390.14	-623.04	
Cluster-Glycerol		-1425.41	-799.01

Fig. R4. Molecular dynamic (MD) simulations on the evolution of calcium phosphate cluster starting from dissolved ions in water solvent, glycerol-dominated (glycerol + water, water content 16.7 v/v%) and glycerol solvent. (A)

Snapshots of the spatial distributions of ions and formed clusters (marked with dashed circles) at 0 ns and 20 ns. Solvent molecules are omitted for clarity. Atoms Ca, P and O are shown in blue, brown and red, respectively. (B) Interaction energy between Ca^{2+} and PO_4^{3-} ions in water, glycerol-dominated and glycerol solvent. (C) The interaction energy between Ca^{2+} or PO_4^{3-} with glycerol or water in the solvents at the beginning 0-2 ns. (D) The interaction energy between a primary cluster (constituted by one Ca^{2+} and one PO_4^{3-}) and solvent molecules in the time range of 15-20 s. (E) The mean square displacement (MSD) of Ca^{2+} and PO_4^{3-} in water, glycerol-dominated and glycerol solvents.

2. In my previous comment #3, after storage at room temperature for 2 weeks, the GCPC solution presents white turbidity as shown in Figure S1E in the original paper, and can exclude the interference of the blue background plate. In response, the authors use ACTEM to confirm that the solution contains mainly 1-2 nm clusters along with very few larger particles. Under this state, the solution should be present the light blue, resembling the one shown in Fig. R4. Therefore, there is a contradiction between the result shown in Figure S1E and the ACTEM result. The authors should explain these results, in particular why the white turbidity is presented, to avoid misleading the reader.

We appreciate the comment regarding the optical appearance of GCPC after storage at room temperature for 2 weeks, which reminded us to analyze the liquid cell-ACTEM data again more carefully. We realize that, upon the liquid cell-ACTEM characterization, the clusters of the 2-weeks are not uniformly distributed in the solution. Instead, they are concentrated in some domains but diluted in others as shown in **Fig. R5** (concentrated domains are outlined by dotted lines). This indicates that clusters have begun to aggregate after such long time, although a macroscopic phase separation has not been observed. We think that such inhomogeneous distribution of the clusters in solutions causes the white appearance in **Supplementary Fig. 1E**. We note that there are no distinct interfaces around the cluster-concentrated domains, thus, they should be the weak aggregations (not solid particles) composed of both clusters and very much glycerol molecules. Therefore, if being diluted in glycerol, a significant blue scattering can still be observed under a strong white light source (**Fig. R6**).

Therefore, in the revised manuscript, we have polished the discussion of the ACTEM data of 2-weeks GCPC on **page 6**:

"Liquid cell-ACTEM further reveals that the GCPC, after a storage at room temperature for 2 weeks, are still 1-2 nm sized clusters predominantly along with very fewer bigger particles, although their distributions in the solution become heterogeneous (weak aggregation).

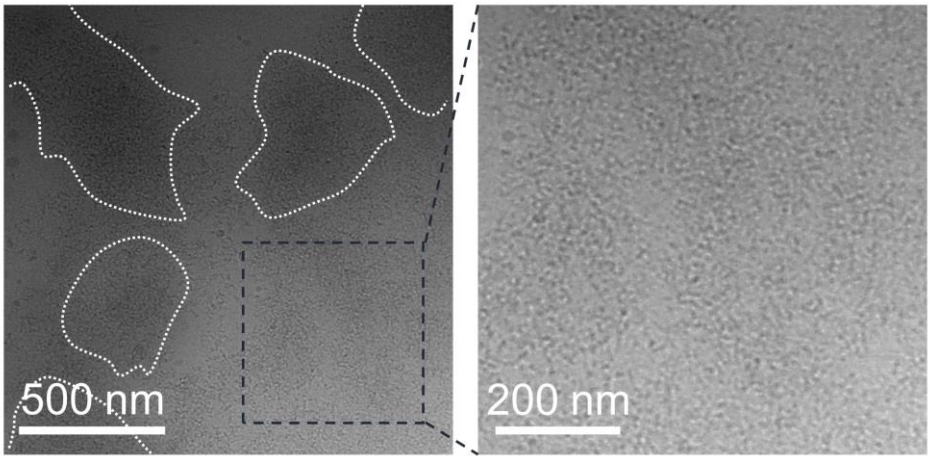


Fig. R5. Liquid cell-ACTEM images of GCPC solution after storage at room temperature for 2 weeks. The white dotted lines outline the cluster-concentrated areas.

