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论文题目： Optimization of a Multifunctional Photothermal Hydrogel for Melanoma Post-surgical Therapy

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Optimization of a Multifunctional Photothermal Hydrogel for Melanoma Post-surgical Therapy

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Abstract

Malignant melanoma is associated with a high risk of post-surgical recurrence, partly due to the difficulty of completely removing residual tumor cells through surgery and conventional chemotherapy alone. In this study, a CPG hydrogel system composed of quaternary ammonium chitosan, chitosan, genipin, and polyvinyl alcohol was formulated and optimized as a potential postoperative photothermal therapeutic platform for residual melanoma cell ablation. Concentration ranges for each component were determined based on reported literature and preliminary experiments, taking into consideration both photothermal responsiveness and practical hydrogel properties such as solubility, gel formation, and structural integrity. An L₉ (3⁴) Taguchi orthogonal array was employed to systematically evaluate four formulation factors at three concentration levels while reducing the total number of experimental trials required. Photothermal performance was assessed under repeated 808nm near-infrared irradiation cycles using a 240s heating window. The analysis focused on short-time temperature-response metrics, including control-corrected ΔT_{240s} , mean heating rate, mean $\Delta T_{peak240s}$, cooling half-life, and coefficient of variation. These parameters were used to evaluate photothermal conversion ability, heat retention ability, and cycling stability, respectively. The orthogonal analysis was then used to identify the relative influence of each component on different photothermal traits and to determine the formulation combinations that maximized short-time heating performance, thermal retention, and repeated-cycle stability. This study provides a preliminary formulation-optimization framework for CPG hydrogels and supports their further development as postoperative photothermal materials for localized melanoma treatment.

Keywords: photothermal hydrogel, chitosan, genipin, NIR radiation, neoadjuvant therapy, malignant melanoma

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1. Introduction

Malignant melanoma is an aggressive form of skin cancer arising from melanocytes, the pigment-producing cells of the skin [1]. It is considered the most lethal form of skin cancer because of its capacity for deep tissue invasion and metastatic spread, and it accounts for the majority of skin cancer-related deaths [2]. Globally, melanoma caused approximately 57,000 deaths in 2020 and 58,667 deaths in 2022, indicating that the annual worldwide mortality burden remained close to 57,000–59,000 deaths in recent global estimates [3]. Global melanoma deaths are projected to rise to about 96,000 per year by 2040 if current rates stay the same, mainly because of aging and population growth [3]. The disease commonly develops from excessive UV exposure or repeated stimulation. Surgery is currently the main treatment for melanoma [4]; however, it does involve risks of infection, scarring, and recurrence.

Neoadjuvant therapy refers to a preoperative treatment strategy used to reduce tumor burden and improve surgical outcomes [5]. In melanoma, neoadjuvant therapy has been investigated in clinical studies [6]. Our previously published study investigated a systematic neoadjuvant chemotherapy (NAC) is designed for malignant melanoma, which contains two parts [7]. The first part is ROS-responsive micelles chemotherapy that reduces tumor size before surgery, makes the operation easier, and reduces bleeding during the surgery. The second part is chitosan-based hybrid hydrogel, consisting of chitosan/PVA/genipin-based hydrogel system (CPG), which we applied post-surgery to reduce the recurrence rate in the melanoma cells existing environment. I participated in this study, and contributed to establishing suitable CPG hydrogel preparation conditions by evaluating how acetic acid content and genipin concentration affected solubility, dissolution time and gelation time. I also assisted with the experiments assessing hydrogel viscosity, shape retention, recovery behavior, and mechanical strength, and performed the in vivo hemostasis experiments. This CPG hydrogel has strong adhesion, high injectability, high hemostasis, efficient biodegradation, and notable photothermal properties, and has been proven to be helpful and effective in the post-surgery curing process with in vivo experiments. These properties were characterized in our previous study, published in *ACS Applied Bio Materials* [7]. Building on this work, the present study investigates how variations in CPG hydrogel composition influence its photothermal performance.

A remarkable NIR laser triggered photothermal performance in CPG, which is important for killing residual melanoma cells and could facilitate post-surgical recovery from melanoma. Cells initiate a heat-shock response at 41 °C. Irreversible tissue damage starts at 42 °C; 10

minutes at 42–46 °C induces necrosis, while temperatures above 60 °C kill cells almost instantly [8]. Because hyperthermia can kill potential remaining cancer cells and denature proteins, it effectively prevents recurrence of tumors [9]. We expect a rapid elimination of residual cancer cell by implementing adhesive CPG hydrogel with NIR radiation at the post-surgical tumor site. However, excessive heat created by photothermal response might potentially cause a local inflammatory response [10].

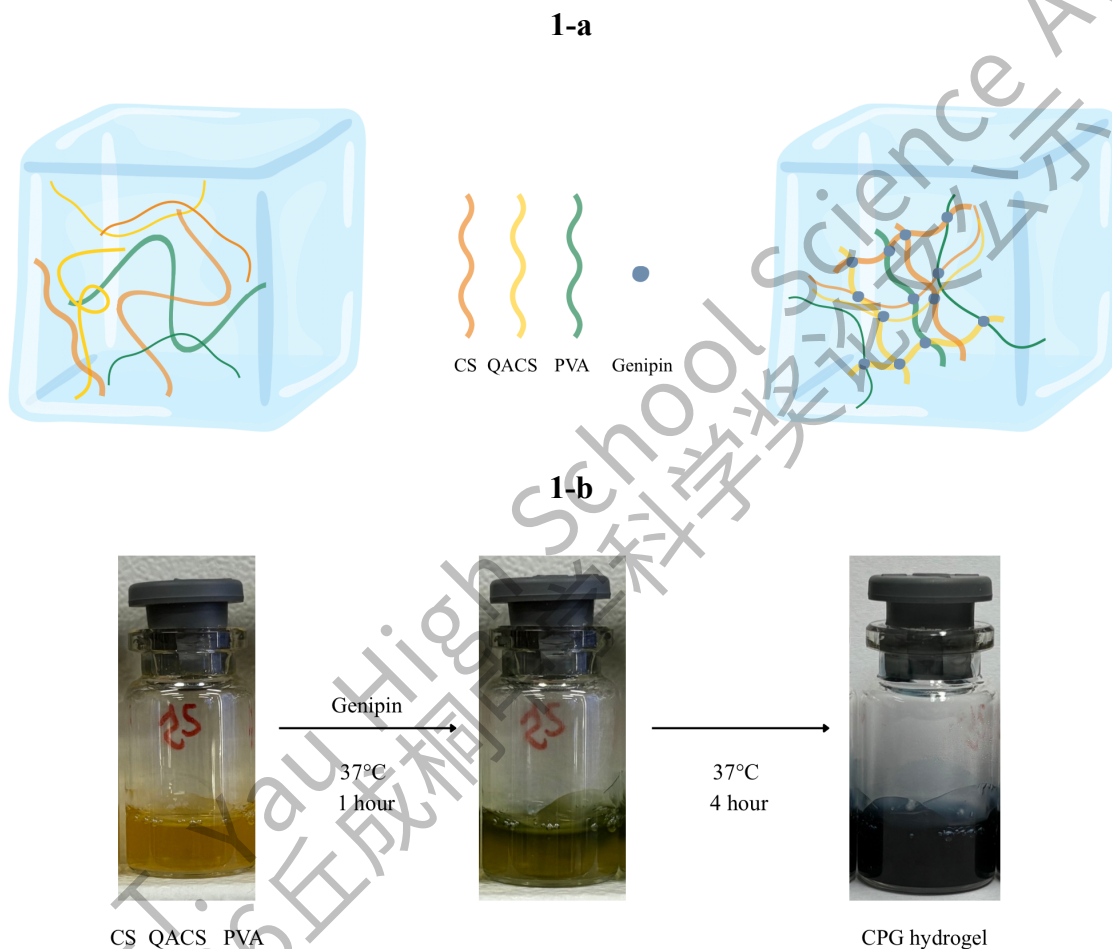


Figure 1. (a): Proposed mechanism of hydrogel crosslinking. (b): Last step of CPG hydrogel formation.

Our previous study demonstrated the photothermal response and retention over several heating-cooling cycles [7]. However, how variations in hydrogel composition influence temperature rise, heat retention, and cycling stability remains insufficiently understood. These characteristics are relevant to the performance during post-surgery photothermal treatment. The photothermal characteristic in the CPG system (Quaternary Ammonium Chitosan/Chitosan/Genipin/PVA) is intriguing because none of the individual components are classic strong photothermal agents. The effect likely arises from the genipin-crosslinked

quaternary ammonium chitosan network, which can form dark-blue pigments with extended conjugation that absorb NIR light. The role of each component would be interpreted within a predefined concentration range of this study (Figure 1). Analyzing the most influential factor in the system is therefore necessary for identifying formulation-dependent effects and providing preliminary insight into the possible mechanism behind the observed photothermal behavior and optimize a final constitution. The primary objective of this study is to optimize the formulations of the CPG hydrogel to improve photothermal performance and to identify the dominant contributing factor among the four constituent components through systematic experimental trials. Each component—quaternary ammonium chitosan, chitosan, genipin, and polyvinyl alcohol—is treated as an independent variable in the experimental design.

2. Experimental section

2.1. Materials

Chitosan (CS) and quaternary ammonium chitosan (QACS) were provided by Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Poly(vinyl alcohol) 1799 (PVA) were purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Genipin (GNP) was obtained from Aladdin Industrial Co., Ltd. (Shanghai, China). Glacial acetic acid provided by Shanghai Yien Chemical Technology Co., Ltd. (Shanghai, China).

2.2. Rationale for Selecting Formulation and Irradiation Parameters

The concentration ranges, laser parameters, and corresponding maximum temperatures for the four experimental factors were collected from existing literature. These empirical data, together with parameters obtained from prior preliminary experiments, were used as references for designing the experimental conditions in this study (Table S1). The preliminary hydrogel experiments showed that higher genipin content increased hydrogel fragility (Figure S2), while higher PVA content reduced solubility (Figure S3). Based on these observations, modifications were made on top of this and the final operating ranges for all variables were selected.

