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3D-Printed Ultratough Hydrogel Structures with Titin-like Domains

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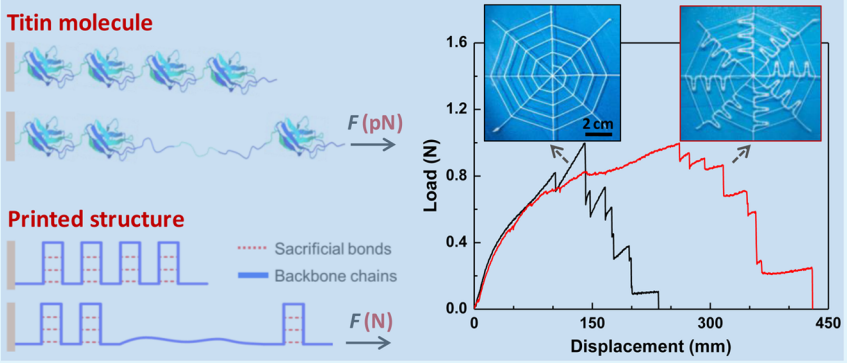
S *Supporting Information*



ABSTRACT: Titin is composed of repeated modular domains which unfold and dissipate energy upon loading. Here we employed such molecular-level paradigm to fabricate macroscopic ultratough hydrogel structures with titin-like domains, enabled by three-dimensional printing with multiple nozzles. Under stretch, the relatively thin and weak gel fibers in the printed structures break first and the hidden lengths postpone the failure of the main structures, mimicking the toughening principle in titin. These titin-like folded domains have been incorporated into a synthetic spider-web, which shows significantly enhanced extensibility and toughness. This work provides a new avenue of topological design for materials/structures with desired properties.

KEYWORDS: *tough hydrogel, 3D printing, biomimetic structure, folded domain, hidden length, titin*

Nature has demonstrated elegant paradigms and strategies for the design of versatile materials such as the silk, bone, and muscle.[1,2] These sophisticated natural systems form intricate multiscale and multiphasic structures to achieve specific functionalities.[3,4] For instance, the adhesive protein layers play a crucial role in resisting the separation of laminated tablets in nacre or mineralized filaments in bone, affording the natural composites with excellent mechanical properties even in the presence of flaws.[5−7] These proteins are often composed of structured domains that achieve extraordinary toughness by reversible breaking and healing of sacrificial bonds (Figure 1a), which dissipate a large amount of energy under loading and thus avoid catastrophic failure.[8] Such stepwise unfolding of molecular-level domains/loops releases the hidden length and relaxes the resultant force, which has been observed and quantified by single-molecule experiments.[7−10] This scenario is widely adopted in natural materials and endows them with remarkable toughness, extensibility, healing, adhesion, etc.

Inspired by nature, scientists have designed biomimetic polymers with modular folded domains at single-molecule level, providing stepwise release of hidden length under pulling.[11−14] These biomimetic polymers have been used as building blocks to prepare bulk materials; however, the characteristic behaviors of protein domains in response to force become vanishingly small. In particular, the stepwise release of molecular-level hidden length cannot be observed at the macroscopic level because of the separated length scales. It remains a challenge to

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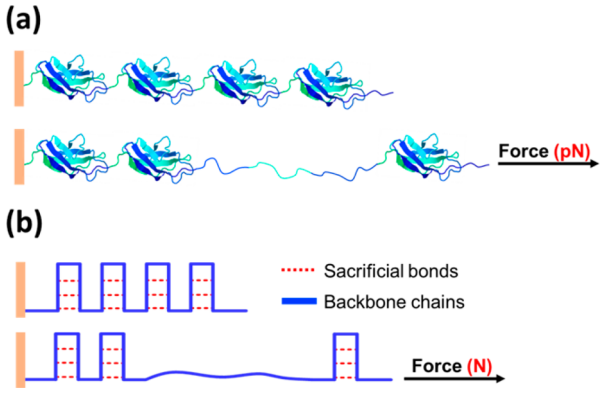

Figure 1. (a) Modular structure of single proteins (such as titin) and unfolding of repeated domains by breaking internal noncovalent bonds under force. Adapted from ref 13 with permission. Copyright 2004 American Chemical Society. (b) Biomimetic macroscopic structure with folded domains. Under pulling, the sacrificial bonds break to release the "hidden length", thereby relaxing the resultant force in the backbone chains.

directly correlate such molecule-level structures to the mechanical performance of bulk materials.[11−13] In addition, the concept of sacrificial bond and hidden length has been applied to the development of ultratough double-network hydrogels, which consist of brittle and ductile networks; under loading, the brittle network gradually fractures to effectively dissipate energy and thus toughen the integrated gels.[15] However, this bioinspired strategy needs elaborate molecular designs in the intact materials with hierarchical structure and programmed breaking of sacrificial bonds.