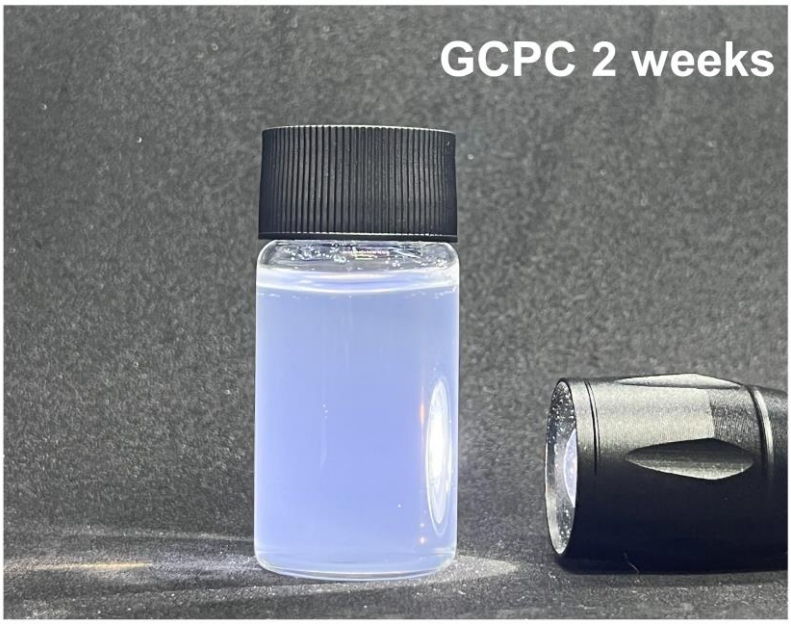


Fig. R6. The digital image of the light scattering of the glycerol-diluted GCPC after storage for 2 weeks.

3. In FTIR (Figure 1E), the authors focus on the analysis of the GCPC-ACP rather than the GCPC which is a core material to use to repair the enamel. Can you please explain this?

We thank the reviewer for this comment.

We would like to explain that the data in **Fig. 1E** is a TGA-FTIR analysis, where the FTIR was to characterize the gas (instead of GCPC-ACP) evaporated from GCPC during the heating of TGA. In this case, we tested the content (up to

23.3 wt%) of glycerol in GCPC-ACP, and further corroborated that glycerol has a strong interaction with inorganic component in GCPC-ACP (also reasonably in GCPC).

We also would like to explain that it is very difficult to directly collect the FTIR spectrum of GCPC. We really tried this many times, but failed as GCPC cannot be separated from the solvents without destroying its structures. Even using ultracentrifugation (20000 rpm), only a gel-like product with large content of solvent can be obtained, and removing the solvents by vacuum or heating can only lead to amorphous or crystalline calcium phosphate particles. Thus, during the measurement of FTIR (or Raman), the signal of the solvents is very strong but that of inorganic part is very weak, leading to a failure to collect the meaningful data (**Fig. R7**).

As an alternative, the solid state ^{31}P NMR spectrum of GCPC was measured and displayed in **Fig. 1B**, which does confirm that a new species (clusters) is formed in the solution.

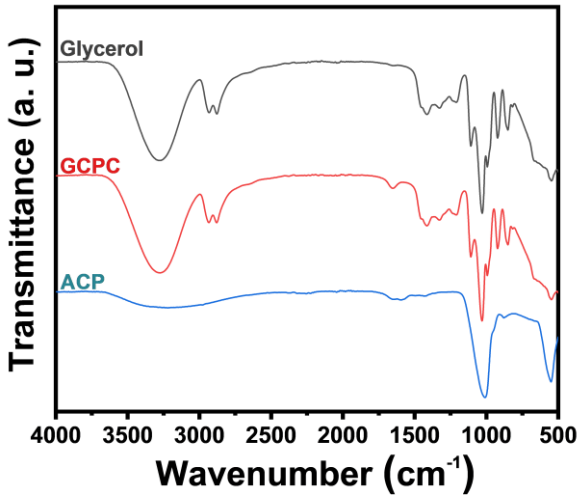


Fig. R7. The FTIR spectra of glycerol, GCPC and normal amorphous calcium phosphate (ACP, prepared by simply mixing the solutions of calcium chloride and sodium phosphate).