Table 1. Selected operating range of each factor with 3 discrete levels.

Factor	Variable	Level 1 (Low)	Level 2 (Medium/Optimum)	Level 3 (High)
A	CS (w/v%)	3.0%	3.5%	4.0%
B	QACS (w/v%)	0.5%	1.0%	1.5%

Factor	Variable	Level 1 (Low)	Level 2 (Medium/Optimum)	Level 3 (High)
C	Genipin (w/v%)	0.03%	0.07%	0.10%
D	PVA (w/v%)	3.0%	5.0%	7.0%

Within these ranges, three discrete concentration levels were designated for each factor to enable systematic evaluation of their individual and interactive effects. The Taguchi orthogonal array method was then applied to substantially reduces the number of required experimental trials while preserving the ability to evaluate the main effects of each variable. Since this study involved four factors, each at three levels, an L_9 (3^4) orthogonal array was selected. Based on this design, a trial chart was constructed to clearly list the composition of each experimental group.

Table 2. Allocated concentrations according to the appropriate array.

Trial No.	A: CS (%)	B: QACS (%)	C: Genipin (%)	D: PVA (%)
1	3.0	0.5	0.03	3.0
2	3.0	1.0	0.07	5.0
3	3.0	1.5	0.10	7.0
4	3.5	0.5	0.07	7.0
5	3.5	1.0	0.10	3.0
6	3.5	1.5	0.03	5.0
7	4.0	0.5	0.10	5.0
8	4.0	1.0	0.03	7.0
9	4.0	1.5	0.07	3.0

2.3. Preparation of CPG hydrogel

CS and QACS were accurately weighed and transferred into a reaction vessel, followed by the addition of 10 mL of a 1.14% (v/v) acetic acid solution prepared from glacial acetic acid ($\geq 99.9\%$, HPLC grade). The mixture was then placed in a magnetic heating apparatus preheated to 65°C and stirred for 2 hours. After complete dissolution of the solids, a clear pale-yellow solution was obtained (Figure S1). Subsequently, the pre-weighed PVA was introduced into the solution, and the mixture was continuously stirred on a hot plate maintained about 60°C for 48

hours until all polymer granules were completely dissolved, forming a homogeneous precursor solution (Figure 2).

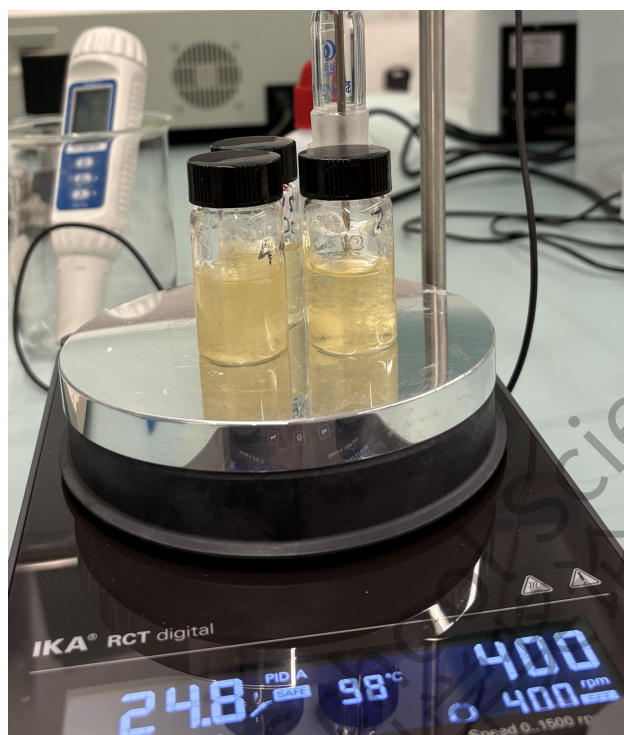


Figure 2. PVA-containing precursor solution on magnetic stirring heating apparatus.

The system was then cooled to ambient temperature, and the pre-weighed genipin was added. The resulting mixture was properly sealed with sealing film and transferred to a 37°C incubator for 48 hours. During incubation, a gradual color change to blue was observed, accompanied by the formation of a gel-like substance, indicating the occurrence of covalent cross-linking (Figure 3). It was also noted that an increase in genipin concentration reduced the time required to complete the cross-linking process (Figure S4).



Figure 3. Completed cross-link final CPG hydrogel.

2.4. Photothermal measurements

To evaluate the short-term photothermal response of each hydrogel, the samples were exposed to repeated near-infrared (NIR) irradiation cycles at 808nm and 2 W. The irradiation apparatus was obtained from Linsen Radiation Co., Ltd. (Hunan, China). Real-time temperature was monitored using PASCO Capstone software connected to a PASCO wireless thermometer, and the probe sensor was inserted into the tested CPG hydrogel. The NIR irradiation source was positioned approximately 5 cm away from the tested hydrogel, while direct irradiation of the probe sensor was avoided (Figure 4). Each sample underwent five heating–cooling cycles. In each cycle, the sample was irradiated for 4 minutes and then allowed to cool naturally for another 4 minutes, which simulates real life application on post-surgical tumor site. A 5.0% PVA control group was included to establish a calibration baseline for subsequent calculation of temperature changes.

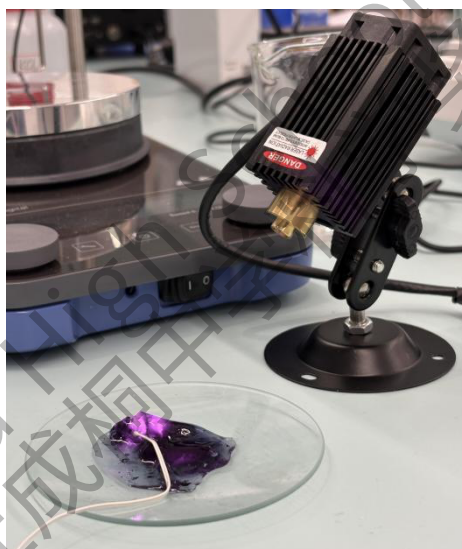


Figure 4. Monitoring real-time temperature change of CPG hydrogel with NIR radiation (808nm, 2W).

2.5. Response metrics and data analysis

Photothermal effectiveness was evaluated based on the capacity of each hydrogel to convert light irradiation into thermal response. In this study, photothermal performance was assessed using short-time temperature-response metrics rather than conventional photothermal conversion efficiency. The completed datasets were analyzed using a 240 s heating window, corresponding to the full laser-on period in each irradiation cycle. For each valid cycle, the cycle starts temperature, maximum temperature within 240 s, raw ΔT_{240s} , mean heating rate, cooling half-life, and coefficient of variation were calculated. Since the 240 s irradiation period did not establish a verified steady-state temperature, the cycle peak temperatures were not used

as substitutes for the equilibrium temperature required by the steady-state energy-balance method. Therefore, control-corrected ΔT_{240s} was used as a comparative indicator of heating response under the specified experimental conditions, rather than as a measure of intrinsic energy-conversion efficiency.

Each time–temperature curve was divided into individual irradiation–cooling cycles (Figure 6). For each cycle, the first 240 s of laser exposure was selected as the main comparison window. The key parameters included peak temperature within 240s, control-corrected ΔT_{240s} , mean heating rate, cooling half-life, and the coefficient of variation of ΔT_{240s} . Control-corrected ΔT_{240s} was defined as the 240 s temperature increase of a hydrogel sample after subtracting the temperature increase produced by the 5.0% PVA control group under the same irradiation conditions. Heat retention ability was evaluated using cooling half-life ($\ln 2/k$), where a longer half-life indicates slower cooling and stronger heat retention. Cycling stability was evaluated using the coefficient of variation of ΔT_{240s} , calculated as the standard deviation divided by the mean ΔT_{240s} across repeated cycles.

3. Result and Analysis

3.1 Main-Effect Analysis

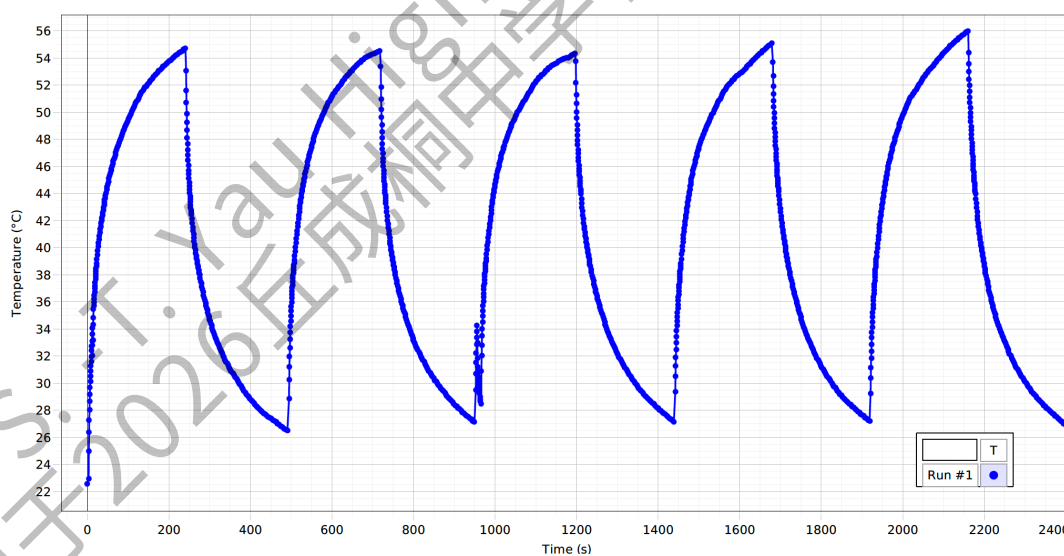


Figure 5. Representative real-time temperature profile of the Trial 7 hydrogel under five repeated NIR irradiation – cooling cycles.