Alternatively, the concept of sacrificial bond and hidden length could be directly applied to the design of structured materials with desired toughness at macroscopic level.[16,17] Here we demonstrate the design of gel structures with relatively weak parts that break ahead of the stronger ones under loading, accompanied by the consecutive release of intentionally buried length that toughens the integrated structures (Figure 1b). These gel constructs with complex structures were fabricated by three-dimensional (3D) printing of plasticized polyion complex (PIC) solution in deionized water, where fast solidification occurred to form tough physical hydrogels.[18] Heterogeneous 3D printing with multiple nozzles was used to fabricate gel fibers of different diameters, corresponding to the backbone and sacrificial portions of a structure. The mechanical performance of the printed gel structures with multiple hairpins or folded domains was tested; under pulling, the thinner fibers broke ahead the thicker ones as expected, serving as the "sacrificial bonds" to release the "hidden length" without impairing the structural integrity, thereby postponing the catastrophic fracture of the main structure.[8] As a result, a typical sawtooth force−displacement curve was observed at the macroscopic level, indicating a similar dissipation process to the unfolding of biomacromolecules such as titin. Furthermore, these titin-like domains were incorporated into a synthetic structure mimicking spider-webs, with significantly enhanced toughness when compared to the counterpart without folded domains. This work may provide a new avenue in designing tough materials and structures to meet different loading demands, and also extend the use of hydrogels as load-bearing materials in various applications.[19−22]

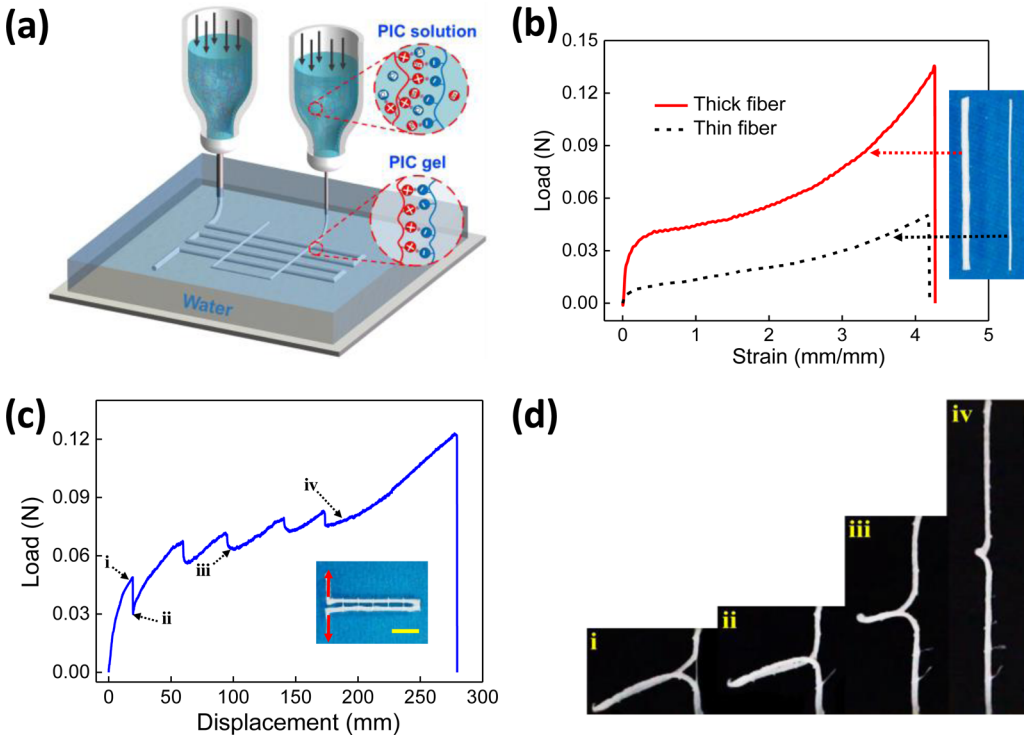

Figure 2. (a) Customized 3D printing system with multiple syringes on the basis of sol−gel transition of plasticized PIC solutions. (b) Tensile behaviors of a printed thin fiber (diameter: 0.20 ± 0.05 mm) and a thick fiber (diameter: 0.40 ± 0.05 mm). (c) Force−displacement curve and (d) snapshots of a hairpin structure containing five "sacrificial" thin fibers. The insets in b and c show the morphology of the tested samples. The red arrows in c indicate the stretching directions, and the scale bar is 1 cm.

