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Peer Review File

Multiscale integral synchronous assembly of cuttlebone-inspired structural materials by predesigned hydrogels

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

This paper presents a design and preparation method for a bioinspired "rigid cavity-wall" material based on the structure of cuttlebone. The authors successfully constructed a complex structure with excellent energy absorption performance by layering various organic and inorganic micro-nano building units. They designed two pre-designed hydrogels and utilized hydrogen bonds, covalent bonds, and electrostatic interactions to layer them into brick walls and brick-mortar structures. Through meticulous analysis and design, they achieved the orderly assembly and arrangement of organic and inorganic micro-nano building units, forming a "rigid cavity-wall" material similar to the structure of cuttlebone. This material exhibits excellent crack resistance, high strength, and energy absorption characteristics, superior to typical energy-absorbing materials with similar densities. The innovation of the paper lies in proposing a novel pre-designed hydrogel integral synchronous assembly strategy, successfully realizing the integrated preparation of complex multi-scale structures of biological structural materials. This research provides new insights into the design of bioinspired materials and demonstrates potential advantages in practical applications. However, the experimental results in the paper mainly focus on the mechanical properties and structural characteristics of the material, and further research is needed to explore its performance and long-term stability in practical applications. Additionally, while the paper mentions the potential applications of the material in the automotive and aerospace industries, specific application cases and experimental validations have not been provided, necessitating further research and validation. A major revision is needed. Specific issues are as follows:

1. Many reports already exist for cuttlebone-like structures (Adv. Mater, 2021, 33, 2007348; Proc. Natl. Acad. Sci., 2020, 117, 23450; THIN-WALLED STRUCTURES, 2023, 186, 110693), the present work seems to lack innovation, and the authors lacked a statement of the cuttlefish bone portion in the intro section to highlight the necessity of this work.

2. The biomimetic effect is not clear. The SEM image in Figure 1c differs significantly from the natural cuttlebone in Figure 1a, and there is occlusion in Figure 1, resulting in incomplete display.

3. The material preparation process is quite complex. What are the advantages of this work compared to the relatively simple 3D printing methods?

4. 4. In Figure 2a Although structural densification can be achieved through the dehydration process, it does not seem to result in the formation of brick-mortar structures as claimed by the authors due to the random orientation of the fillers in the hydrogel framework. Similarly, in Figure 2d, Does the uneven build-up of hollow glass vesicles affect the strength and energy-absorbing capacity of the material?

5. On what scales can this integrated structure remain ordered? From the point of view of architectural requirements, it seems that the micro-nano scale is difficult to meet the needs of

practical applications. The authors should build this structure on a larger scale to prove its feasibility. Alternatively, the authors should provide suitable application concept scenarios to demonstrate its feasibility.

6. Has there been in-depth understanding and research on the interactions between building units of different scales, especially between organic and inorganic components at the micro and nano scales? How do these interactions affect the material's performance and stability?

7. While the paper mentions the mechanical properties and energy absorption performance of the material, has there been research on durability and long-term stability? For example, how does the material perform under different environmental conditions, and what is the performance degradation after long-term use?

8. The paper mentions various external factors affecting material performance, such as hydrogen bonds, covalent bonds, and electrostatic interactions, but is the mechanism of these factors fully understood? Is it possible to further optimize these factors to improve material performance?

9. The authors show the compression resistance of the material in the vertical direction, which is indeed very important. But what about its compressive properties in the horizontal direction?

Reviewer #2 (Remarks to the Author):

The authors reported an integral synchronous assembly strategy of cuttlebone-inspired structural material by hierarchical predesigned hydrogels. This is an important work about the integral fabrication of bio-inspired lamellar multiple sophisticated architectures represented by the “rigid cavity-wall” exquisite structure of cuttlebone. By layer-by-layer assembly and then under directional pressure, a “rigid cavity-wall” structure can be prepared orderly and robustly. The cuttlebone-inspired structural material has excellent strength and energy absorption. The authors also reported a new chemical bonding phenomenon of surface covalent interaction between PVA and hollow glass bubbles, which is promising for the fabrication of bio-inspired micro-cavity structures. The manuscript is well-organized and clearly presented with adequate data. Therefore, I recommend publishing this interesting work in Nature Communications.

A few minor points for the authors to consider:

1. The hierarchical predesigned hydrogel integral synchronous assembly strategy is impressive. During this assembly process, how to achieve the robust interlayer bonding between different structures? I suggest the authors add relevant interpretation to fully display this novel assembly strategy of bioinspired structural materials with multiple different micro-nano architectures.

2. During the solid-state crosslinking interaction process of GB/PVA/qCNF initial gel, the modulus changes largely. How does the viscosity change in this process?

3. The total amount and addition form of crosslinking CaCl2 solution should be introduced in detail.

4. In situ X-ray microtomography coupled with compression testing is used to interpret the failure process of the RCWSM under static compression. It is an attractive visualization of the destruction process, but more details should be provided on why the compressive strains of 0%, 10%, 30%, 65%, and 80% are the representative statuses.

5. Supplementary Movies are suggested to add more texts to further describe the contents of movies.

6. Some improvements should be made in image presentation. For example, the abscissa axis labels of Figure 1e-g should be more clarified by fine-tuning the position of the figure edge.

Reviewer #3 (Remarks to the Author):

In this manuscript, the authors reported a new type of bio-inspired composite hydrogel with promising crack growth resistance and high compressive strength, which I believe is the less studied area in hydrogel. The manuscript aligns with the scope of Nature Communications while its novelty will inspire the future development of hydrogel with high mechanical integrity where compressive strength is of the prime importance. I recommend publishing this manuscript after the author providing more information about the hydrogel based on the question below.

1. In this manuscript, the authors reported a composite hydrogel with layered structure and claimed that the embedded dovetail-like structure was observed at the interface of the two layers. I suggest the authors to provide more information about the bonding strength between two layers as the delamination could a big concern for layered composite.

2. After compression, it is worth to investigating the reversibility and durability of the hydrogel. It could be important for the hydrogel to reverse to its original state after deformation.

The point-to-point answers to the Nature Communications reviewers' comments

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"Multiscale integral synchronous assembly of cuttlebone-inspired structural materials by predesigned hydrogels"

We have carefully considered all the concerns of the three independent reviewers, and have made suitable revision accordingly. For clearness reason, the answers were marked with **BLUE** color and started with **"**"** and the revision parts in the revised manuscript were noted in highlight color.

Reviewer comments:

Reviewer #1:
This paper presents a design and preparation method for a bioinspired "rigid cavity-wall" material based on the structure of cuttlebone. The authors successfully constructed a complex structure with excellent energy absorption performance by layering various organic and inorganic micro-nano building units. They designed two pre-designed hydrogels and utilized hydrogen bonds, covalent bonds, and electrostatic interactions to layer them into brick walls and brick-mortar structures. Through meticulous analysis and design, they achieved the orderly assembly and arrangement of organic and inorganic micro-nano building units, forming a "rigid cavity-wall" material similar to the structure of cuttlebone. This material exhibits excellent crack resistance, high strength, and energy absorption characteristics, superior to typical energy-absorbing materials with similar densities. The innovation of the paper lies in proposing a novel pre-designed hydrogel integral synchronous assembly strategy, successfully realizing the integrated preparation of complex multi-scale structures of biological structural materials. This research provides new insights into the design of bioinspired materials and demonstrates potential advantages in practical applications. However, the experimental results in the paper mainly focus on the mechanical properties and structural characteristics of the material, and further research is needed to explore its performance and long-term stability in practical applications. Additionally, while the paper mentions the potential applications of the material in the automotive and aerospace industries, specific application cases and experimental validations have not been provided, necessitating further research and validation. A major revision is needed. Specific issues are as follows:

**We thank you for the positive evaluation and the constructive comments on the manuscript. In this work, we developed a robust hierarchical predesigned hydrogel assembly strategy to integrally synchronously assemble organic and inorganic micro-nano building blocks to bioinspired structural materials with multiple architectures. Based on this strategy, we have achieved the macroscopic-scale preparation of our "rigid cavity-wall" structural material (RCWSM) at the centimeter scale in the laboratory. The obtained cuttlebone-inspired structural materials gained crack growth resistance, high strength, and energy absorption characteristics beyond typical energy-absorbing materials with similar densities.

With the help of your valuable suggestions, besides focusing on the mechanical properties and structural characteristics of the material, we further explore its performance and long-term stability under different practical application scenarios, such as ultraviolet (UV) irradiation and cyclic load. The details of the above exploration experiments are in question 7, showing the durability of RCWSM under UV irradiation and cyclic load. Additionally, as a lightweight, strong, and energy-absorbing material, RCWSM has the potential applications as lightweight and energy-absorbing protective structural materials in intelligent cars and aerospace devices. To visibly illustrate the practical application potential, we evaluated the behavior of our RCWSM in simple application scenarios, including the rolling of car wheels and the impact of the steel ball. In the above-mentioned specific application cases, the RCWSM sample can still maintain the integrity of the overall structure. The details of the above exploration experiments are in question 5. According to your comments, we have carried out sample preparation and exploration experiments of specific application cases on the lab scale, but further industrial production and application verification will require a long time to continue exploring in future works.

After careful revision, we believe that all the concerns have been addressed, and the quality of this manuscript has been improved.

1. Many reports already exist for cuttlebone-like structures (Adv. Mater., 2021, 33, 2007348; Proc. Natl. Acad. Sci., 2020, 117, 23450; THIN-WALLED STRUCTURES, 2023, 186, 110693), the present work seems to lack innovation, and the authors lacked a statement of the cuttlefish bone portion in the intro section to highlight the necessity of this work.

**Thank you for your valuable suggestions. We have further complemented and emphasized the necessity of this work, highlighted in the “Introduction” section on page 3 of the manuscript. Meanwhile, we would like to further elucidate the innovation and advances of our work as follows.

Natural cuttlebone internal microscale structure has the superior capability of mechanical energy absorption over the stainless-steel foam due to its unique hierarchical “rigid cavity-wall” structure in microscale (*Proc. Natl. Acad. Sci., 2020, 117, 23450*). Nowadays, previous works for the structure analysis and design for advanced engineering materials mainly focus on the macroscale design, which usually achieves cuttlebone-inspired structures via 3D printing by resin (*Adv. Mater., 2021, 33, 2007348*; *Thin Wall Struct., 2023, 186, 110693*). However, for the fabrication of cuttlebone-inspired structure, current works only achieve the preparation at the millimeter-scale stage limited by the accuracy of 3D printing, which does not reach the micro-nano scale and multiple organic/inorganic complex components of the original overall natural architecture.