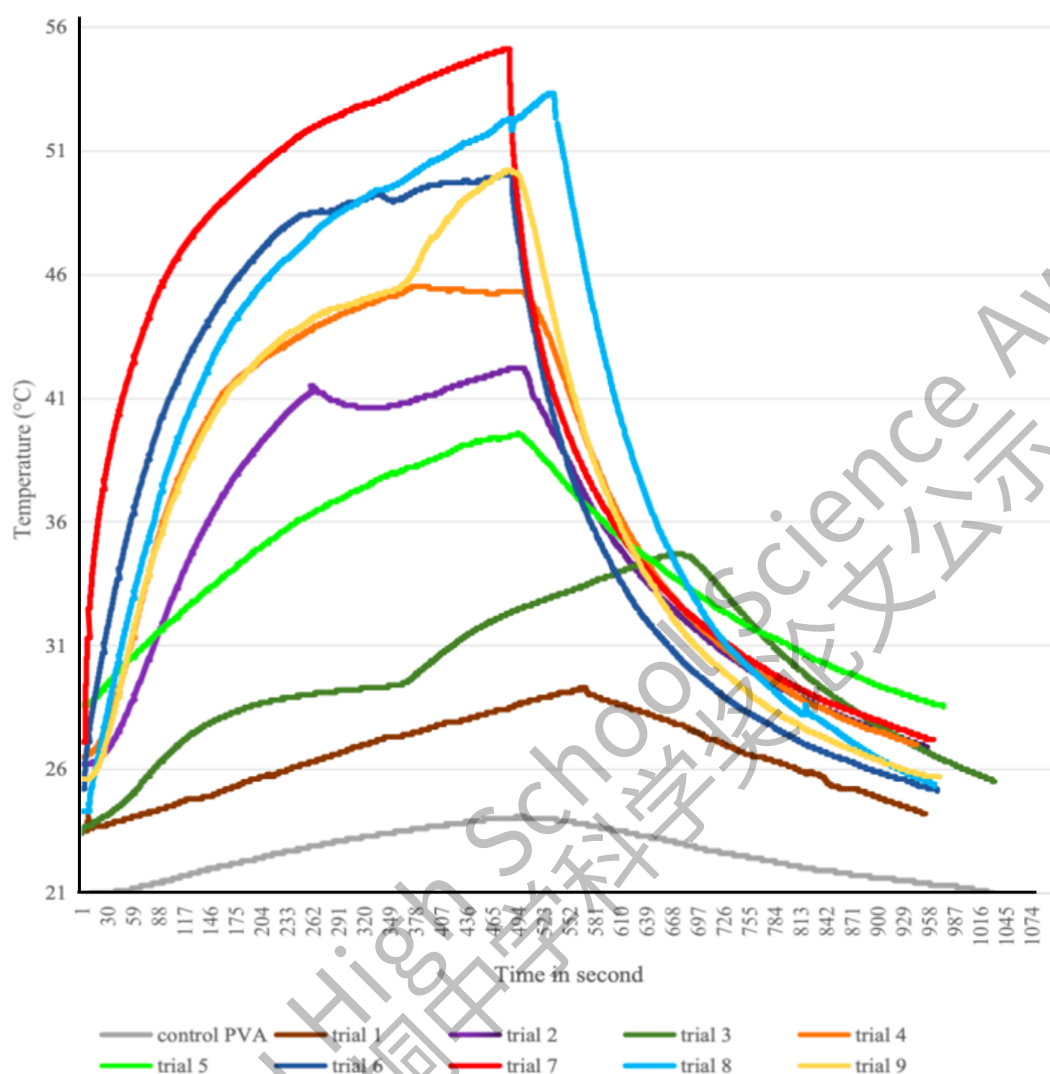


Figure 6. Comparison of temperature changes during the 4th irradiation-cooling cycle across all hydrogel trials. The 4th cycle was selected for comparison because the samples had already undergone three prior cycles, allowing the temperature response to become more stabilized while avoiding early-cycle variation.

Each trial was prepared using the corresponding composition in Table 2 and the CPG hydrogels were tested under NIR for 5 heating and cooling cycles (Figure S5 in supporting information).

Raw ΔT_{240s} was calculated as the difference between the maximum temperature reached during the 240 s period and the temperature at the start of each cycle: $T_{peak,240s} - T_{start}$. The control-corrected temperature rise was calculated by subtracting the mean raw temperature rise of 5.0% PVA control group: $\Delta T_{240s} = \Delta T_{240s, sample} - \Delta T_{240s, PVA control}$. The PVA control showed a mean raw ΔT_{240s} of 2.56°C which was used as the calibration baseline (Figure S6 in supporting

information). This corrected temperature rise value was used to compare the photothermal conversion ability because it reflects the net heating response of different hydrogel formulation under the same irradiation conditions. A comparison of ΔT_{240s} across each group is shown in Figure 7.

Mean heating rate was calculated to evaluate the average rate of temperature increase during the 240 s irradiation period.

$$\text{Mean heating rate} = \frac{1}{n} \sum \frac{\Delta T_{240s}}{4\text{min}}$$

For each cycle, the temperature increase ΔT_{240s} was divided by 4 min, and the mean value across all valid cycles was then calculated. A higher mean heating rate indicates a faster photothermal response under NIR irradiation (Figure 7).

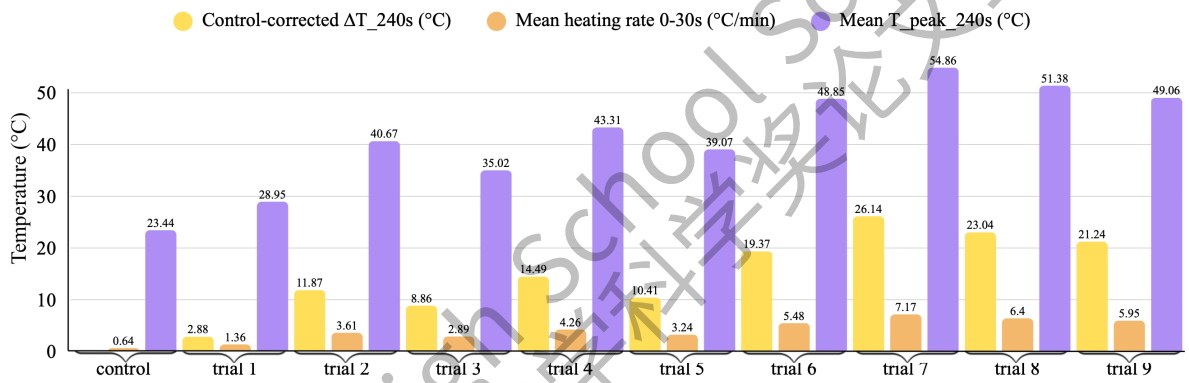


Figure 7. Chart of calculated control-corrected ΔT_{240s} , mean heating rate, and mean $\Delta T_{\text{peak},240s}$ for CPG samples. Control-corrected ΔT_{240s} is defined as the 240-second temperature increase of a hydrogel sample after subtracting the temperature increase caused by the PVA control under the same irradiation condition. Heating rate 0–30 s refers to the temperature increase rate during the first 30 seconds after laser irradiation begins. It reflects the initial photothermal response speed of the material. Mean $\Delta T_{\text{peak},240s}$ is the average maximum temperature reached by the hydrogel across repeated irradiation cycles within the 240-second laser exposure window.

Cooling half-life was used to evaluate the heat retention ability of each hydrogel formulation after laser irradiation was stopped. The cooling phase was fitted using the Newton cooling model, where $\ln(T(t)-T_{\text{baseline}})$ was plotted against cooling time to obtain the cooling constant k .

$$\ln(T(t)-T_{\text{baseline}})=\ln(\theta_0)-kt$$

The cooling half-life was then calculated as $t_{1/2}=\ln 2/k$, with a longer half-life indicating slower cooling and stronger heat retention. A comparison of cooling half-life is shown in Figure 8.

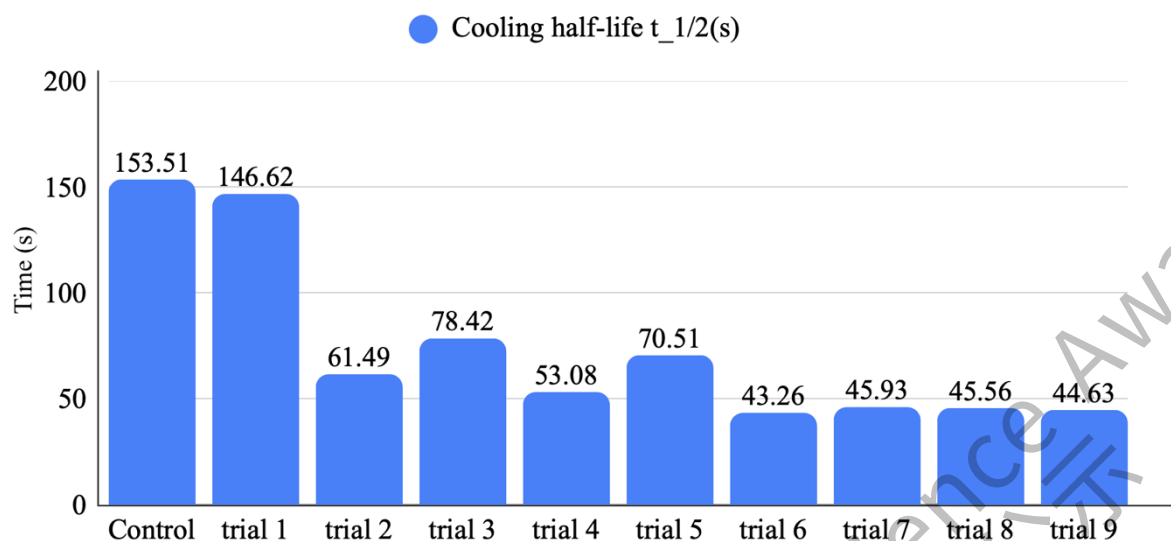


Figure 8. Chart of calculated cooling half-life of the sample CPG hydrogels, calculated from Newton cooling fits as $t_{1/2} = \ln 2/k$, where a longer half-life indicates slower cooling and stronger heat retention ability.