4.As stated by the authors, there is no difference between the repaired layer after 30 min and after 24 h (according to the morphology of the repaired layer and GIXRD results). Thus, the authors claimed that all GCPC crystallized after 30 min. Furthermore, based on the SEM result (Figure S20), the authors concluded that the artificial saliva had a very low capacity for enamel remineralization. It is reasonable to conclude that the newly formed minerals in the repaired layer are mainly attributable to GCPC. However, the cross-section of the enamel coated with GCPC shows that the constructed repaired layer is in the range of 400 - 600 nm (Figure 2B1). After 24 h of remineralization, the authors concluded that the thickness of the repaired layer was around 1 um. HOW??? Where do the extra minerals come from?

Even after 48 h of remineralization, the thickness of the repaired layer reached 3 to 4 um, indicating that each time treatment with GCPC formed a layer of at least 1.5 to 2 um at the initial stage. These results also contradict the results from Figure 2. This is

incomprehensible. The authors should explain this carefully.
In addition, the authors should clearly indicate the interface between natural enamel and repaired enamel in Figure 2E1 and Figure S19 (24 h) with a magnified SEM image, because it is hard to distinguish the newly formed mineral from the natural enamel.

We thank the reviewer for raising this issue.

Regarding the repair data in **Fig. 3B2** (Fig. 2B1 in the previous version), as mentioned in the experimental methods, the enamel samples were flushed with ethanol before SEM characterizations. This step ensures a total quenching of reactions and the removal of the glycerol (glycerol cannot be dried and thus does not facilitate the SEM observation) at early time. However, it should also result in a loss of the materials that have not been strongly fixed on the enamel surface but contribute to enamel repair, especially at the early time of enamel remineralization. Therefore, in **Fig. 3B2**, as well as **Fig. 3C2 and 3D2** (Fig. 2B1, 2C1 and 2D1 in the previous version), we can only observe a thin early repair layer.

To corroborate this explanation, we herein gently immersed the GCPC-coated enamel samples in ethanol for quenching the reactions instead of flushing. SEM images (**Fig. R8**) demonstrate that GCPC can form a repair layer of ~1.5 μm at 0-10 min. Hence, it is reasonable that the repair layer with a thick of ~1 μm at 24 h and 3-4 μm at 48 h (treated by GCPC for twice) can be obtained. We would like to note that, the variations in thickness of repair layer within a reasonable range are expected in such vitro experimental condition, which could stem from several factors including slight differences in sample preparation, variations in the treatment process and even enamel surface. Therefore, despite our efforts, minor variations may still occur due to the inherent complexities of the experimental procedures.

Further details regarding the data have been added to the Result and discussion section (**Page 10**) and Supporting Information (**Supplementary Fig. 14**).

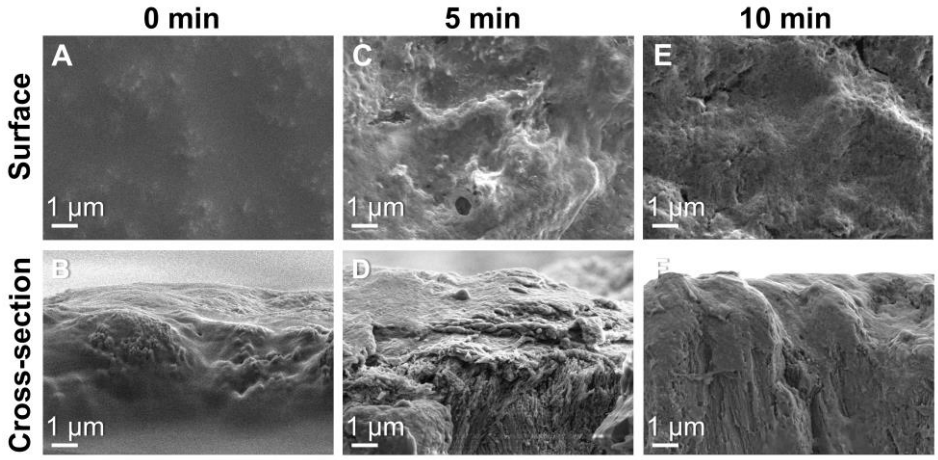


Fig. R8. SEM images of top and cross-sectional views of the acid etched enamel repaired by GCPC at 0, 5, 10 min.