In this work, we extract the cuttlebone-inspired “rigid cavity-wall” feature and report a hydrogel integral assembly strategy based on predesigned composite hydrogels, which achieve the bioinspired synchronous fabrication of different microstructure at the micrometer scale. Predesigned hydrogel is a suitable medium for the robust assembly of micro-nano building blocks via surface multi-interactions, including hydrogen, covalent bonding, and electrostatic interactions. Through the ordered and robust combination of two types of predesigned hydrogels, we achieve the integral preparation of two different microscopic meta-structures within the cuttlebone-inspired structural material, which is much closer to the scale of natural cuttlebone. Thus, our work has innovation in the hydrogel assembly strategy, the assembly and interaction regulation of multiple organic/inorganic complex components, and the micro-nano scale structure, which has notable differences and development from other works of cuttlebone-like structures.

We hope that these distinctions can effectively interpret the novelties of this work.

2. The biomimetic effect is not clear. The SEM image in Figure 1c differs significantly from the natural cuttlebone in Figure 1a, and there is occlusion in Figure 1, resulting in incomplete display.

**Thank you for the valuable comments. For the structure design and fabrication of high-performance bioinspired composite material, core structure features and the working mechanism of the prototype are vital considerations. Natural cuttlebone has outstanding energy absorption performance due to its unique “rigid cavity-wall” internal microstructure (Figure R1a). Under the external force perpendicular to the intrinsic layer direction, a network-like rigid cavity layer within the cuttlebone occurs to crushing failure so as to dissipate the energy inside the material. The horizontal wall structure can effectively disperse the stress concentration to protect the overall bulk from catastrophic failure.

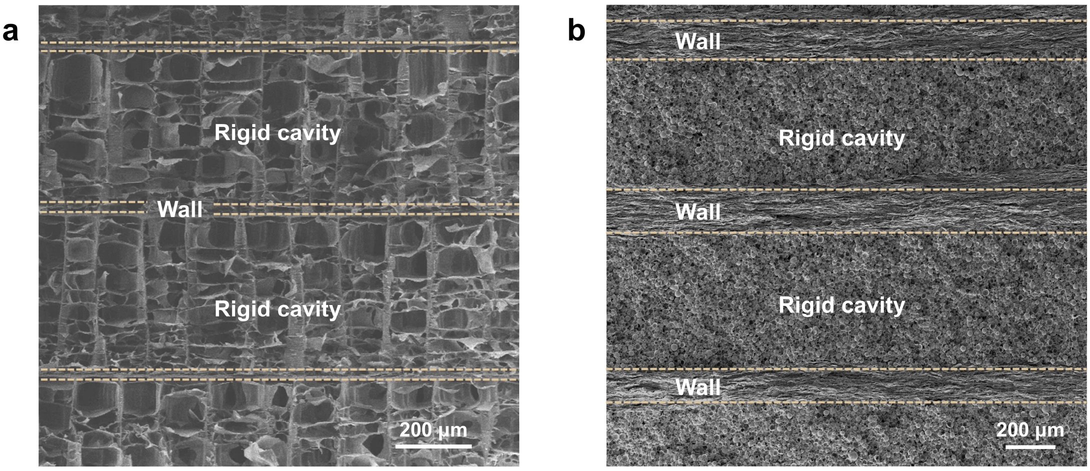


Figure R1. Comparison of structure feature between (a) natural cuttlebone and (b) “rigid cavity-wall” structural material (RCWSM).

Based on the structure feature and mechanism of the cuttlebone structure, in this work, we extract the “rigid cavity-wall” feature of the cuttlebone and choose glass bubble (GB) to assemble the rigid cavity layer by the micro-scaled intrinsic rigid hollow sphere structure, and choose mica platelet and cellulose nanofiber (CNF) to fabricate the brick-and-mortar structure as the rigid wall (Figure R1b). In the rigid cavity layers we constructed, GB could occur to be crushed and densified for effectively absorbing the mechanical energy (Figure R2), whose behavior is similar to the rigid cavity layers of natural cuttlebone. Meanwhile, brick-and-mortar wall layers can disperse the stress concentrations to maintain the overall stability of materials (Figure R3 and

Supplementary Movie 5), like the wall layers in natural cuttlebone. Moreover, the rigid cavity and wall in this work are at the micrometer scale, closely resembling the scale of the actual cuttlebone. Thus, our fabrication strategy has replicated the core structure features and mechanism of cuttlebone, realizing the preparation of cuttlebone-inspired structural materials.

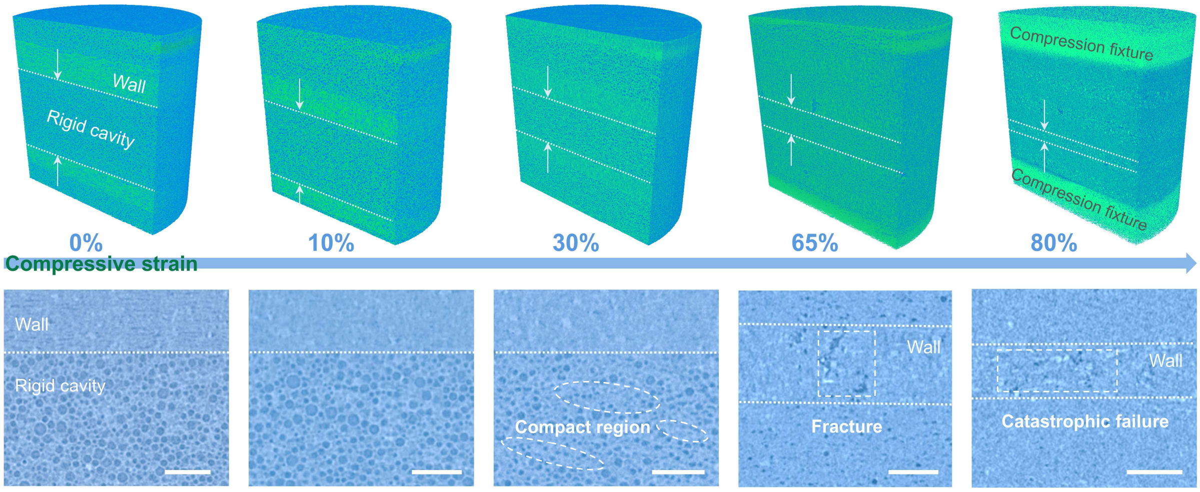


Figure R2. Failure process of the RCWSM under static compression coupled with *in situ* X-ray microtomography. 3D reconstructions and corresponding cross sections of the RCWSM under various compressive strains are shown to demonstrate the details of the failure process. Scale bar: 100 μm.

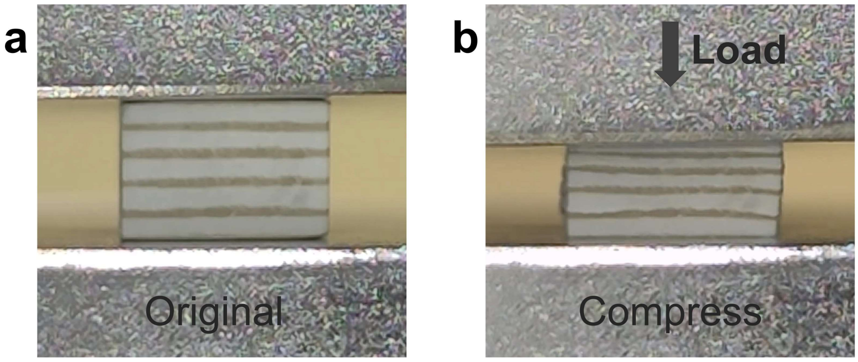
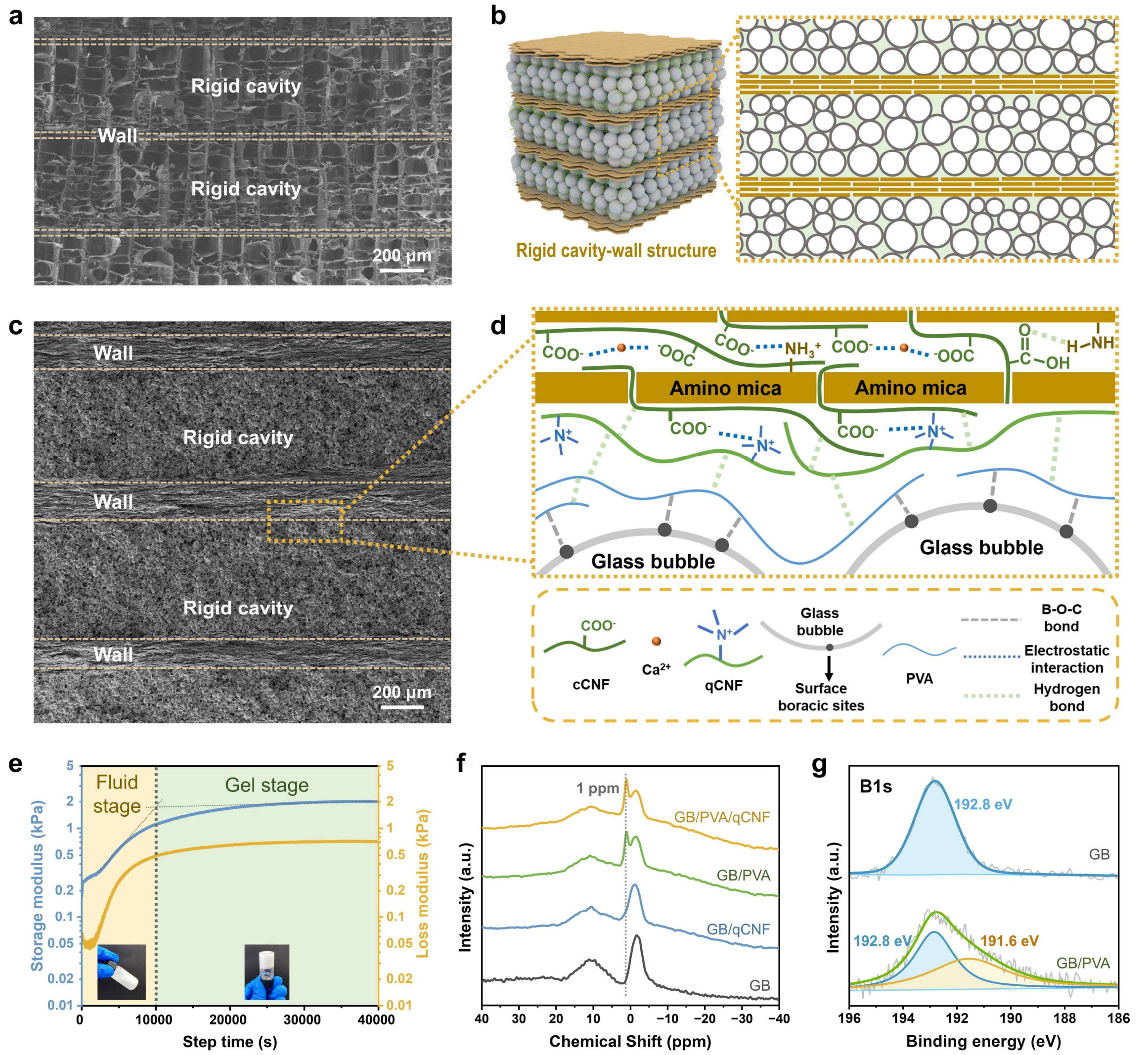