The coefficient of variation (CV%) was used to evaluate the cycle-to-cycle stability of each hydrogel formulation. CV% was calculated by dividing the standard deviation of ΔT_{240s} across all valid cycles by the mean ΔT_{240s} , followed by multiplication by 100%. A lower CV% indicates smaller variation between repeated irradiation cycles and therefore better photothermal cycling stability.

$$CV(\%) = \frac{SD(\Delta T_{240s})}{Mean(\Delta T_{240s})} \times 100\%$$

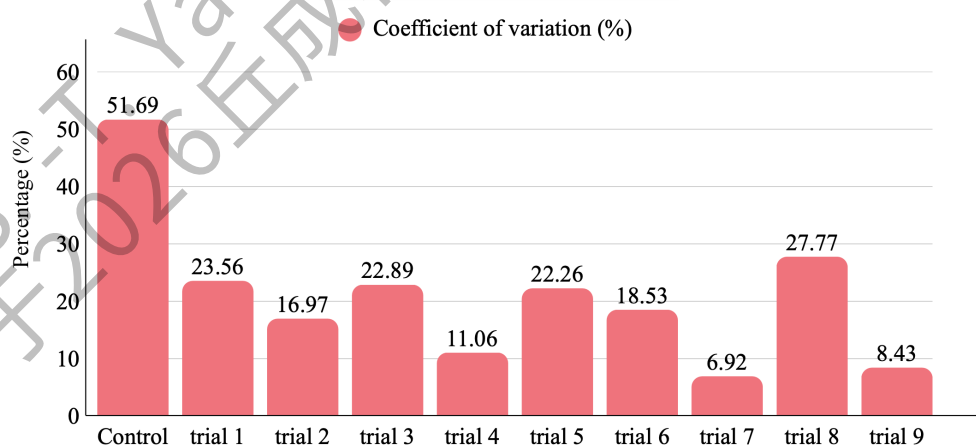


Figure 9. Calculated percentage of coefficient of variation (CV) for CPG samples. Calculated as the standard deviation dividing mean ΔT . Measuring the relative variation of ΔT across multiple cycles within the same group. A smaller value shows a better cycling stability.

3.2 Prediction of Optimal Formulations

The L₉ orthogonal design was analyzed using factor-level means and range analysis. The four factors were CS (A), QACS (B), genipin (C), and PVA (D), each tested at three concentration levels. (Table 1) Each level appeared in three trials.(Table 3)

Table 3. Trials used to calculate each factor-level mean

Factor	Trials at Level 1	Trials at Level 2	Trials at Level 3
A	1, 2, 3	4, 5, 6	7, 8, 9
B	1, 4, 7	2, 5, 8	3, 6, 9
C	1, 6, 8	2, 4, 9	3, 5, 7
D	1, 5, 9	2, 6, 7	3, 4, 8

For each response variable, the mean response at a given factor level was calculated as:

$$y_{F,j} = \frac{1}{3} \sum y_i$$

For example, the mean control-corrected temperature rise (ΔT_{240}) at CS level A1 was calculated from Trials 1, 2, and 3: $y_{A1} = \frac{2.88+11.87+8.86}{3} = 7.87^\circ\text{C}$

Therefore, the predicted optimal combination for highest temperature rise (ΔT_{240}) is A3B3C2D2, corresponding to 4.0% CS, 1.5% QACS, 0.07% genipin, and 5.0% PVA (Table S2 in supporting information).

For cooling half-life, higher means are preferred for maximizing heat retention. The combination predicted to maximize cooling half-life was therefore A1B1C1D1, corresponding to 3.0% CS, 0.5% QACS, 0.03% genipin, and 3.0% PVA (Table S3). This combination corresponded to Trial 1, which also exhibited the longest observed cooling half-life among the nine formulations, at 146.62 s.

For cycling stability, the lowest mean CV values are preferred. The combination predicted to minimize CV was thus A3B1C2D2, corresponding to 4.0% CS, 0.5% QACS, 0.07% genipin, and 5.0% PVA.

Among the nine tested formulations, Trial 7 exhibited the highest control-corrected temperature rise over 240 s (26.14 °C) and the lowest cycling CV (6.92%), whereas Trial 1 showed the longest cooling half-life (146.62 s). Within the investigated concentration ranges, CS exhibited the largest main-effect range for temperature rise, CS and PVA showed the largest ranges for cooling half-life, and genipin showed the largest range for CV. However, a large main effect does not necessarily indicate that the corresponding component is the primary heat-

generating molecule, as the observed temperature response reflects the combined effects of formulation composition and material properties. Similarly, the formulation with the highest temperature rise cannot be directly considered the optimal formulation for postoperative treatment, since therapeutic performance depends on multiple factors beyond photothermal heating. Main-effect analysis identified A3B3C2D2, A1B1C1D1, and A3B1C2D2 as candidate combinations for maximizing temperature rise, maximizing cooling half-life, and minimizing CV, respectively.

3.3 Result Analysis of the Validation Experiment of Predicted Optimal Formulations

The three predicted optimized formulations were synthesized and tested under repeated 240 s NIR irradiation followed by 240 s natural cooling as described in Experimental Section 2.4, results shown in Figure 10.

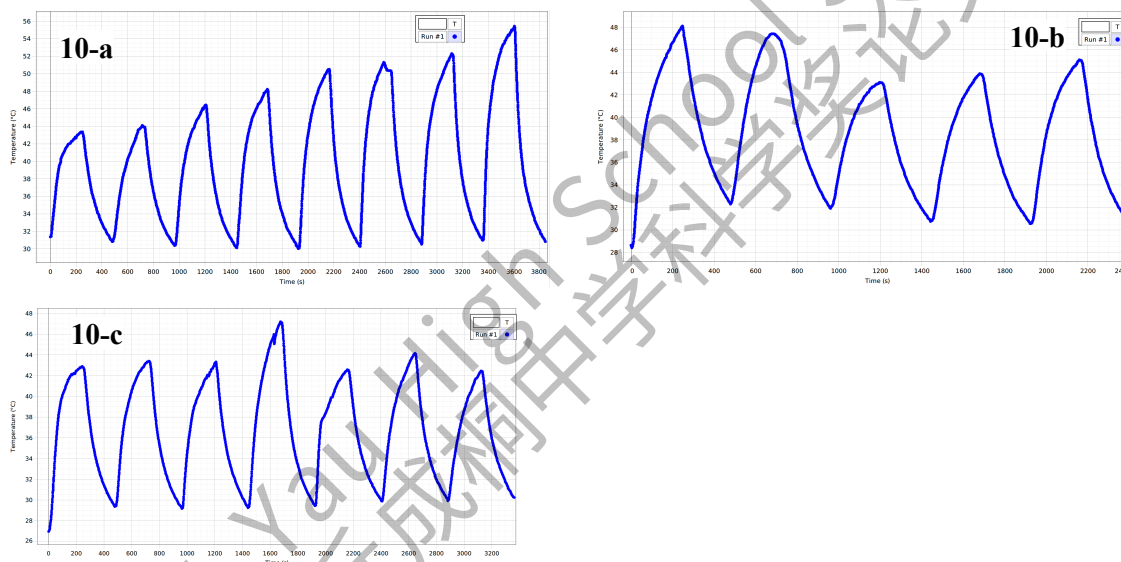


Figure 10. Real-time temperature profile of each trial of hydrogel under repeated NIR irradiation–cooling cycles. (a) Combined optimum: the combination predicted optimal combination for highest temperature rise (ΔT_{240}) is A3B3C2D2 (b) Retention optimum: the combination predicted to maximize cooling half-life was therefore A1B1C1D1 (c) Cycling optimum: the combination predicted to minimize CV% was thus A3B1C2D2.

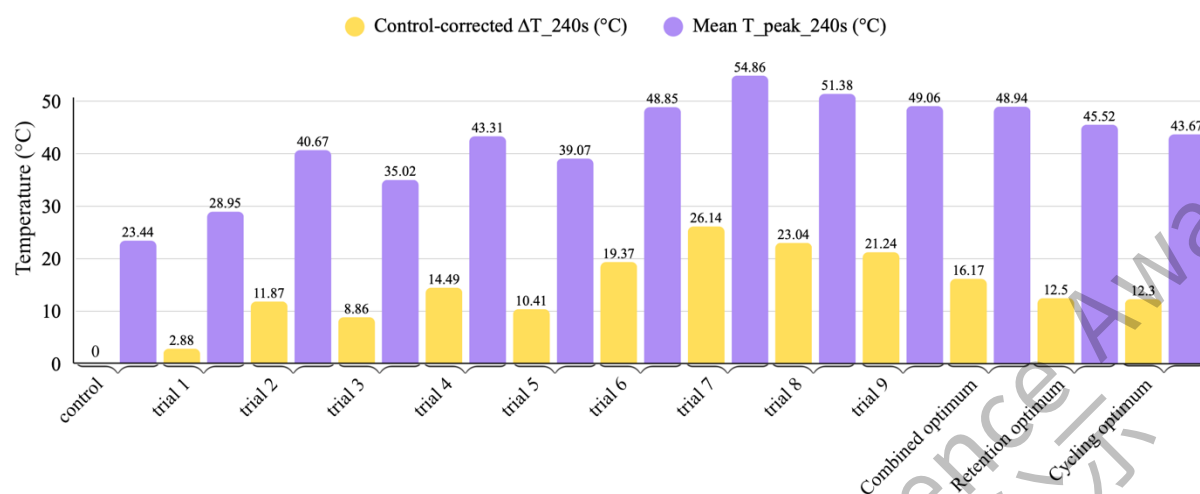


Figure 11. Comparison of control-corrected ΔT_{240s} , and mean T_{peak} for validation experiments.