Regarding the interface between repair layer and enamel. Following the suggestion, we have clearly marked the interface between the newly formed crystals and the natural enamel substrates (**Fig. R9**).

Due to the lack of clear high-magnified SEM images of the previous **Supplementary Fig. 19** at the initial capture, we prepared the same samples and conducted SEM characterizations again. The corresponding magnified SEM images are here displayed in **Fig. R10**.

Further details regarding the data have been added to the Result and discussion section (**Page 11-12, Fig. 3; Page 12-13**) and Supporting Information (**Supplementary Fig. 21**).

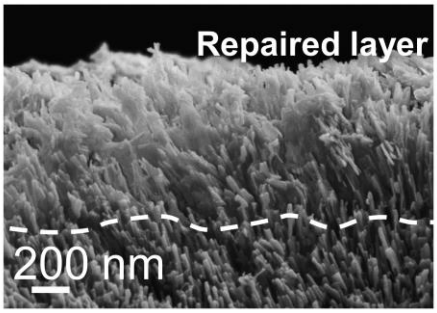


Fig. R9. SEM images from cross-sectional view of etched enamel after treatment by GCPC for 30 min. The dotted line indicates the boundary between the repair layer and the natural enamel.

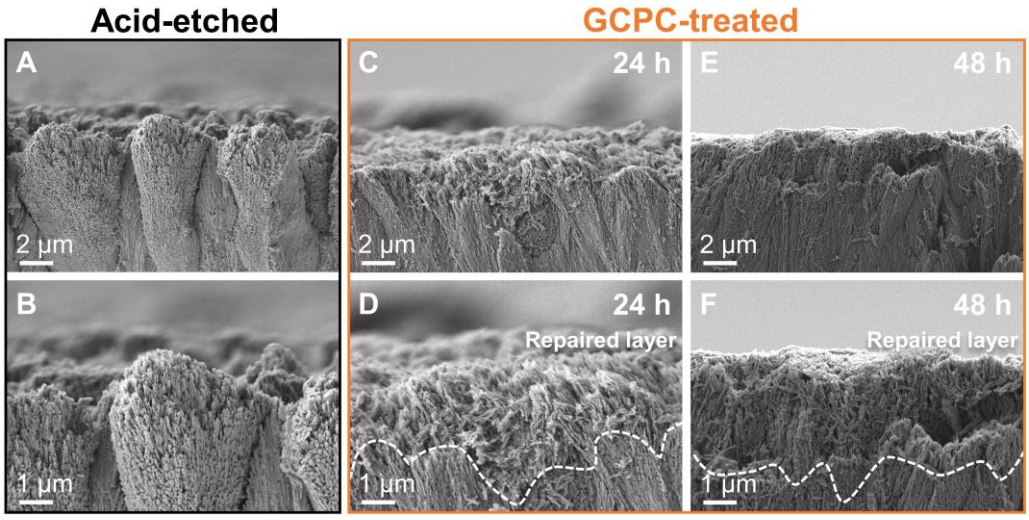


Fig. R10. SEM images of the cross-sectional view of etched enamel repaired by GCPC for 24 h and 48 h. The dotted line indicates the boundary between the

repair layer and the natural enamel.

5. When the authors respond to my previous comment #8, they weaken the crucial role of ACP nanowires in enamel repair in the revised manuscript. In the original paper (version 1), the authors believe that ACP nanowires play a critical role. What are the reasons that lead the authors to believe that ACP nanowires do not play a decisive role? Just because the ACP nanoparticles were detected after more than 10 repetitions or for other reasons. Authors should provide a detailed scientific explanation rather than replacing the original description. And it is also better to explain how the ACP nanowires are formed.
In addition, the characterization of the ACP nanowires was carried out and the results are shown in Supplementary Figures 11, 12, and 13. The authors described the diameter of the ACP nanowires as 5-10 nm, which is inconsistent with the result in Figure 12, which shows that the diameter is at least 40 nm in some parts, and which is consistent with the result shown in Supplementary Figure 13. The authors need to clearly confirm this and explain the cause of the difference between the Supplementary Figures 12 and 13.