Figure R3. Photographs of the RCWSM before (a) and after (b) the compression test.

The image occlusion problem in Figure 1 is encountered when converting images to PDF. We've solved that problem and modified Figure 1, which has been updated in the revised manuscript on Page 5.



Modified Figure 1. Design, structure, and interaction characterization of "rigid cavity-wall" structural material (RCWSM).

3. The material preparation process is quite complex. What are the advantages of this work compared to the relatively simple 3D printing methods?

**We appreciate your interest in the work. As a useful strategy, 3D printing can achieve the programmed construction of diverse exquisite structures. Up to now, 3D printing has developed towards multi-material and multi-process (*Macromol. Mater. Eng. 2024, 309, 2300272; Macromol. Biosci. 2022, 22, 2100332*). However, for the preparation of high-performance bioinspired structures, the current 3D printing method mainly focuses on the design of macroscopic structures (*Adv. Mater. 2021, 33, 2007348*; *Thin Wall Struct. 2023, 186, 110693*; *Thin Wall Struct. 2024, 198, 111768*), which is a far cry from the actual micro-nano scaled bio-structure and limits the performance advantages derived from biomimetic structural design.

In order to achieve the structure construction on a similar microscopic scale to the bioinspired structure, a promising strategy is to manufacture delicate bioinspired structures by robustly combining organic-inorganic components. However, for traditional 3D printing, it is challenging to use ink with organic-inorganic multiscale building blocks. The reason is that the rheological properties of this sort of ink are usually unsuitable for injection and stable molding, and the robust combination of the organic-inorganic components in ink will affect the printable state. Furthermore, constructing the cuttlebone-inspired structure via organic-inorganic building blocks needs densification inducement, such as the specific molding pressure substantially higher than regular atmospheric pressure, which makes it difficult for the relatively simple 3D printing method to meet this requirement. Thus, the 3D printing methods can hardly realize the fabrication of a cuttlebone-inspired micro-nano scale “rigid cavity-wall” structure like our method.

To further demonstrate the advantages of our predesigned hydrogel integrated assembly method compared with 3D printing, we tried to prepare the cuttlebone-inspired composite by 3D printing. We used our predesigned aqueous dispersion as the ink of each layer to integrally assemble the composite through 3D printing layer-by-layer and dry it in natural conditions (Figure R4a). Due to the excessive water content of the ink and lack of combination, integral material occurs to obvious collapse and creates lots of interstices during the forming process (Figure R4b), which fails to achieve the precision and practical fabrication of the cuttlebone-inspired exquisite structure. We found that traditional 3D printing fails to construct the close-packed rigid cavity layers because of many loose interstices (Figure R4c). The brick-and-mortar wall structure also fails to be built with lots of obvious loose interstices (Figure R4d). Moreover, interlayer interface bonding is far from robust enough, which embodied the visibly insufficient interlayer adhesion (Figure R4d). Thus, compared 3D printing with our method, 3D printing is only equivalent to the step of predesigned hydrogel in our material preparation process, and the combination of the organic-inorganic components of our predesigned hydrogel method is better than the 3D printing method.

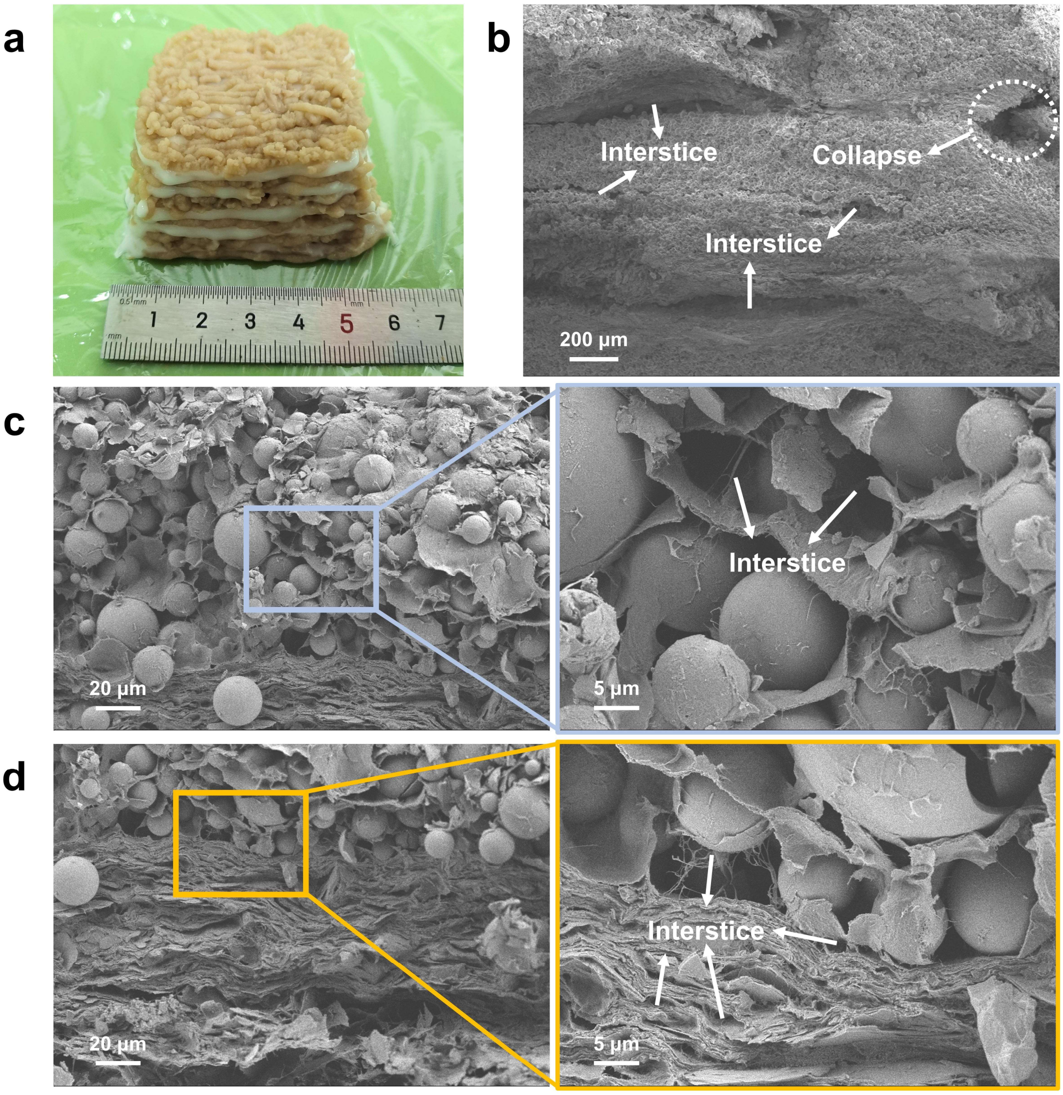


Figure R4. Attempted preparation of cuttlebone-inspired composite through 3D printing. a, Photograph of the cuttlebone-inspired composite just finished 3D printing. b, Scanning electron microscopy (SEM) image of the cross-section of this cuttlebone-inspired composite after sufficient drying. c, SEM images of attempted-prepared rigid cavity layer. d, SEM images of attempted-prepared wall structure and interlayer interface.

We hope our statement and experiment evidence can effectively interpret the advantages of the predesigned hydrogel integrated assembly method in this work compared with the 3D printing method.

4. In Figure 2a Although structural densification can be achieved through the dehydration process, it does not seem to result in the formation of brick-mortar

structures as claimed by the authors due to the random orientation of the fillers in the hydrogel framework. Similarly, in Figure 2d, Does the uneven build-up of hollow glass vesicles affect the strength and energy-absorbing capacity of the material?

**Thank you for the valuable suggestions. To achieve the construction of highly ordered brick-and-mortar structure, we designed the process of dramatically reducing the thickness of the hydrogel while the size in in-plane directions remains unchanged. By directly pressing the hydrogel of mica platelets and carboxylated cellulose nanofiber (cCNF), the mica platelets (bricks) can achieve highly uniform orientations, and cCNF (mortars) can evenly distribute between the bricks (Figure R5a).