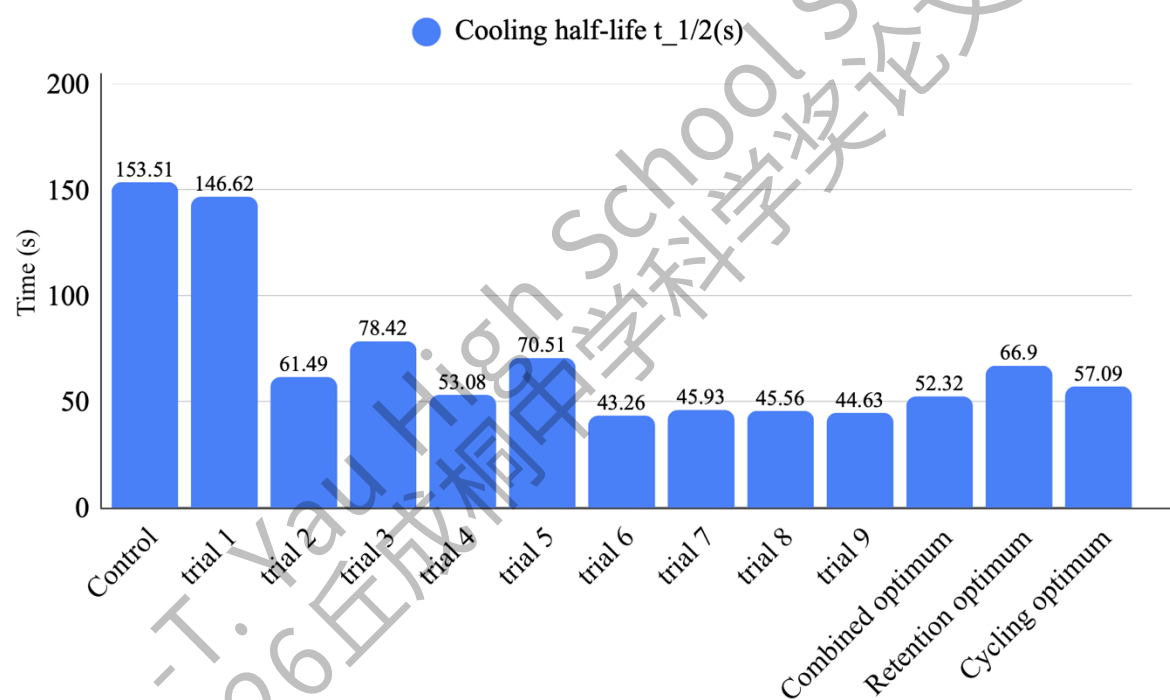


Figure 12. Comparison of cooling half-life for validation experiments, where a longer half-life indicates slower cooling and stronger heat retention ability.

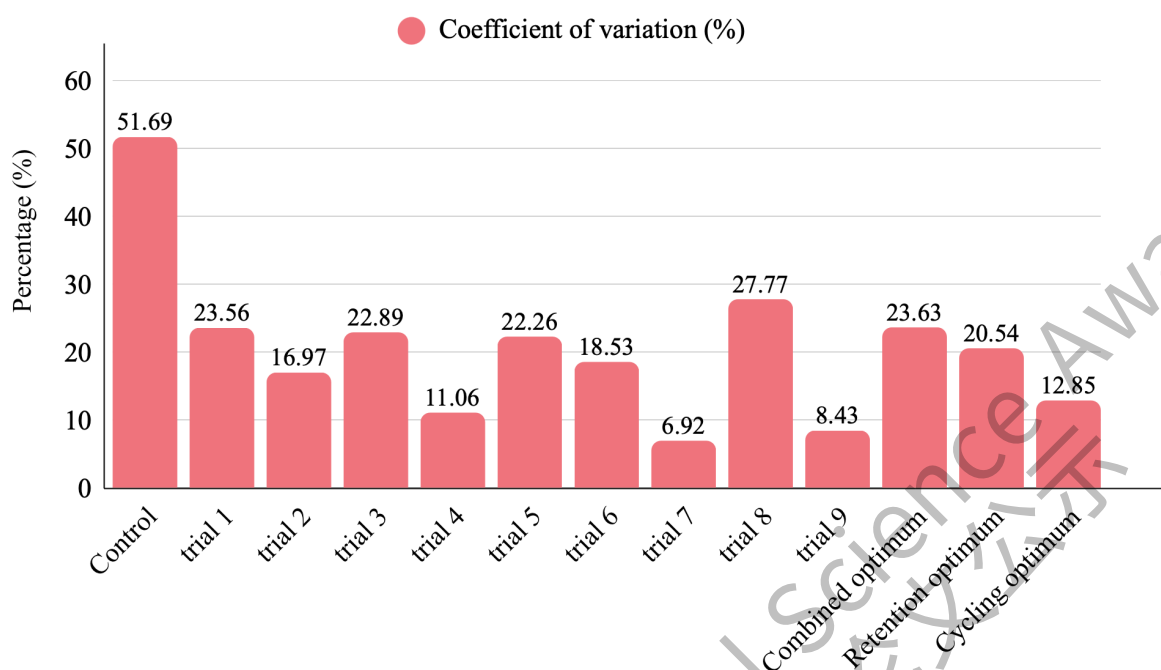


Figure 13. Comparison of percentage of coefficient of variation (CV) for validation experiments. Lower CV reflects higher stability across heating-cooling cycles.

The formulations predicted to optimize temperature rise, cooling half-life, and cycling CV performed favorably in the validation experiments but did not outperform the best-performing L₉ formulations respectively. These results suggest that main effect analysis can guide the selection of promising formulations, but the predicted combinations should be regarded as candidates rather than confirmed optima.

Variations in hydrogel shape and texture made it difficult to maintain consistent contact between the temperature probe and the sample with various trials. The distance between the probe and the irradiated region also varied slightly among measurements. These differences may have affected the recorded temperature profiles and contributed to variability in the performance metrics. Slight shrinkage after several heating-cooling cycles, possibly due to dehydration, may also have altered sample geometry and probe contact.

The formulation predicted to maximize temperature rise, referred to here as the combined-optimum formulation, exhibited relatively strong heating performance in the validation experiment. Another interesting feature we found is its peak temperature increased over successive heating-cooling cycles (Figure 10a). This trend cannot be explained by residual heat accumulation between cycles because it returned to approximate the same temperature before start of each cycle. Changes in sample-temperature probe contact, water redistribution or partial dehydration may have contributed, but these possibilities were not independently verified.

Overall, the predicted formulation showed favorable performance in the validation testes. The main contributor of each train has been supported by the results.

4. Conclusion

This study examined how the composition of CPG hydrogels influences temperature rise, heat retention and cycling stability using an $L_9 (3^4)$ orthogonal experimental design. With in the tested concentration ranges, the main-effect analysis indicated that CS had the greatest influence on temperature rise, CS and PVA had the greatest influence on heat retention, and genipin had the strongest influence on cycling stability. Different combinations indicate that formulation selection should depends on the intended heating and cooling treatment requirements. The final validation tests supported the use of orthogonal array analysis to guide formulation optimization. These finding provide an experimental basis for further refinement of CPG hydrogel formulations. Even within the relatively narrow concentration ranges tested, variations in CPG hydrogel composition were associated with marked differences in photothermal performance. These findings highlight the importance of formulation control and provide an experimental basis for further investigation and optimization of multicomponent hydrogels. The adhesion, injectability, and biocompatibility of the candidate formulations remain to be evaluated, and their therapeutic efficacy requires further biological validation. Overall, this study identifies promising formulations combining strong heating performance with prolonged heat retention and provides a basis for its further development for postoperative photothermal applications.

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Acknowledgement

This research was independently completed by the participating student during their time at school. Ms. Zhenzi Mi, the student's course instructor, provided detailed guidance and support throughout the development of the project. Her assistance primarily focused on the practical implementation of the experimental procedures, clarification of questions regarding experimental operations and technical details, and systematic review of the outline for the research paper. She provided supervision and guidance, while the student carried out the experiments, responding to the student's questions at different stages of the project and helping ensure that the research progressed in a steady and organized manner.

Professor Yao Fu provided important guidance at the strategic and conceptual levels of the project. At the early stage of the research, Professor Fu participated in discussions concerning the overall research direction and helped establish the chitosan-based system as the focus of the study. During the experimental design stage, Professor Fu provided key recommendations regarding the overall technical approach, including the strategy for screening polymer concentrations. During the preparation of the research paper, Professor Fu reviewed the scientific validity of the conclusions and the coherence of the overall logical framework. All guidance and support provided by both instructors were offered without compensation and did not involve any form of payment, financial benefit, or exchange of interests.

The topic of this study originated from the author's earlier exploration of a related research area. The preliminary work focused on the synthesis and application of tannic-acid-based hydrogel systems. With guidance from the instructors, this research was subsequently expanded to a related material system based on chitosan. All literature sources used in the study were obtained from publicly available academic journals. The experimental data were independently generated and collected by the participating student in a high school laboratory. The data, figures, and written analysis presented in the research paper were also prepared by the participating student.

Several practical challenges were encountered during the course of the research. One of the initial difficulties was the lack of a clearly established concentration range for the polymers used in the formulation. To address this issue, approximately six months of preliminary experiments were conducted to systematically investigate how different polymer concentrations

affected the final solubility and pH of the formulations. These experiments helped establish appropriate concentration ranges for subsequent formulation and optimization.