We thank the reviewer for this comment, and feel sorry for the confusions regarding the role of nanowires in remineralization. In fact, after carefully analyzing the characterization data (repeated for many times) of the enamel during early remineralization, we found that the ratio of ACP nanowires in the repair layers is much lower than that of nanoparticles according to our observation (e.g., in **Fig. 3C1 and C2**). Thus, we think that, while that the nanowire exists as special intermediate species during GCPC transformation, there is no clear evidence that they mainly contribute to the enamel remineralization. In this version, we have added this explanation in **Page 10**.

Regarding the diameter of the ACP nanowires, we would like to explain that the statement of “5-10 nm” was based on the nanowires in **Fig. 3C1** and **Supplementary Fig. 11** (as we discussed on **page 9** of the manuscript). After a careful analysis, we agree that the statement of “5-10 nm” is not very correct, and we apologize for it. Such mistake was caused by our confusion on whether some nanowires are single or aggregated/fused ones as shown in **Fig. R11** (originated from **Fig. 3C1** in the manuscript) below. Thus, based on further measurements in **Fig. 3C1** and **Supplementary Fig. 11, 12 and 13** without distinguishing the aggregated/fused nanowires, we think “tens of nanometers” should be more appropriate. Such correction is displayed in **Page 9** of the revised manuscript.

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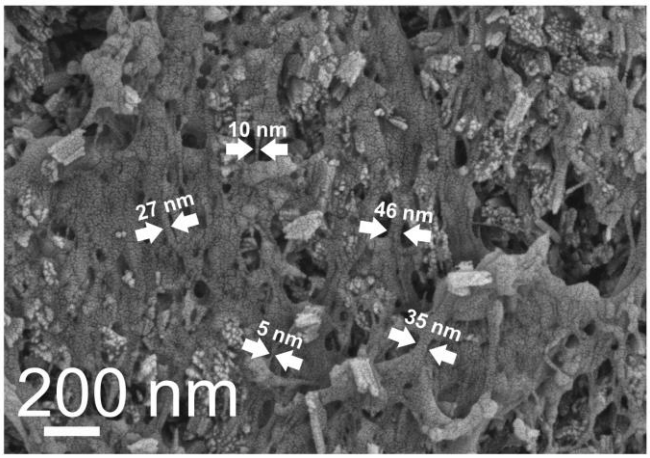

Fig. R11. The SEM images from the top surface view of etched enamel after treatment for 5 min. The white arrows indicate nanowires of different diameters.

For the formation mechanism of the nanowires, we have employed the cryo-TEM (**Fig. R12**) to explore it. Specifically, synthesized HAP nanorods (to mimic the HAP nanorods in enamels; see preparation methods in the experimental section) were mixed with GCPC, then incubated in artificial saliva, and collected at a certain time point for direct observation by cryo-TEM. The cryo-TEM images do find thin nanowires in the solution along with the (aggregated) nanoclusters in the sample collected at 3 min. Their diameters are (indicated by red arrows) ~2 nm, similar to the size of GCPC. Besides, some sections of the nanowires are constituted by clusters (indicated by white arrows), which suggests that they are probably formed by the assembly of the clusters. We note that the diameter of the nanowires in cryo-TEM images is much smaller than that in normal SEM. It is possible that the drying process for SEM observation, which is not needed for cryo-TEM, causes the shrinkage, aggregation and fusion of the thin nanowires.

It is also interesting to further study the driven force of nanowire formation in the future. We hypothesize that the interface between GCPC and saliva at the early incubation time may induce an oriented aggregation of the clusters, thus resulting in the formation of the nanowires.

Further details regarding the data have been added to the Result and Discussion section (**Page 11**) and Supporting Information (**Supplementary Fig. 20**).

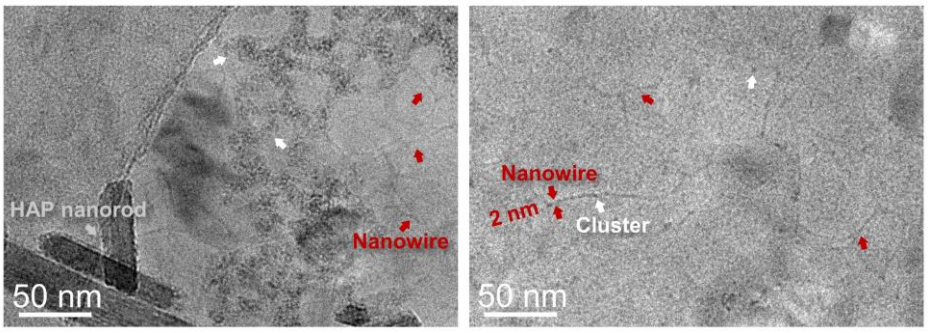

Fig. R12. Cryo-TEM image of nanowires formed in artificial saliva at 3 min. The red arrows indicate the nanowires with the diameter of ~ 2 nm. The white arrows indicate independent clusters, or those in nanowires and synthesized nanorods.