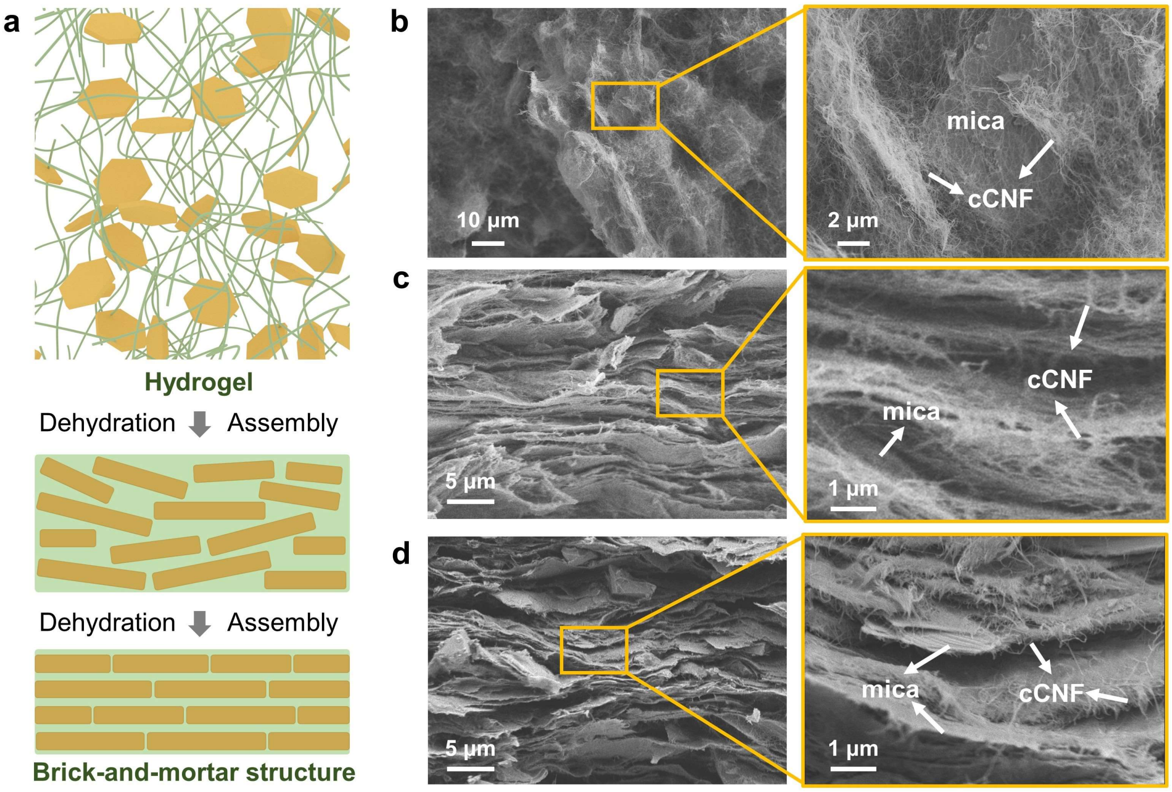


Figure R5. Synchronous assembly of brick-and-mortar wall structure in the RCWSM. a, Schematic of the brick-and-mortar wall structure from the initial gel to the dense state. Amino mica, yellow platelet; carboxylated CNF (cCNF), green curve. b, SEM images of the irregular arrangement of mica platelets in the initial gel. c, SEM images of the oriented arrangement of mica platelets in the partially dehydrated initial gel. d, SEM images of the brick-and-mortar wall in the RCWSM.

For the brick-and-mortar wall structure, we have further refined the characterization to the intermediate process of the densification assembly. Brick-and-mortar wall structures are constructed via dehydration under the directional pressure from the initial gel to the ordered brick-and-mortar structure. In the initial gel, mica platelets are evenly distributed in cCNF nanonetwork (Figure R5b). Just as your comments, the fillers are random orientations in the hydrogel framework. Under the directional deforming stress, mica platelets and cCNF in the hydrogel begin to stack and densify, which form the preliminary oriented arrangement (Figure R5c). With further densification and dehydration during the hot-pressing process, mica platelets gradually reduce the gaps between them and exhibit a regular arrangement in the cCNF nanonetwork, resulting in the fabrication of a brick-and-mortar wall structure (Figure R5d). During the dehydration process, the cCNF 3D nanonetwork assembles the mica platelets from an irregular to the ordered arrangement state. Therefore, through directional deforming pressure, mica platelets and cCNF in the hydrogel framework can effectively and evenly construct a regular and robust brick-and-mortar structure.

For the rigid cavity layers, to demonstrate the effect of evenness of the GB distribution on mechanical property and energy absorption, we prepared GB composites that had the same content ratio as rigid cavity layers in RCWSM, with GB distributed evenly and unevenly in separate samples. In the composite with even-distributed GB, GB is distributed evenly and packed densely in the polyvinyl alcohol (PVA)/quaternized cellulose nanofiber (qCNF) assembly matrix (Figure R6a). In contrast, the composite with uneven-distributed GB exhibited visibly compact and loose regions (Figure R6b). GB is robustly combined with the PVA/qCNF matrix in the compact regions, while the combination is much looser in the loose regions (Figure R6b). We further evaluated the mechanical properties and energy absorption of GB composites with different GB distributions. We performed the static compression test with a Shimadzu AGX-V universal testing machine at a loading rate of 1 mm min^{-1}. Due to the close-packing and robust assembly of GB with the PVA/qCNF matrix, the composite with an even GB distribution showed higher strength than the unevenly distributed sample (Figure R6c). Moreover, the even distribution of GB allows for effective energy absorption through dense packing extrusion, fully leveraging excellent mechanical properties of GB. Consequently, the GB composite with the even distribution of GB exhibited the remarkable higher compressive strength and energy absorption (Figure R6d). The results above-mentioned demonstrated that the uneven build-up of GB affect the strength and energy-absorbing capacity of the material.

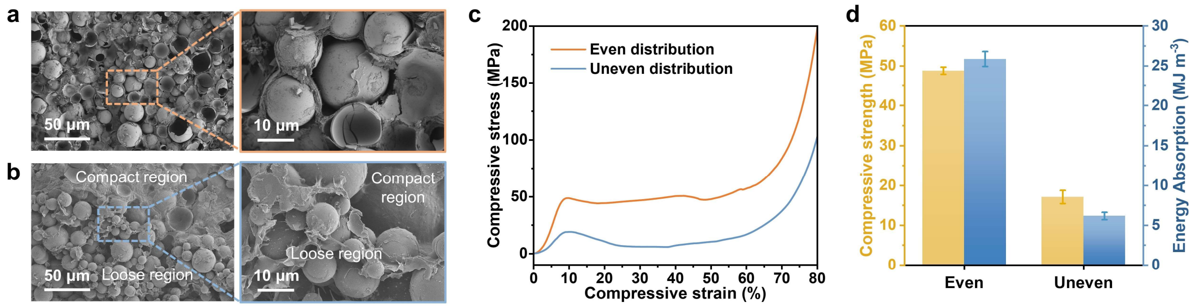


Figure R6. Comparison of internal structure, mechanical property, and energy absorption about different evenness of the GB distribution in GB composites. a, SEM images of GB composite with even-distributed GB. b, SEM images of GB composite with uneven-distributed GB. c, Compressive stress-strain curve of GB composites with even-distributed GB and uneven-distributed GB. d, Comparison of compressive stress and energy absorption about GB composites with even-distributed GB and uneven-distributed GB.

5. On what scales can this integrated structure remain ordered? From the point of view of architectural requirements, it seems that the micro-nano scale is difficult to meet the needs of practical applications. The authors should build this structure on a larger scale to prove its feasibility. Alternatively, the authors should provide suitable application concept scenarios to demonstrate its feasibility.

**Thank you for the valuable suggestions. Our approach can enable simultaneous assembly, and the internal structure is not significantly affected by the increase in size. Therefore, our materials can have ordered integrated micro-nano scale structures at the macroscopic scale. As you said, the micro-nano scale is difficult to meet the needs of practical applications, so we built the micro-nano scale structure on a larger scale to prove its feasibility. We have achieved the macroscopic-scale preparation of RCWSM at the centimeter scale in the laboratory (Figure R7). We have added Figure R7 as Supplementary Figure 23 in Supplementary information and supplemented the relevant text on page 8 of the manuscript.

Figure R7. Photograph of RCWSM. The sample size is about 60 mm × 60 mm × 4 mm.

To further and visibly illustrate the practical application potential in daily application scenarios, we evaluated the weight capacity of our RCWSM to visually assess the damage to the RCWSM after load bearing (Figure R8). We found that after supporting the weight of the 1594 kg car, the RCWSM sample could still maintain the integrity of the overall structure (Figure R8). It illustrates the excellent mechanical strength and satisfactory energy absorption of the RCWSM. Moreover, we found that the RCWSM can effectively absorb the energy from the impact of the steel ball (~36 g of the weight) dropped from the height of ~0.4 m, which can remain intact in the meantime (Figure R9). The results above could provide suitable application concept scenarios to demonstrate feasibility in the potential practical application of RCWSM.

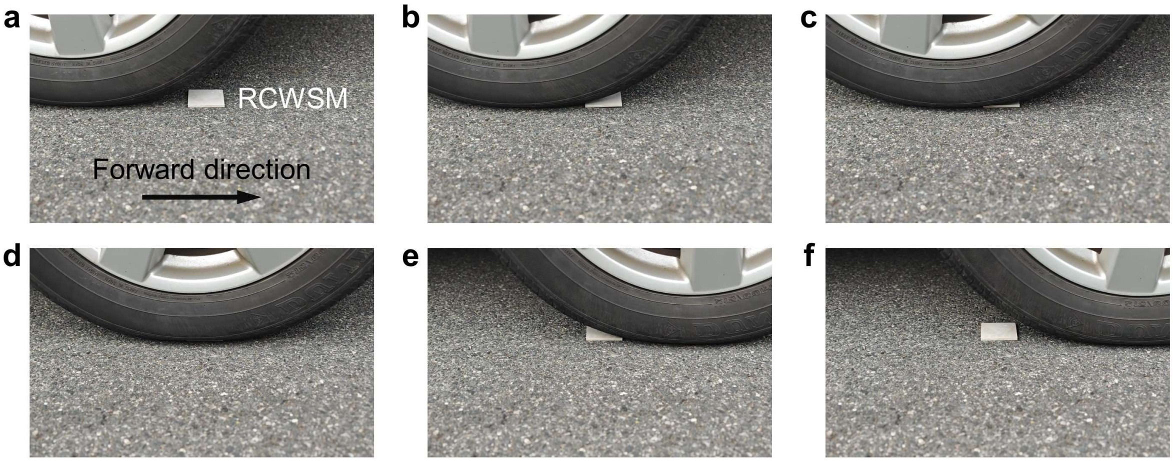


Figure R8. Photographs of the RCWSM supporting the weight of a car. Overall structure of RCWSM remained intact after loading.