The experimental procedure also encountered issues related to the stability of the temperature. Temperature fluctuations during synthesis resulted in hydrogels that were relatively heterogeneous and unstable. To improve the consistency and stability of the synthesis process, a magnetic stirring hot plate was subsequently introduced instead of the water bath to assist with dissolution and temperature control. This modification provided a more stable environment for preparing the hydrogel formulations.

Another challenge occurred during the photothermal testing procedure. The original experimental design involved directing the near-infrared (NIR) laser onto the temperature probe through a layer of hydrogel. However, inadequate temperature control caused the outer coating of the temperature probe to become scorched during the experiment. The experimental setup was therefore modified so that the NIR laser was directed toward the hydrogel from a different angle rather than directly toward the temperature probe. This adjustment reduced the risk of excessive localized heating of the probe and allowed the photothermal experiments to proceed more reliably.

Overall, the research process involved not only the execution of the planned experiments but also repeated troubleshooting and methodological refinement. The concentration-screening experiments, modifications to the heating and stirring procedure, and adjustment of the NIR irradiation geometry were all implemented in response to experimentally observed problems. These iterative adjustments allowed the student to improve the reliability and consistency of the experimental procedure while independently carrying the project forward.

While doing the research, the author used ChatGPT-4 to help choose and understand details of data analysis method, such as how to use L_9 Taguchi orthogonal array. Chat GPT 4 is also being used before the experiment to predict the optimal formulations, and the original anticipation was 1% CS, 1.3%QACS, 0.2% Genipin, and 5% PVA, which is completely different from the real experimental data. The was one of the original directions before I start this project, to test how well can ChatGPT predict the result of experiment just by reading existed publications.

During the preparation of this work, ChatGPT 5.6 is used for language editing and proofreading. After using this tool, the author reviewed and revised the content as needed and takes full responsibility for the final publication.

Supporting information

Table S1. Concentration ranges, laser irradiation parameters, and corresponding maximum temperature responses were compiled from published studies for each of the four constituent factors. Rows marked in blue are data from our previous work.

Component	Concentration (w/v%)	Laser Parameters	Max Temp / ΔT	Observations/Ref
QACS	0.8%	808nm, 1.36 W/cm ²	$\Delta T > 10^{\circ}\text{C}$	Base heating effect (Our group's work) [7]
QACS	3.59%	-	-	Chang et al. [11]
CS	4.2%	808nm, 1.36 W/cm ²	$\Delta T > 10^{\circ}\text{C}$	High conc. heating baseline (Our group's work) [7]
CS	2.0%	1.0 W/cm ² , 790s, 808 nm	45°C	Pan et al. [12]
CS	1.0%	1.0 W/cm ² , 790s, 808 nm	$\Delta T = 24.1^{\circ}\text{C}$	Zhang et al. [13]
CS	0.8%	1.0 W/cm ² , 5 min, 808 nm	49.7°C	High efficiency at low conc. [14]
CS	0.357%	1.0 W/cm ² , 10 min, 808 nm	49.5°C	Liang et al. [15]
CS	0.91%	2.0 W/cm ² , 10 min, 808 nm	48.1°C	Requires higher power [16]
CS	0.87%	2.0 W/cm ² , 5 min, 808 nm	23.0°C	Low efficiency variant [16]
Genipin	1.0%	808nm, 1.36 W/cm ²	$\Delta T > 10^{\circ}\text{C}$	High concentration (Our group's work) [7]
Genipin	0.01%	6.0 W/cm ² , 10 min	57.9°C	Requires extreme power [17]
PVA	4.0%	808nm, 1.36 W/cm ²	$\Delta T > 10^{\circ}\text{C}$	(Our group's work) [7]
PVA	5.0%	1.0 W/cm ² , 10 min	51.0°C	Park et al. [18]

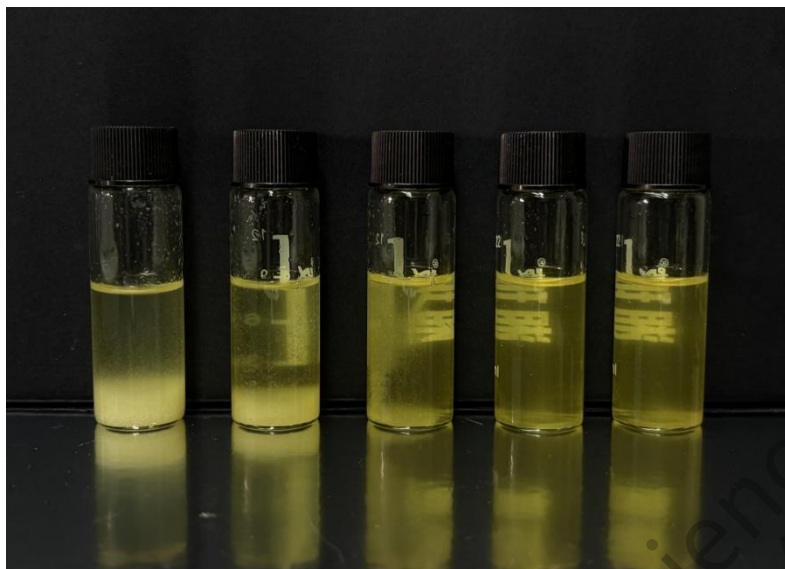


Figure S1. Comparison of the solubility of CS and QACS correlation to different acetic acid concentration, with fixed concentrations of CS, QACS, and acetic acid.

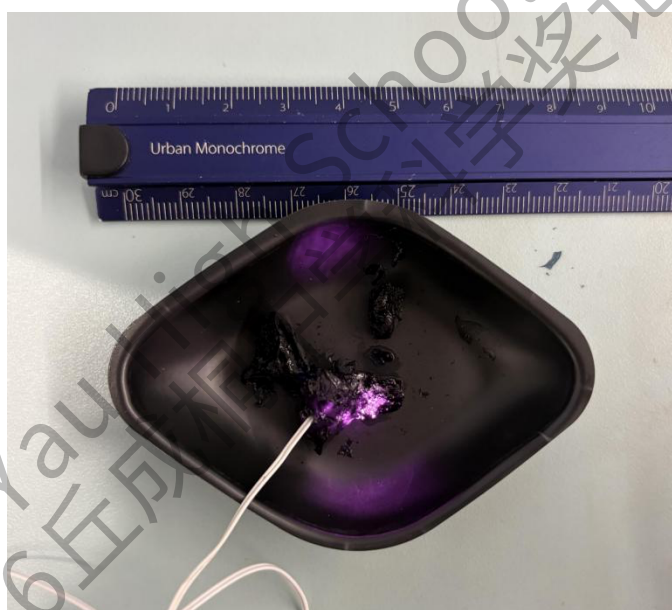


Figure S2. Preliminary synthesized CPG hydrogel demonstrating fragility with high genipin content.

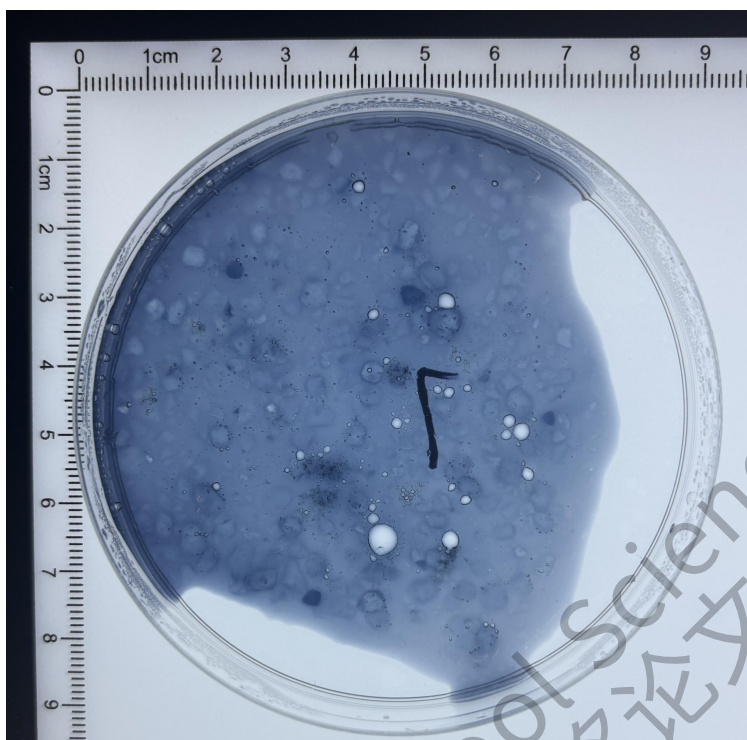
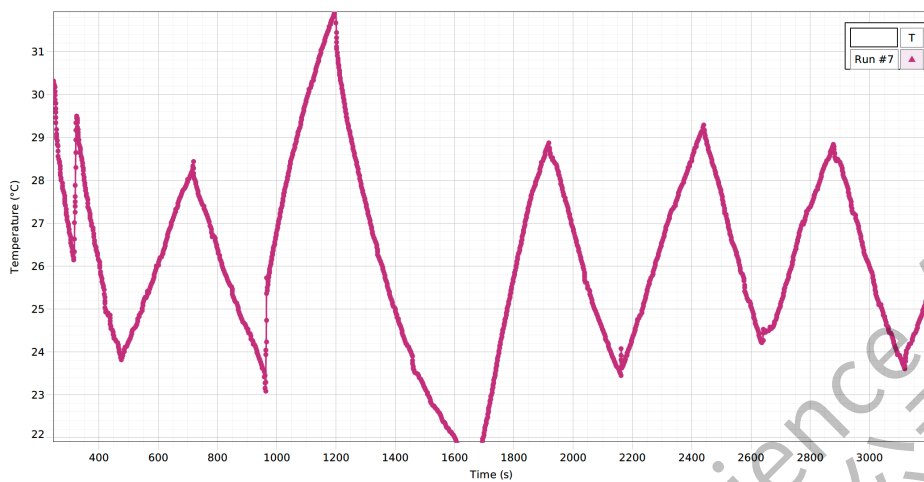


Figure S3. Preliminary synthesized CPG hydrogel decrease in solubility with high PVA content.