6. In the authors' response to my previous comment #9, they stated that the artificial saliva had a very poor performance in remineralizing enamel, as demonstrated by Supplementary Fig. 20. An equally magnified SEM image as the insets in Figures 4A and D should be included for comparison with that repaired with the GCPC.

We thank the reviewer for this comment.

To address this concern, SEM images (**Fig. R13**) of the etched enamel immersed in artificial saliva for 24 h at the different magnification are now provided to compare with that of GCPC. Consistent with the previous conclusion, artificial saliva exhibits a weak remineralization capability on enamel blocks.

Further details regarding the data have been added to the Result and discussion section (**Page 13**) and Supporting Information (**Supplementary Fig. 22**).

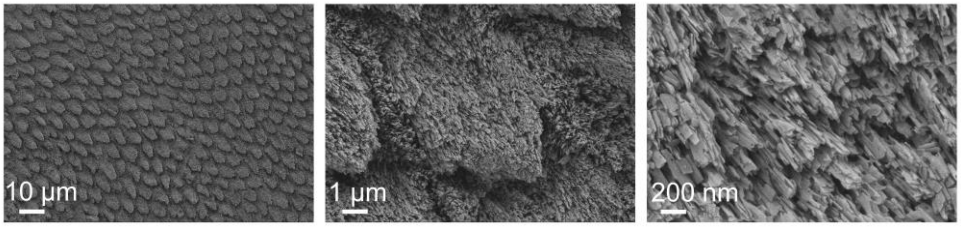

Fig. R13. SEM images of the etched enamel after being incubated in artificial saliva (without treatment by repair materials) for 24 h.

7. The method of preparing glycerol containing calcium and phosphate ions should be provided in the Experimental section.

We would like to express our apologies for missing the detailed methods

about glycerol containing calcium and phosphate ions. Specifically, 1.00 mmol of Na_3PO_4 and 1.50 mmol of $CaCl_2$ were added separately in 20 mL and 10 mL of glycerol, then undergoing vigorous stirring at 60 ºC until complete dissolution.

We have revised the Experimental section (**Page 22**) to include a description of the methods employed.

Other Changes:

1. HAP nanorods, with the similar morphology and structure to the HAP crystals in natural enamel, can be used as a simplified in vitro model to study enamel remineralization. Herein, in order to further understand the growth mechanism of HAP during enamel repair, we mixed HAP nanorods with GCPC solution, then incubated them in artificial saliva, and collected at a certain time point for observation. **Fig. R14A and B** show that, comparing to the original HAP nanorods, the ones treated by GCPC adhere clusters on the top and side (indicated by white arrows) after incubation in artificial saliva at 3 min. Furthermore, the nanowires (indicated by red arrows) with the diameter of ~ 2 nm, similar to the size of GCPC, are also found in the solution. At 10 min, the HAP nanorods grow longer with newly formed minerals at the ends instead of the sides (**Fig. R14C**), which is kept at 30 min (**Fig. R14D).** Therefore, GCPC does have an affinity to the HAP nanorods in the artificial saliva, and the new minerals originated from GCPC prefer to grow from the ends (not the sides) of the nanorods. This preference essentially favors the restoration of the demineralized enamel by GCPC, and more importantly, avoids a total disordered arrangement of the formed HAP crystals during enamel repair.

   The results above have been added on **Fig. 3** and displayed in **page 11-12** of the revised manuscript.

2. Grazing incidence X-ray diffraction spectra of enamel surface repaired by GCPC for different durations has been moved from Supporting Information (previous Supplementary Fig. 16) to the of the main text of the manuscript (Fig. 3F).