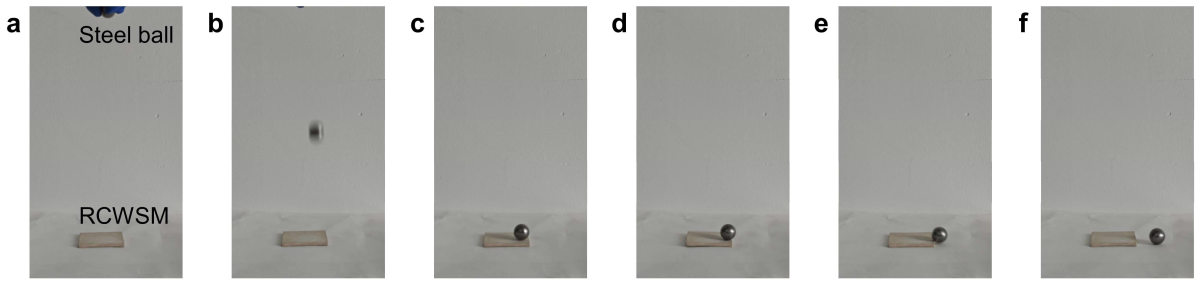


Figure R9. Photographs of the RCWSM bearing impact from the steel ball. RCWSM can absorb the impact energy and remain intact after the impact.

6. Has there been in-depth understanding and research on the interactions between building units of different scales, especially between organic and inorganic components at the micro and nano scales? How do these interactions affect the material's performance and stability?

**Thank you for the valuable suggestions. The effective interactions between building units of different scales, especially between organic and inorganic components at the micro and nano scales, are the precondition to fabricating composite materials.

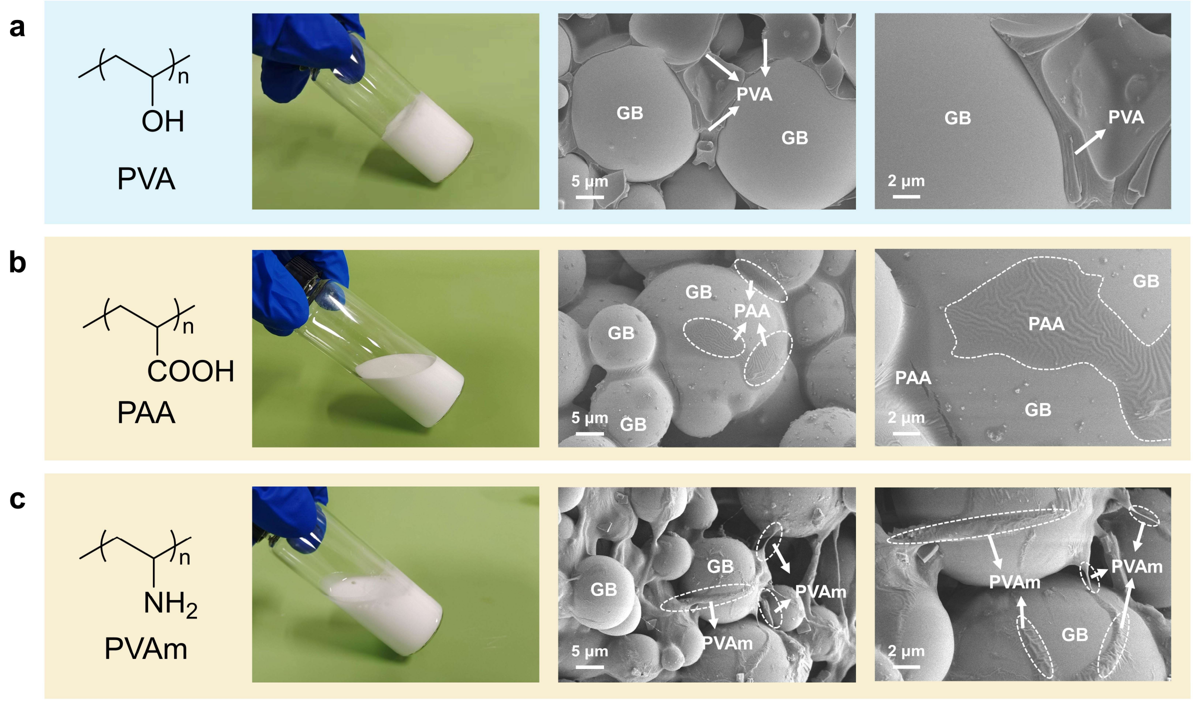


Figure R10. Photographs and corresponding internal cross-section SEM images of GB assembled with the aqueous solutions of (a) polyvinyl alcohol (PVA), (b) polyacrylic acid (PAA), and (c) polyethyleneamine (PVAm).

In order to figure out the interactions between organic and inorganic components, we chose polyvinyl alcohol (PVA), polyacrylic acid (PAA), and polyethyleneamine

(PVAm) as the typical model owing to their functional groups (hydroxyl, carboxyl, and amino), and mixed them with GB to form the mixed gels, respectively. We mixed 5 wt% aqueous solutions of these three polymers with twice the solute mass of GB to visually illustrate the surface interaction between GB and polymer. Due to the B-O-C covalent bond, after mixing with GB, PVA occurs with rapid gelation and a robust surface bonding on the surface of GB (Figure R10a). Due to the difficulty of forming a strong surface interaction between the carboxyl group of PAA or amino groups of PVAm and GB surface, the mixed system cannot gelate like the GB-PVA system. Meanwhile, the PAA or PVAm on the GB surface appeared to have a phase separation effect, resulting in an inability to bond with GB as strongly as PVA (Figure R10b,c).

In summary, surface interaction significantly affects the material's molding, performance, and stability. Because of the critical influence of surface functional groups on the assembly of GB, we chose PVA as an essential assembly element for the robust assembly of the rigid cavity layer in RCWSM, considering its chemical interactions with the GB surface.

7. While the paper mentions the mechanical properties and energy absorption performance of the material, has there been research on durability and long-term stability? For example, how does the material perform under different environmental conditions, and what is the performance degradation after long-term use?

**Thank you for the valuable suggestions. To evaluate the application potential in the daily application scenarios, we performed the UV accelerated aging test, in order to evaluate the potential for practical applications after long-term use by comparing the difference in energy absorption properties of materials before and after UV accelerated aging treatment. After UV aging treatment for 20 days under UV irradiation intensity of 2.00 W m^{-2} (Figure R11), we performed the static compression test with a Shimadzu AGX-V universal testing machine at a loading rate of 1 mm min^{-1}. Shape and behavior of compressive stress-strain curve of the sample before and after UV aging has little difference (Figure R11b). After 20 days of UV aging treatment, RCWSM can maintain similar compressive strength and energy absorption (Figure R11c).

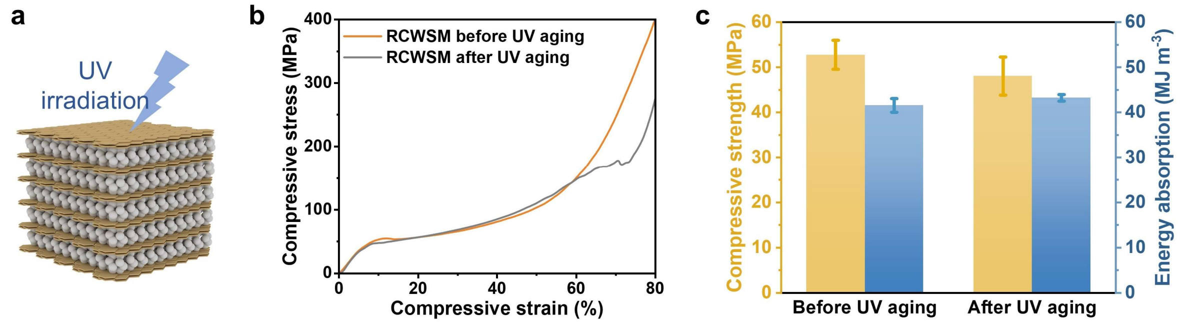


Figure R11. Performance changes of RCWSM after 20 days of UV aging treatment. a, Schematic of the UV aging treatment. b, Compressive stress-strain curve of the RCWSM before and after UV aging treatment. c, Compressive strength and energy absorption of the RCWSM before and after UV aging treatment. Error bars show standard deviation with 4 repeats.

Additionally, we performed the cyclic compression test to simulate the practical application scenario under the condition of cyclic stress. Figure R12 shows the result of the cyclic compression test. After 200 loading-unloading cycles, the maximum stress of RCWSM has no significant change, demonstrating the long-term stability of cyclic compressive stress resistance for RCWSM.

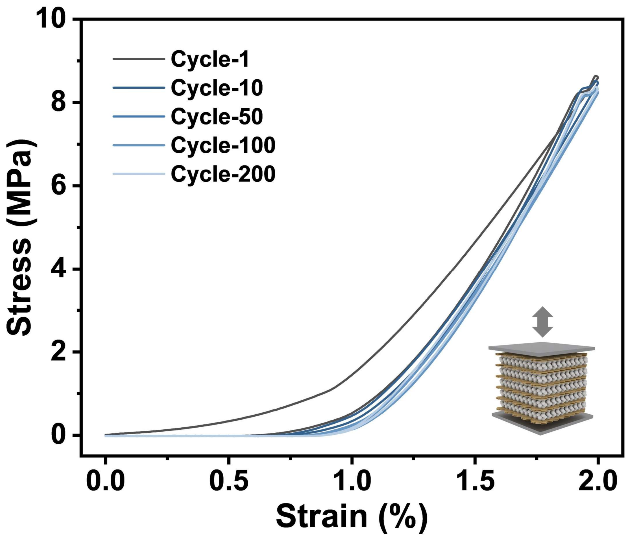


Figure R12. Cyclic compressive stress-strain curve of the RCWSM. Inset: schematic of the cyclic compression test for RCWSM, marked the compressive stress direction of the test.

Therefore, our RCWSM is resistant to UV irradiation and cyclic compressive stress, showing durability in practical environmental conditions. We have added Figure

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R11 and Figure R12 as Supplementary Figure 27 and Supplementary Figure 28, respectively, on page 13 of Supplementary information. We have also supplemented the relevant introduction on page 12 of the manuscript. UV aging treatment condition and cyclic compression test methods have been added in the Method section on page 17 of the manuscript.