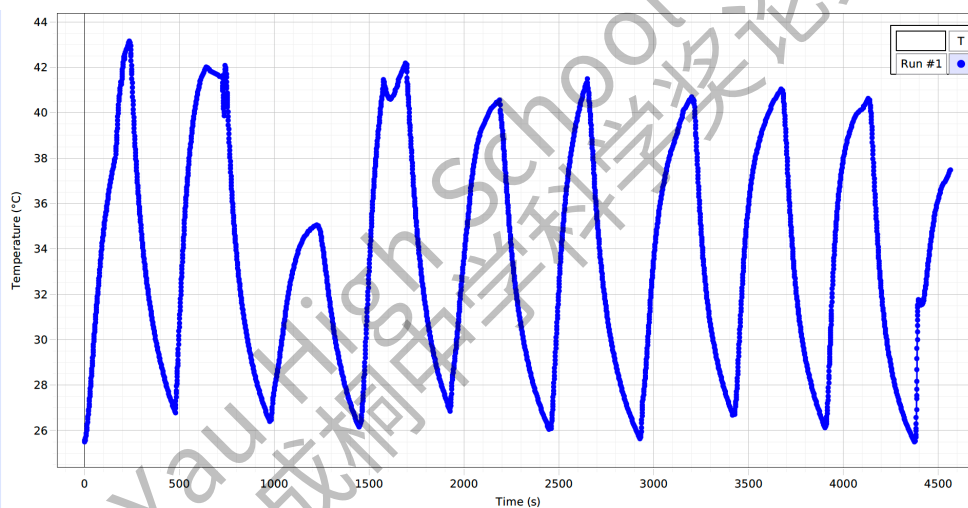


Figure S4. Comparison of the effects of different concentration of genipin on the color and state of hydrogels over time, with fixed concentrations of CS, QACS, and acetic acid.

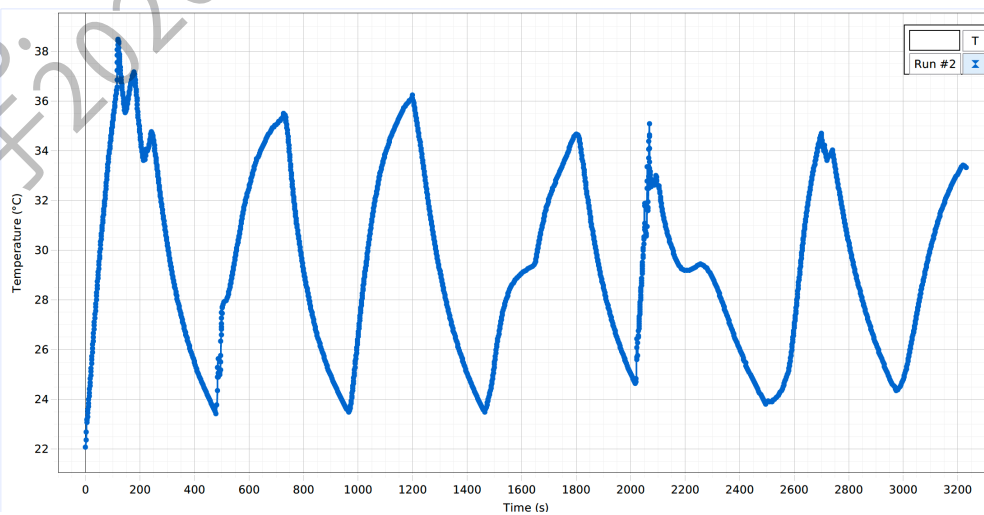
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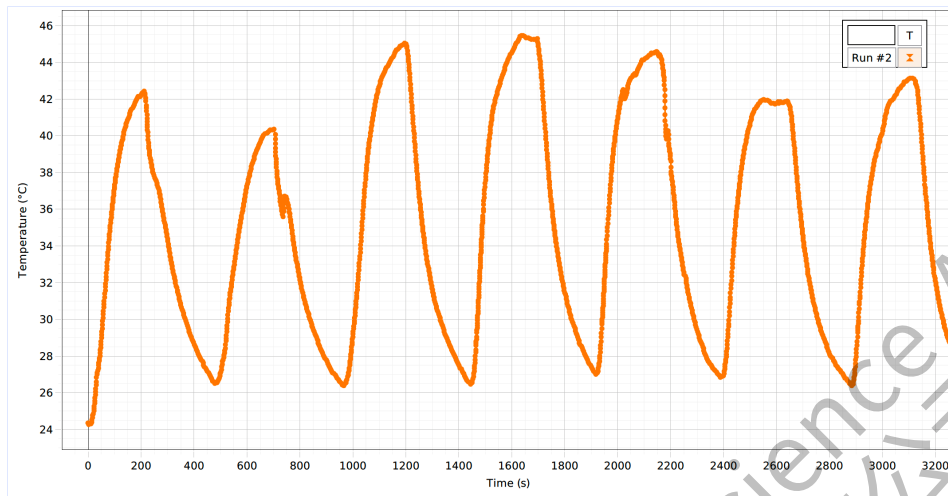
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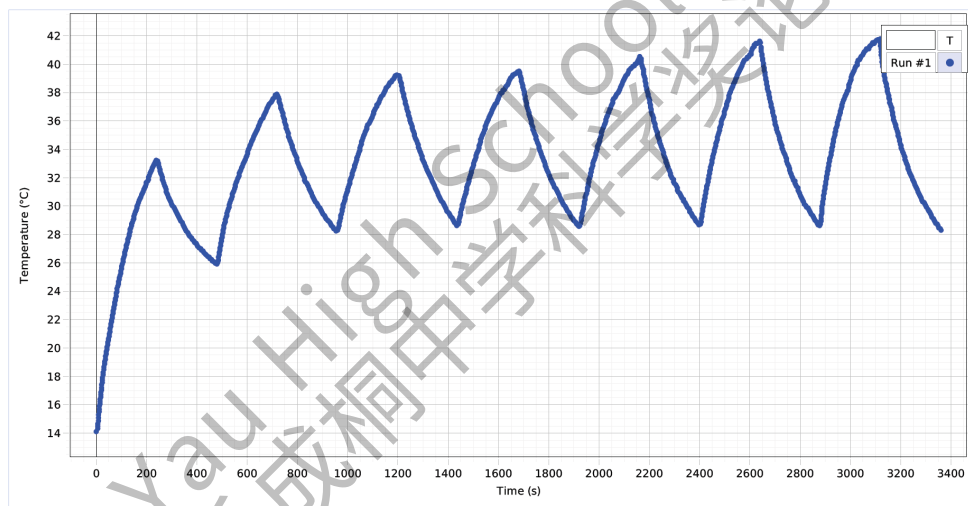
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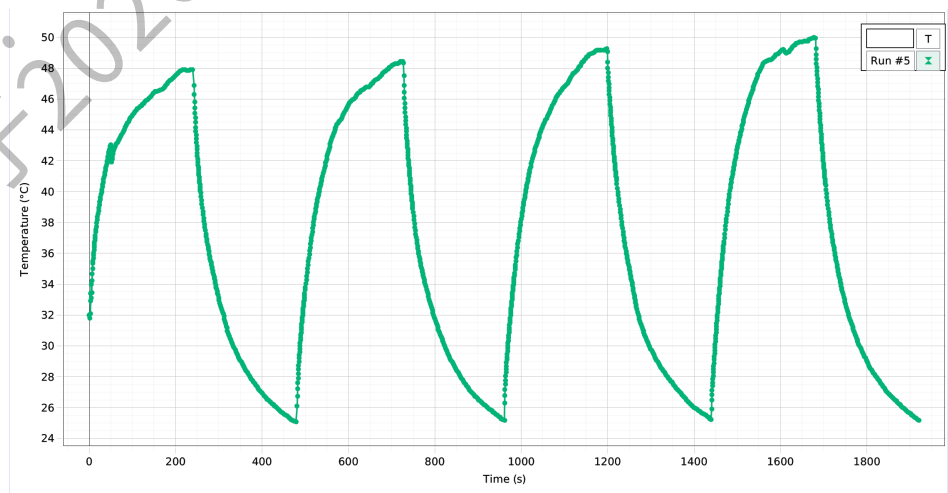
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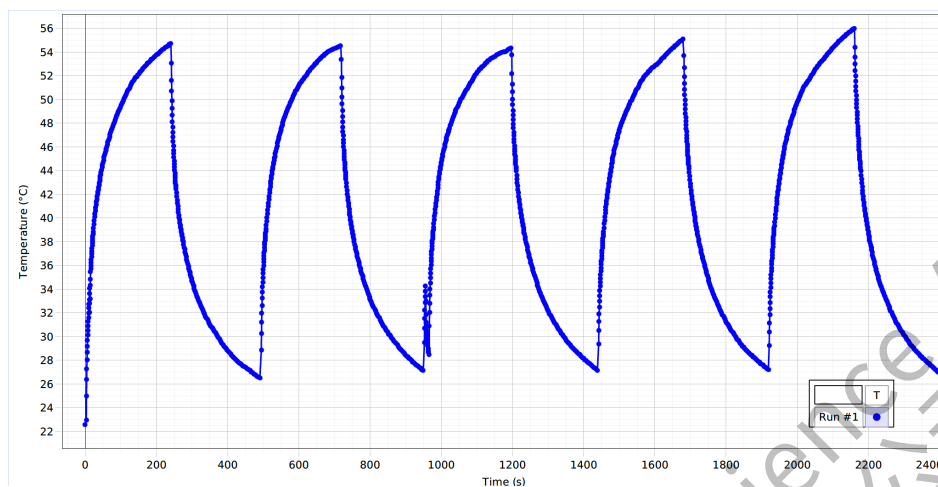
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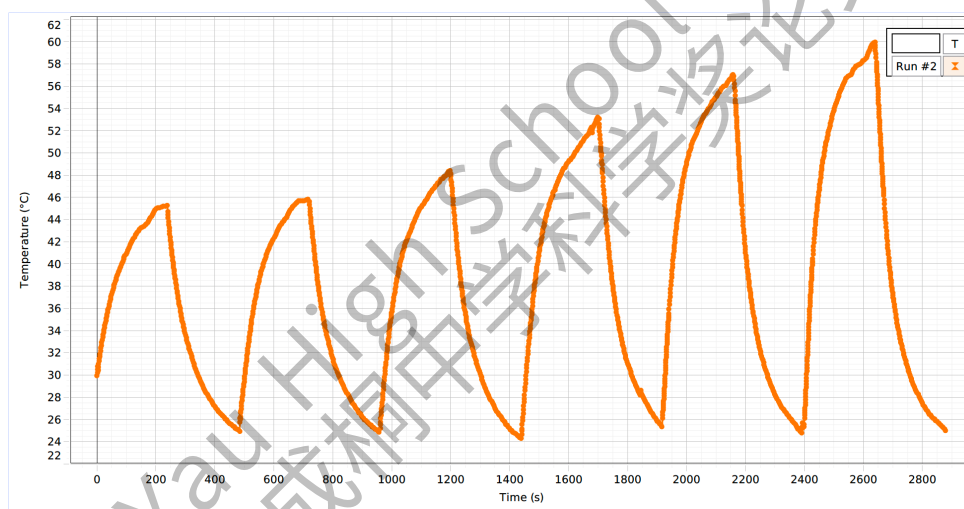
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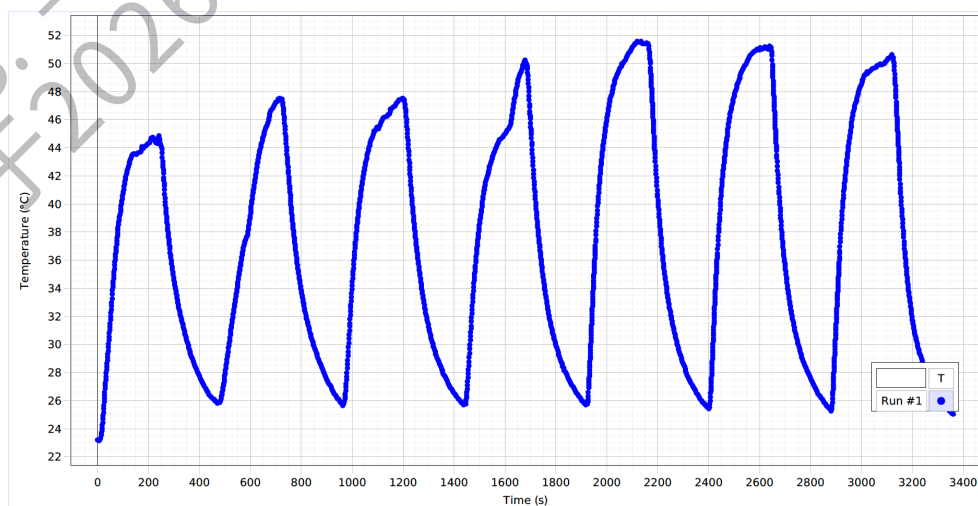