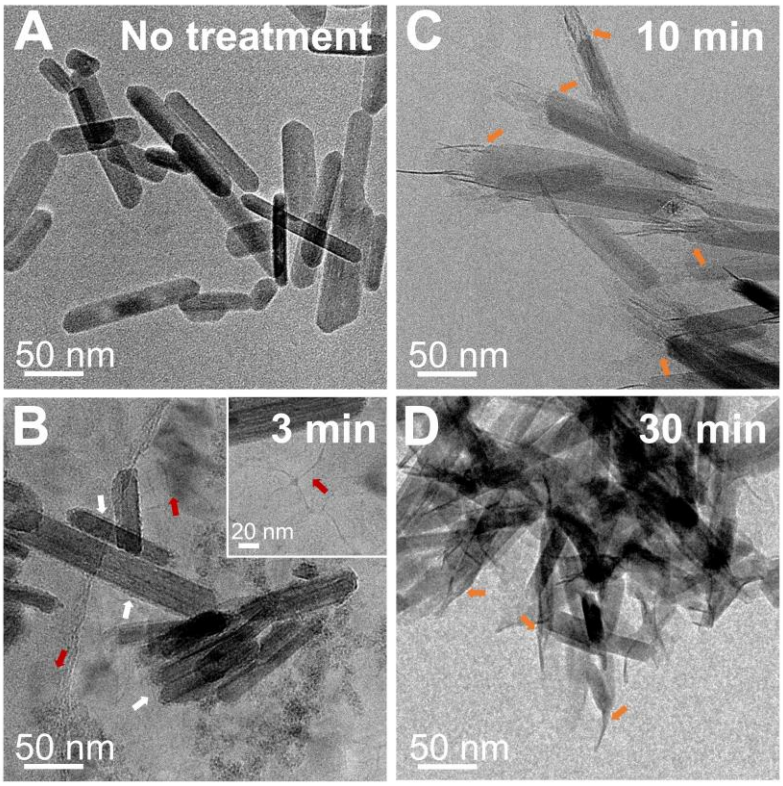

Fig. R14. The evolution of GCPC-treated HAP nanorods in artificial saliva. (A) TEM image of the HAP nanorods without treatment. (B, C) Cryo-TEM images of the GCPC-treated HAP nanorods at 3 min (B) and 10 min (C). Clusters are indicated by white arrows and the newly formed nanowires are marked by red arrows in (B). (D) TEM images of the GCPC-treated HAP nanorods at 30 min. Newly formed structures at the ends of HAP nanorods are marked by orange arrows in (C, D).

Response to reviews

We appreciate the important and constructive comments provided by reviewers, based on which this manuscript has been carefully revised. Our responses to the comments, as well as explanations of the changes in our manuscript, are explicated as follows. We hope that the revised manuscript is now acceptable for publication, and look forward to the further evaluation.

Our replies appear indented, and corresponding changes in the manuscript are highlighted in yellow.

Reviewer: In this revised manuscript, the authors have addressed almost all the issues I raised. However, there is one very important issue that is not well addressed. The issue is the confirmation of the thickness of the repaired layer, including the initial GCPC deposited onto the enamel surface and the final newly formed mineral layer. Although the author has shown the SEM results (Figs. R2, R8, R9 and R10), I don't think that the dotted line is the boundary between the natural enamel and the repaired layer. The SEM results presented here show that the newly formed mineral has a structure that is similar to that of natural enamel (Figs. R2, R9 and R10). Therefore, if this is the case, the SEM image is inaccurate for confirming the boundary. Here, the authors should provide the basis behind their determinations of the boundary the repaired layer and natural enamel. Additionally, employing alternative technologies (such as AFM) to validate the thickness measurements and further confirms SEM findings would enhance the robustness of the study. In Fig. R8, the enamel is repaired by GCPC at an early stage. From the cross-sectional SEM images provided by the authors, I cannot even distinguish the repaired layer from the natural enamel, although they are significantly different in structure. The authors should provide an SEM image to clearly show the boundary, especially for 0 min.

We appreciate your comments and suggestions. Regarding the boundary between the natural enamel and the repaired layer, we would like to clarify that it was identified through the observed changes in the continuity and alignment of HAP nanorods along the enamel cross-section in the SEM images (a shift in continuity, alignment or orderliness indicates the interface between the natural enamel and the repaired region), a method that has been commonly adopted in the literature.