8. The paper mentions various external factors affecting material performance, such as hydrogen bonds, covalent bonds, and electrostatic interactions, but is the mechanism of these factors fully understood? Is it possible to further optimize these factors to improve material performance?

**Thank you for the valuable suggestions. Robust assembly within the building blocks is the precondition for fabricating bioinspired composite materials effectively. For the fabrication of brick-and-mortar wall structure, multi-interactions are effectively employed to construct, including electrostatic interaction between carboxyl of cCNF and Ca^{2+}, as well as hydrogen bond and electrostatic interaction between carboxyl of cCNF and amino of amino mica. For the fabrication of a rigid cavity layer, as shown in Figure R10 of question 6, we tried various polymers to form the predesigned hydrogel of rigid cavity layers to determine the assembly capability of multiple polymers and GB. We chose the PVA, PAA, and PVAm as the typical model owing to their functional groups (hydroxyl, carboxyl, and amino). Due to the B-O-C covalent bond, PVA, after being mixed with GB, occurs in rapid gelation and a robust surface bonding on the surface of GB (Figure R10a). On the contrary, PAA and PVAm cannot robustly assemble with GB owing to the lack of strong surface interaction (Figure R10b,c). Except for PVA, which has a B-O-C covalent bond with GB, other polymers cannot mold stably to form the predesigned hydrogel of rigid cavity layers after being mixed with GB.

We then assembled two predesigned hydrogel layers and studied their interfacial bonding. After external forces break the interlayer interface, GB in the rigid cavity layer adheres to the surface of the wall layer, and mica platelets in the wall layer adhere to the surface of the rigid cavity layer, indicating the robust combination of two different hydrogel layers (Figure R13).

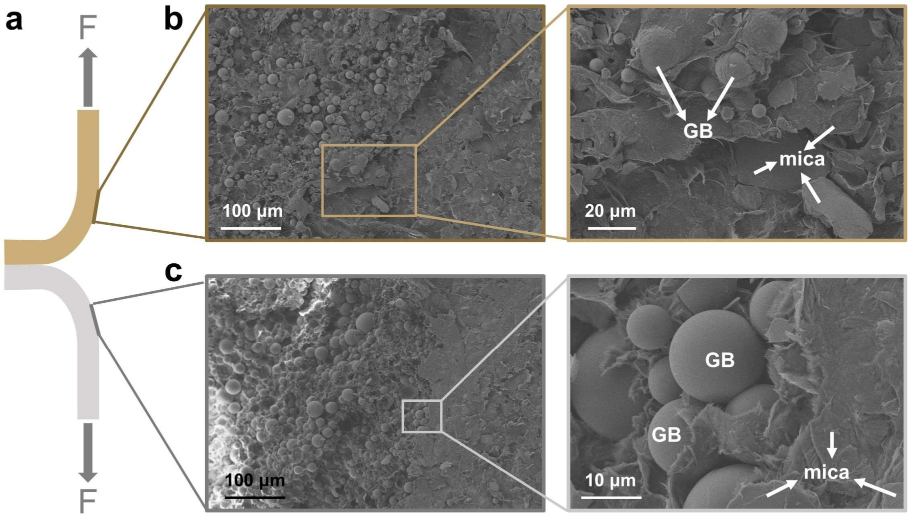


Figure R13. The interfacial bonding of two different initial hydrogel layers. a, Schematic of the 180° peel test. b, SEM images of the rigid wall initial hydrogel layer surface after 180° peel test. c, SEM images of the rigid cavity layer initial hydrogel layer surface after 180° peel test. All the SEM images were obtained from the initial hydrogel after 180° peel test and dried with supercritical CO_2.

In summary, surface interaction has a significant effect on molding. Because of the influence of surface interaction on the assembly of GB, considering the chemical interactions with GB surface and functional groups of GB and polymer, we chose PVA as an essential assembly element for the robust assembly of rigid cavity layer in RCWSM. Regarding the new mechanisms of GB and PVA, there is still a lot of room for future research, and it is possible to optimize these factors further to improve material performance.

We have added Figure R13 as Supplementary Figure 22 on page 10 of Supplementary information. We have also supplemented the corresponding description on page 8 of the manuscript.

9. The authors show the compression resistance of the material in the vertical direction, which is indeed very important. But what about its compressive properties in the horizontal direction?

**Thank you for the valuable suggestions. Based on your suggestion, we have performed the compressive test of the RCWSM in the vertical direction. All cuboid samples (about 4 mm × 4 mm × 4 mm) were tested at room temperature via compressive tests (Figure R14). The compression test was performed with a Shimadzu AGX-V universal testing machine at a loading rate of 1 mm min^{-1}. The compressive stress-strain curve embodies ~61 MPa of compressive strength and ~961 MPa of compressive modulus (Figure R14). The compressive stress-strain curve in Figure R14 shows a remarkable decrease after the elastic deformation, which demonstrates the difference in the curve shape of different directions under the compression stress.

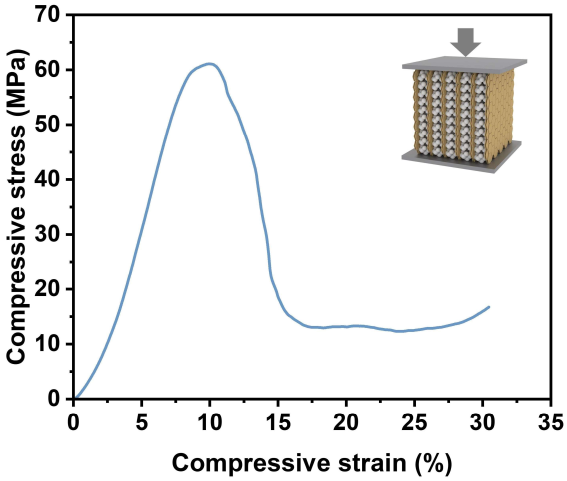


Figure R14. Compressive stress-strain curve of the RCWSM under the horizontal direction stress. Inset: compressive stress direction of the RCWSM.

Reviewer #2:

The authors reported an integral synchronous assembly strategy of cuttlebone-inspired structural material by hierarchical predesigned hydrogels. This is an important work about the integral fabrication of bio-inspired lamellar multiple sophisticated architectures represented by the “rigid cavity-wall” exquisite structure of cuttlebone. By layer-by-layer assembly and then under directional pressure, a “rigid cavity-wall” structure can be prepared orderly and robustly. The cuttlebone-inspired structural material has excellent strength and energy absorption. The authors also reported a new chemical bonding phenomenon of surface covalent interaction between PVA and hollow glass bubbles, which is promising for the fabrication of bio-inspired micro-cavity structures. The manuscript is well-organized and clearly presented with adequate data. Therefore, I recommend publishing this interesting work in Nature Communications.

**We appreciate your positive comments and encouraging words. In this work, we engineered a hydrogel integral synchronous assembly strategy based on predesigned cross-scale organic/inorganic hydrogels to construct hierarchical cuttlebone-inspired “rigid cavity-wall” architectures. After careful revision, we believe that the quality of this manuscript has been improved.

A few minor points for the authors to consider:

1. The hierarchical predesigned hydrogel integral synchronous assembly strategy is impressive. During this assembly process, how to achieve the robust interlayer bonding between different structures? I suggest the authors add relevant interpretation to fully display this novel assembly strategy of bioinspired structural materials with multiple different micro-nano architectures.

**Thank you for recognizing our work and the constructive comments on this manuscript. Interlayer bonding plays an essential part in the synchronous assembly process of two different kinds of structures. Specifically, we utilized electrostatic interactions between the quaternary ammonium group in quaternized cellulose nanofiber (qCNF) and the carboxyl group in carboxylated cellulose nanofiber (cCNF) to enhance interlayer bonding. As hydroxyl-rich building blocks, dense hydrogen bonding within these two kinds of cellulose nanofiber (CNF) and polyvinyl alcohol (PVA) was employed to further improve the interlayer bonding. We further assembled two predesigned hydrogel layers and studied their interfacial bonding. After external forces break the interlayer interface, GB in the rigid cavity layer adheres to the surface of the wall layer, and mica platelets in the wall layer adhere to the surface of the rigid cavity layer, indicating the robust combination of two different hydrogel layers (Figure R1).

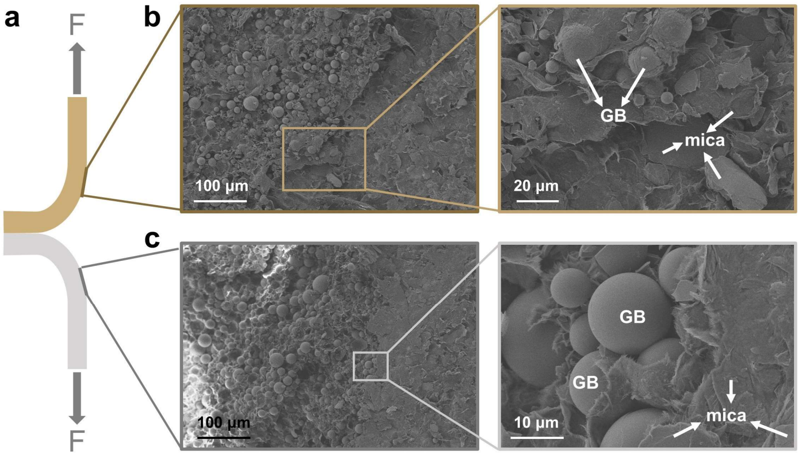


Figure R1. The interfacial bonding of two different initial hydrogel layers. a, Schematic of the 180° peel test. b, SEM images of the rigid wall initial hydrogel layer surface after 180° peel test. c, SEM images of the rigid cavity layer initial hydrogel layer surface after 180° peel test. All the SEM images were obtained from the initial hydrogel after 180° peel test and dried with supercritical CO_2.

We have added Figure R1 as Supplementary Figure 22 on page 10 of Supplementary information. We have also supplemented the corresponding description on page 8 of the manuscript.

2. During the solid-state crosslinking interaction process of GB/PVA/qCNF initial gel, the modulus changes largely. How does the viscosity change in this process?