Figure S5. Real-time temperature profile of the each trial of hydrogel under repeated NIR irradiation–cooling cycles. (a) Trial 1. (b) Trial 2. (c) Trial 3. (d) Trial 4. (e) Trial 5. (f) Trial 6. (g) Trial 7. (h) Trial 8. (i) Trial 9.

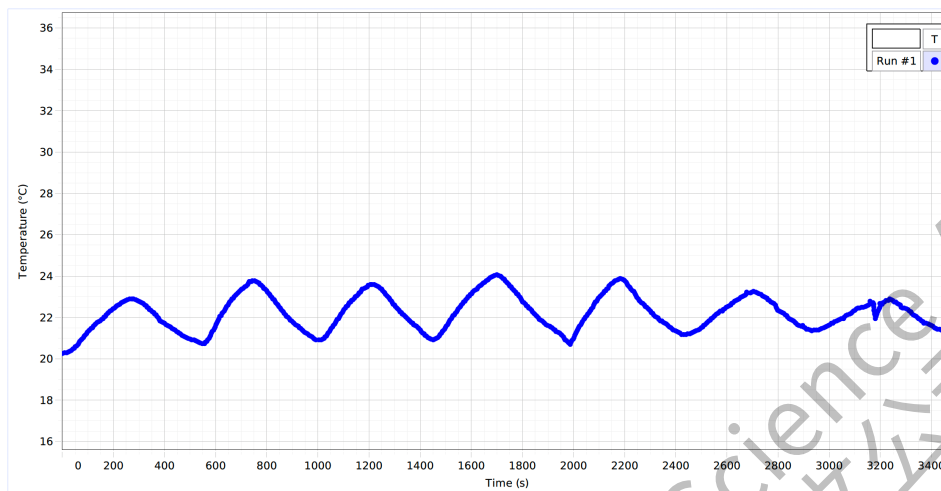


Figure S6. Real-time temperature profile of the control PVA group under repeated NIR irradiation–cooling cycles.

Table S2. Calculation of predicted optimal combination using control-corrected ΔT_{240s} , higher the mean temperature represents stronger the effect.

Factor	Level	Trials	Calculation (°C)	Mean (°C)
CS (A)	A1	1, 2, 3	$(2.88 + 11.87 + 8.86) / 3$	7.87
CS (A)	A2	4, 5, 6	$(14.49 + 10.41 + 19.37) / 3$	14.76
CS (A)	A3	7, 8, 9	$(26.14 + 23.04 + 21.24) / 3$	23.47
QACS (B)	B1	1, 4, 7	$(2.88 + 14.49 + 26.14) / 3$	14.50
QACS (B)	B2	2, 5, 8	$(11.87 + 10.41 + 23.04) / 3$	15.11
QACS (B)	B3	3, 6, 9	$(8.86 + 19.37 + 21.24) / 3$	16.49
Genipin (C)	C1	1, 6, 8	$(2.88 + 19.37 + 23.04) / 3$	15.10
Genipin (C)	C2	2, 4, 9	$(11.87 + 14.49 + 21.24) / 3$	15.87
Genipin (C)	C3	3, 5, 7	$(8.86 + 10.41 + 26.14) / 3$	15.14
PVA (D)	D1	1, 5, 9	$(2.88 + 10.41 + 21.24) / 3$	11.51
PVA (D)	D2	2, 6, 7	$(11.87 + 19.37 + 26.14) / 3$	19.13
PVA (D)	D3	3, 4, 8	$(8.86 + 14.49 + 23.04) / 3$	15.46

Table S3. Calculation of heat retention ability using cooling half life, higher the mean time represents stronger the effect.

Factor	Level	Trials	Calculation (s)	Mean (s)
CS (A)	A1	1, 2, 3	$(146.62 + 61.49 + 78.42) / 3$	95.51
CS (A)	A2	4, 5, 6	$(53.08 + 70.51 + 43.26) / 3$	55.62
CS (A)	A3	7, 8, 9	$(45.93 + 45.56 + 44.63) / 3$	45.37
QACS (B)	B1	1, 4, 7	$(146.62 + 53.08 + 45.93) / 3$	81.88
QACS (B)	B2	2, 5, 8	$(61.49 + 70.51 + 45.56) / 3$	59.19
QACS (B)	B3	3, 6, 9	$(78.42 + 43.26 + 44.63) / 3$	55.44
Genipin (C)	C1	1, 6, 8	$(146.62 + 43.26 + 45.56) / 3$	78.48
Genipin (C)	C2	2, 4, 9	$(61.49 + 53.08 + 44.63) / 3$	53.07
Genipin (C)	C3	3, 5, 7	$(78.42 + 70.51 + 45.93) / 3$	64.95
PVA (D)	D1	1, 5, 9	$(146.62 + 70.51 + 44.63) / 3$	87.25
PVA (D)	D2	2, 6, 7	$(61.49 + 43.26 + 45.93) / 3$	50.23
PVA (D)	D3	3, 4, 8	$(78.42 + 53.08 + 45.56) / 3$	59.02

Table S4. Calculation of cycling ability using mean CV, lower the CV represents better stability.

Factor	Level	Trials	Calculation (%)	Mean CV (%)
CS (A)	A1	1, 2, 3	$(23.56 + 16.97 + 22.89) / 3$	21.14
CS (A)	A2	4, 5, 6	$(11.06 + 22.26 + 18.53) / 3$	17.28
CS (A)	A3	7, 8, 9	$(6.92 + 27.77 + 8.43) / 3$	14.37
QACS (B)	B1	1, 4, 7	$(23.56 + 11.06 + 6.92) / 3$	13.85
QACS (B)	B2	2, 5, 8	$(16.97 + 22.26 + 27.77) / 3$	22.33
QACS (B)	B3	3, 6, 9	$(22.89 + 18.53 + 8.43) / 3$	16.62
Genipin (C)	C1	1, 6, 8	$(23.56 + 18.53 + 27.77) / 3$	23.29
Genipin (C)	C2	2, 4, 9	$(16.97 + 11.06 + 8.43) / 3$	12.15
Genipin (C)	C3	3, 5, 7	$(22.89 + 22.26 + 6.92) / 3$	17.36
PVA (D)	D1	1, 5, 9	$(23.56 + 22.26 + 8.43) / 3$	18.08
PVA (D)	D2	2, 6, 7	$(16.97 + 18.53 + 6.92) / 3$	14.14
PVA (D)	D3	3, 4, 8	$(22.89 + 11.06 + 27.77) / 3$	20.57