Having said that, we acknowledge that the repair thickness recognized by the aforementioned method requires additional validation. Hence, we further used a 3D laser scanning microscope (VK-X3000, Keyence, Japan), which can provide 3D images with a spatial resolution of 20 nm, to visualize surface morphology of enamels repaired by GCPC. Based on the 3D image, the average repair thickness was determined by comparing the heights between the GCPC-treated and non-treated areas. As shown in **Fig. R1,** the thickness of the repair layer reach 1 μm at 30 min and 24 h, and further increase to 3 μm at 48 h (after repeating the treatment), which do corroborate the SEM findings.

Therefore, in the revised manuscript, we describe the dotted (dashed) lines

marking the interface between the natural enamel and the repaired region as the “speculated boundary” instead of “boundary”; besides, the 3D laser scanning microscope images are added to further validate the thickness of the repair layers.

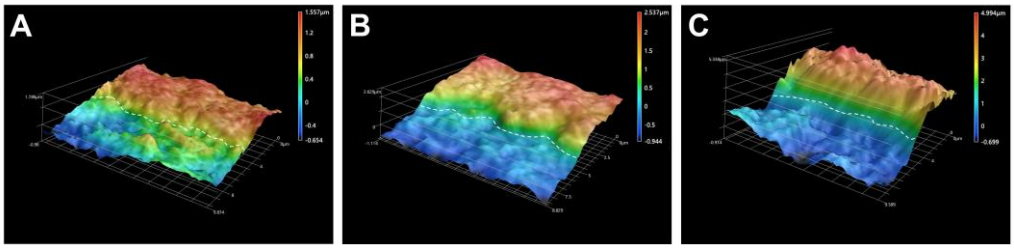

Fig. R1. (A-C) 3D laser scanning microscope images of the etched enamel surfaces after repair for 30 min (A), 24 h (B) and 48 h (C). Each enamel surface consists of the non-treatment area (nail polish coating) showing lower height and the GCPC treatment area exhibiting higher height, which are divided by a dashed line to indicate the boundary between the two areas. The average repair thickness is determined by comparing the heights between the treated and non-treated areas.

Regarding the cross-sectional SEM images of the enamels repaired at early stages, we acknowledge that it is difficult to distinguish the repaired layer from the natural enamel in the previous images, as the GCPC material spread slightly beyond the intended boundary, and covered it during sample preparation due to the high flowability of GCPC before transformation. To address this issue, we prepared the sample again more carefully, and observed the cross section of the repaired enamel one more time (new data are present in **Fig. R2B** and **R2F as below,** and updated in revised **Supplementary Fig. 14)**. As shown in **Fig. R2**, a new mineral layer can now be observe on enamels at 0-10 min.

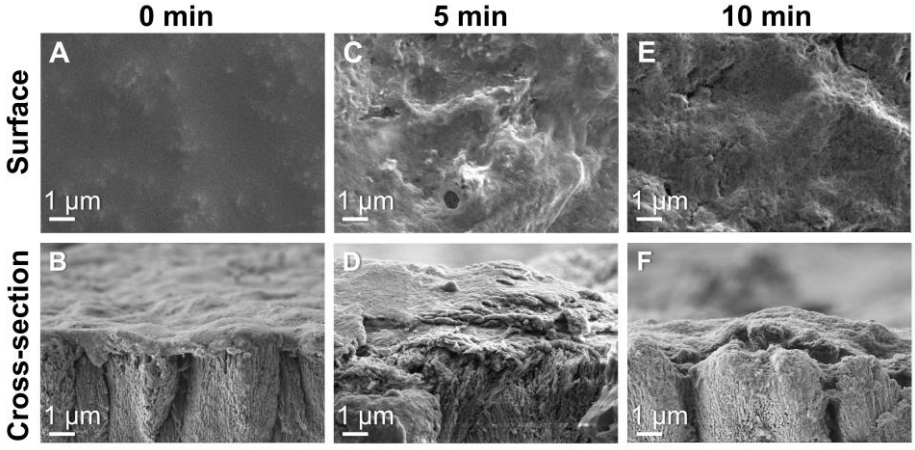

Fig. R2. SEM images of top and cross-sectional views of the acid etched enamel repaired by GCPC at 0, 5, 10 min.

Further details regarding the data have been added to the Result and discussion section (**Page 12-13, Fig. 5**) and Supporting Information

(**Supplementary Fig. 14 and 15**).