**Thank you for the constructive comments. Based on your suggestion, we have performed the rheology test of GB/PVA/qCNF initial gel to analyze the viscosity change during the solid-state crosslinking interaction process. In Figure R2, during the test, the viscosity of the initial gel increases notably. While reaching approximately 10000 seconds, the viscosity of the hydrogel increases slowly and stably with step time. It further demonstrates the apparent interaction within the initial hydrogel of the rigid cavity layer, which is the significant precondition for the robust fabrication of the rigid cavity layers in the “rigid cavity-wall” structural material (RCWSM). We have added Figure R2 as Supplementary Figure 16 on page 7 of Supplementary information and supplemented the corresponding text on page 4 of the manuscript, which has been highlighted. In the rheological test section on page 16 of the manuscript, the rheological test method has been supplemented.

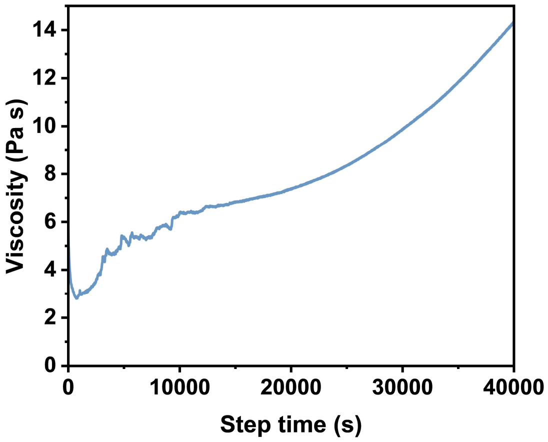


Figure R2. The rheological properties of the GB/PVA/qCNF initial gel of rigid cavity layer, which shows the change in viscosity with the step time.

3. The total amount and addition form of crosslinking $CaCl_2$ solution should be introduced in detail.

**Thank you for the valuable suggestions. For the Ca^{2+} crosslinking process, we achieved the robust assembly between amino mica and cCNF by spraying $CaCl_2$ (200 mL, 1 mol L^{-1}) evenly on the mixed slurry of amino mica and cCNF, and then washed three times with deionized water. We have added corresponding text in the Method section on page 14 of the manuscript.

4. In situ X-ray microtomography coupled with compression testing is used to interpret the failure process of the RCWSM under static compression. It is an attractive visualization of the destruction process, but more details should be provided on why the compressive strains of 0%, 10%, 30%, 65%, and 80% are the representative statuses.

**Thank you for the valuable comments. To interpret the compression failure process of the RCWSM visibly, we used *in situ* compression-X-ray microtomography characterization to show the dynamic change of the internal microstructure of RCWSM (Figure R3). We collected the five representative instantaneous statuses during the compression process to further analyze, corresponding to five different compressive strains. 0% compressive strain represents the beginning of the compression process, while the RCWSM has not been deformed. When the compressive strain reaches 10%, the RCWSM approaches the limitation of elastic deformation, which is demonstrated in the corresponding compressive stress-strain curve (Figure R4). The internal microstructure of RCWSM at this specific status could illustrate the overall structural stability and structure rigidity after deforming elastically.

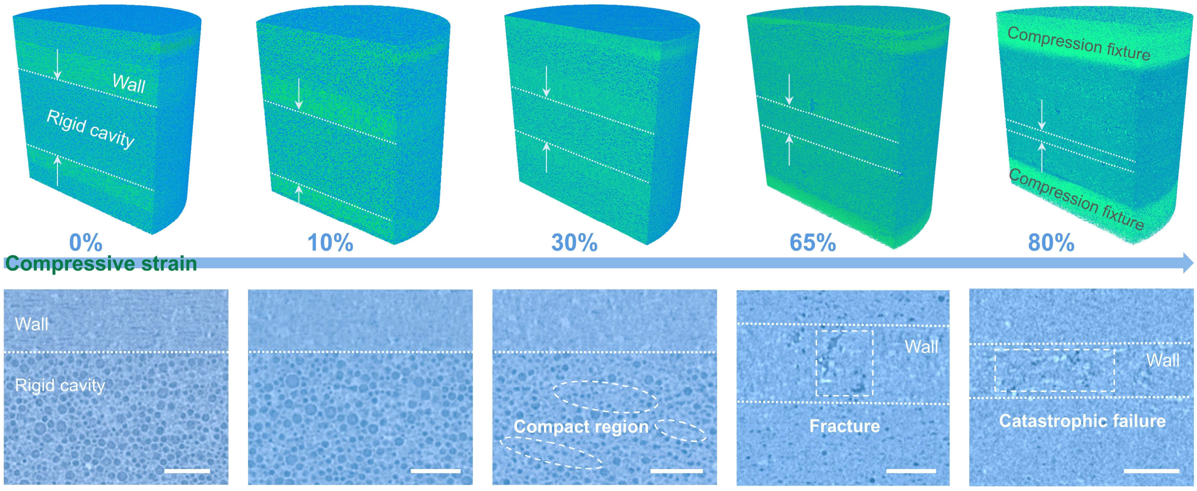


Figure R3. Failure process of the RCWSM under static compression coupled with *in situ* X-ray microtomography. 3D reconstructions and corresponding cross sections of the RCWSM under various compressive strains are shown to demonstrate the details of the failure process. Scale bar: 100 μm.

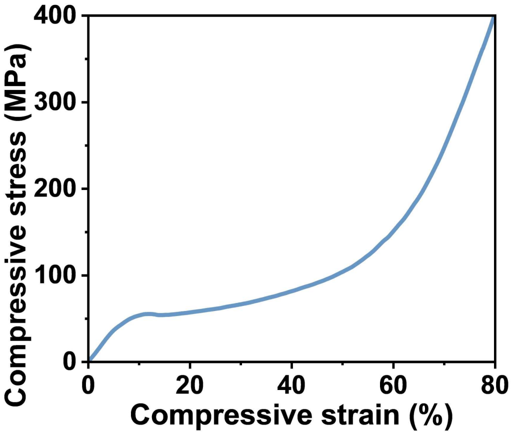


Figure R4. Compressive stress-strain curve of the “rigid cavity-wall” structural material (RCWSM).

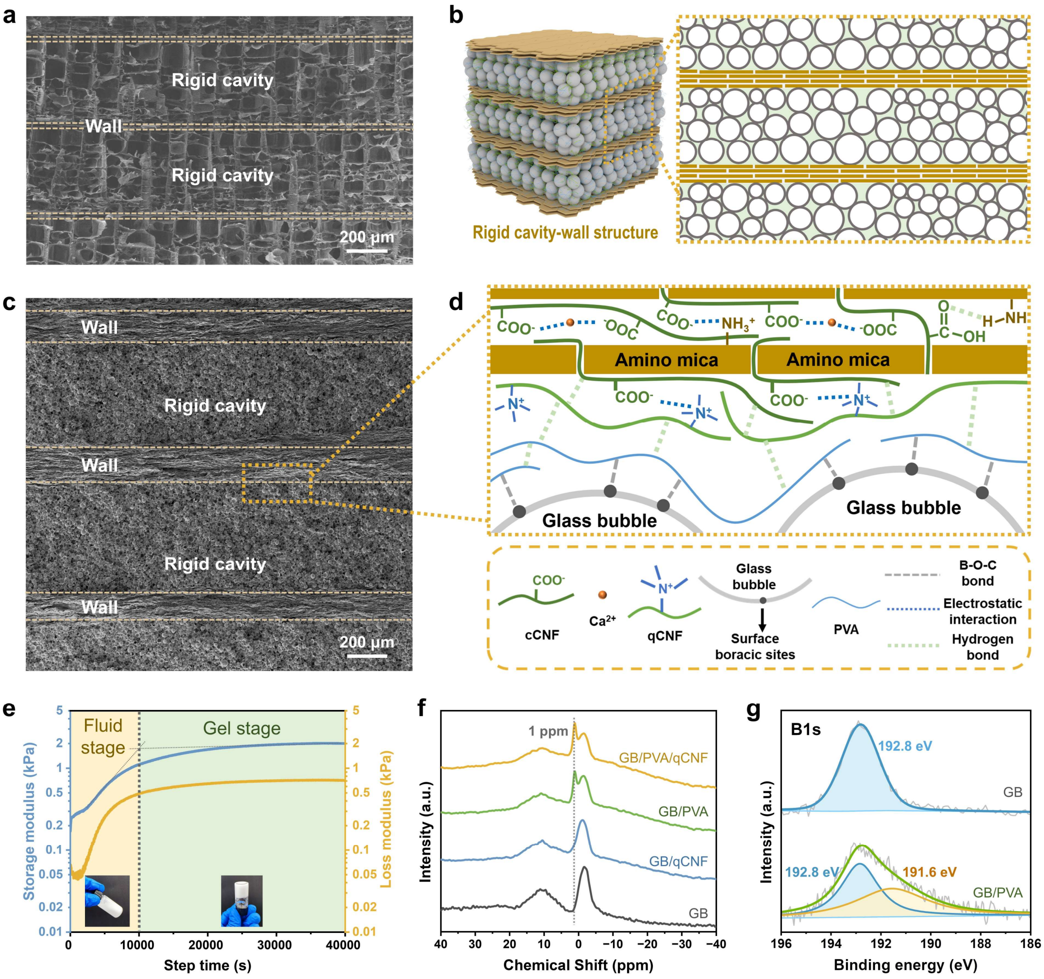
The 30% compressive strain status represents the typical plateau stage for energy absorption. Fragmentation and densification in the rigid cavity layer become evident at this status, while the brick-and-mortar wall layer remains intact. Under 65% compressive strain status, fracture of the brick-and-mortar wall layer can be observed, illustrating the change of failure form of internal microstructure. The 80% compressive strain status can visibly illustrate the catastrophic fracture failure condition of the internal microstructure of the overall material.

5. Supplementary Movies are suggested to add more texts to further describe the contents of movies.

**Thank you for the valuable suggestions. Following your suggestion, we have complemented more texts, including the brief introduction and annotation. The texts have been updated in the revised Supplementary Movies 1-5.

6. Some improvements should be made in image presentation. For example, the abscissa axis labels of Figure 1e-g should be more clarified by fine-tuning the position of the figure edge.

**Thank you for the valuable suggestions. Following your suggestion, we have carefully checked Figure 1 and all the figures in the manuscript. The image occlusion problem is encountered when converting images to PDF. Modified Figure 1 has been updated in the revised manuscript on Page 5.



Modified Figure 1. Design, structure, and interaction characterization of “rigid cavity-wall” structural material (RCWSM).

Reviewer #3:

In this manuscript, the authors reported a new type of bio-inspired composite hydrogel with promising crack growth resistance and high compressive strength, which I believe is the less studied area in hydrogel. The manuscript aligns with the scope of Nature Communications while its novelty will inspire the future development of hydrogel with high mechanical integrity where compressive strength is of the prime importance. I recommend publishing this manuscript after the author providing more information about the hydrogel based on the question below.

**We appreciate your positive comments and encouraging words. In this work, inspired by the cuttlebone's "rigid cavity-wall" with excellent energy absorption, we developed a robust hierarchical predesigned hydrogel assembly strategy to integrally synchronously assemble multiple organic and inorganic micro-nano building blocks to structural materials. With the help of your valuable suggestions, we have provided more information on the experiment and analysis of the predesigned hydrogel, and we believe that the quality of this manuscript has been improved after careful revision.

1. In this manuscript, the authors reported a composite hydrogel with layered structure and claimed that the embedded dovetail-like structure was observed at the interface of the two layers. I suggest the authors to provide more information about the bonding strength between two layers as the delamination could a big concern for layered composite.

**Thank you for the constructive suggestions. In this work, we achieve the integral synchronous preparation of different multiscale architectures through the robust combination of two different kinds of initial hydrogels. In order to characterize the interlayer interfacial bonding between the wall and rigid cavity layers at the hydrogel state, we performed the lap-shear test to measure the bonding strength (Figure R1a), which showed the 855 kPa of bonding strength. We also conducted the 180° peel test to measure the interfacial toughness of two different layers (Figure R1b). After external forces break the interlayer interface, glass bubbles in the rigid cavity layer adhere to the surface of the wall layer, and mica platelets in the wall layer adhere to the surface of the rigid cavity layer, indicating the robust combination of two different hydrogel layers (Figure R1c-e).

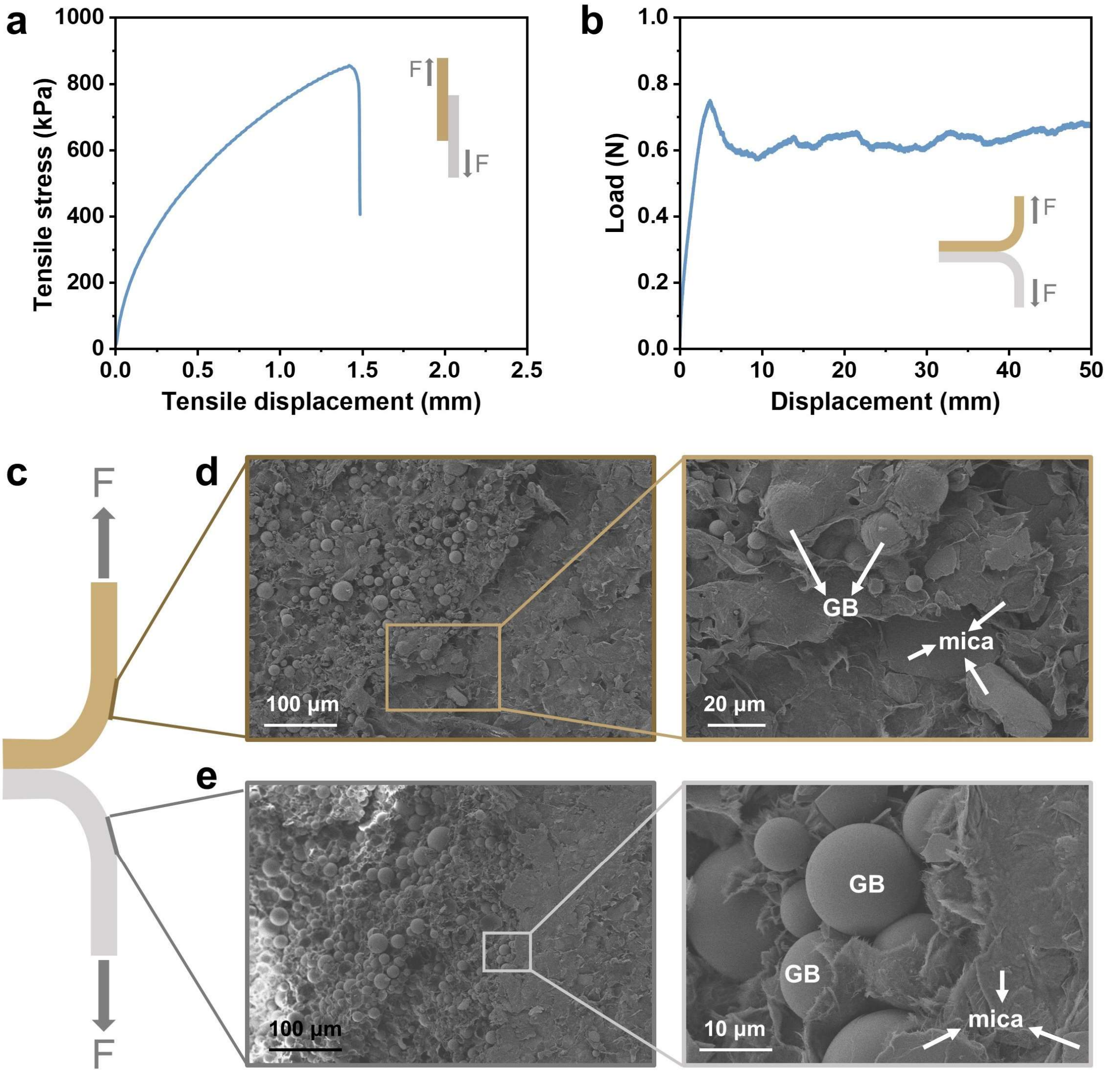


Figure R1. The interfacial mechanical properties of two different initial hydrogel layers. a, Tensile stress-displacement curve for the lap-shear test. b, Load-displacement curve for the 180° peel test. c, Schematic of the 180° peel test. d, Scanning electron microscopy (SEM) images of the rigid wall initial hydrogel layer surface after 180° peel test. e, SEM images of the rigid cavity layer initial hydrogel layer surface after 180° peel test. All the SEM images were obtained from the initial hydrogel after 180° peel test and dried with supercritical CO_2. GB: glass bubble.

We have added Figure R1 as Supplementary Figure 22 on page 10 of the Supplementary information. We have also supplemented the corresponding description on page 8 of the manuscript. Corresponding sample preparation and test conditions have been added in the Method section on pages 16 of the manuscript.

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2. After compression, it is worth to investigating the reversibility and durability of the hydrogel. It could be important for the hydrogel to reverse to its original state after deformation.

**Thank you for the valuable comments. Following your suggestion, we have further explored and interpreted the reversibility and durability of our two kinds of predesigned hydrogels for the assembly of “rigid cavity-wall” structural material (RCWSM).

The predesigned hydrogel of the brick-and-mortar wall layer is formed by the 3D nanonetwork of carboxylated cellulose nanofiber (cCNF) crosslinked with amino mica via amino-carboxyl multi-interactions (Figure R2). The overall structure of the hydrogel network lacks shape reversibility, which is beneficial to fabricating the brick-and-mortar bioinspired microstructure through the dehydration and densification process, as it allows for easier water removal from the gel network under the directional forming.

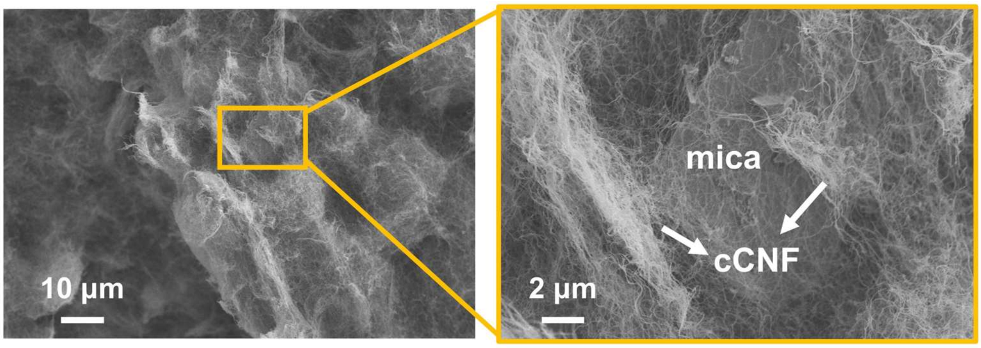

Figure R2. SEM images of predesigned hydrogel of the brick-and-mortar wall layer.

As for the predesigned hydrogel of rigid cavity layer, under compressive stress in the vertical direction, this hydrogel exhibits satisfactory shape reversibility and a certain capability to resist the stress, which is helpful for the molding process. We mixed glass bubble (GB) with polyvinyl alcohol (PVA)/quaternized cellulose nanofiber (qCNF) aqueous dispersion to prepare the predesigned hydrogel. After gelation, we cut the hydrogel sample, and half was dyed blue for a more straightforward presentation (Figure R3a). We put two halves of hydrogels together at room temperature (Figure R3b). Then, we observed that two halves of hydrogels combined back together, making it hard to pull apart the two halves from the original incision (Figure R3c). The above results show that GB-based hydrogel has obvious self-healing properties because of the multi-interactions within the micro-nano building blocks, including B-O-C dynamic covalent bonding and hydrogen bonding.

Figure R3. Predesigned hydrogel of rigid cavity layer. Self-healing process of PVA/qCNF/GB hydrogel, including as slices before healing (a), rapid healing behavior while occurring to sufficient interfacial contact (b), and the robust combination demonstrated under the stretch (c).

In conclusion, the predesigned hydrogel of the brick-and-mortar wall layer has no reversibility, but the predesigned hydrogel of the rigid cavity layer has outstanding self-healing properties, which can reverse to its original state. The properties of these two kinds of predesigned hydrogels play an important role in providing a time window for integrated assembly of RCWSM with cuttlebone-inspired exquisite microstructure.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have made a good revision. And thus, i recommend to accept as it is.

Reviewer #2 (Remarks to the Author):

The revised manuscript is recommended for publication.

Reviewer #3 (Remarks to the Author):

This paper is now ready for publication.