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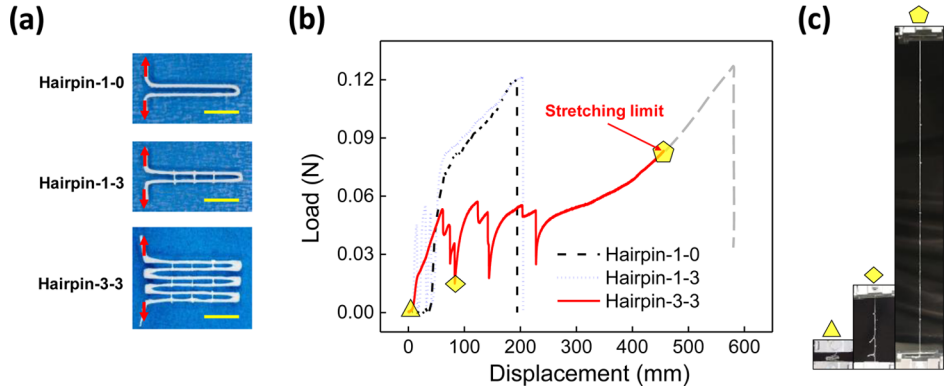

Figure 3. (a) Morphology of the printed hairpin-*m*-*n* structures, *m* and *n* denoting the number of hairpins and the number of sacrificial bonds within each domain. (b) Tensile behaviors of the structures in a. (c) Snapshots of the hairpin-3-3 structure under tension, which can be elongated by more than 50 times of the original length. The tensile curve of hairpin-3-3 is limited by the maximum range of the testing machine, denoted as "stretching limit" in (b), and the ultimate failure of hairpin-3-3 is extrapolated to occur at even larger deformation. The red arrows in panel a indicate the stretching directions, and the scale bar is 1 cm.

To fabricate gel structures with integrated sacrificial and backbone parts, which have disparate mechanical and/or geometric properties, we employed a 3D printing approach to directly "write" the "ink" material into desired architectures.[23−26] On the basis of the distinct strength of ionic bonding in PIC hydrogels in concentrated saline solution and water,[18] we selected the "ink" material as a viscous PIC solution of poly(3-(methacryloylamino)propyl-trimethylammonium chloride) and poly(sodium *p*-styrenesulfonate) (PMPTC/PNaSS), which carry positive and negative charges, respectively. The PIC was plasticized in the presence of concentrated saline solution, where the ionic bonding is screened and the rheology of PIC solution can be precisely regulated.[27,28] Thus, the plasticized PIC solution can be loaded into syringes and easily extruded out of nozzles in the form of fibers in different sizes (e.g., 0.7 and 0.3 mm) (see experimental details in the Supporting Information). After being printed into deionized water, the NaCl and counterions of PIC diffuse out, resulting in fast sol−gel transition and the formation of tough physical PIC hydrogels (Figure 2a). The diameter of thick and thin gel fibers at equilibrium state is 0.40 ± 0.05 and 0.20 ± 0.05 mm, respectively. The precise path control of our printing platform enables the fabrication of complicated structures, and the high interfacial binding strength of different gel fibers results in integrated structures with combined mechanical properties.[18] Titin-like structures can therefore be mimicked at macroscopic level by printing gel structures with folded domains (geometric details of the printed structures are shown in Figure S1).

The prerequisite of certain printed parts breaking ahead of the main structure is that the gel fibers from differently sized nozzles have distinct mechanical properties. As shown in Figure 2b, the printed gel fibers with different diameters exhibit similar breaking strain, ca. 420%. However, the load-bearing abilities are different; the thin gel fiber breaks at a load of 0.05 N, while the thick one can bear a load up to 0.13 N. It can be expected that when being stretched simultaneously, the thin fibers in the printed structures would break ahead of the thick ones, even though they have similar stress−strain curves due to the same constituents (Figure S2).

To demonstrate the concept of heterogeneous breaking, we fabricated and tested a hydrogel structure with five "sacrificial" thin fibers to mimic one folded domain of titin molecule (Figure 2c). The force−displacement curve showed sawtooth behavior with five regularly separated peaks, corresponding to the sequential breaking of the thin fibers under stretch; this feature is analogous to the unfolding of titin domains, despite at different scales. Each drop of the ramping force corresponds to the releasing of hidden length, coinciding with the sequential breaking of thin gel fibers that protect the main structure (Figure 2d). Therefore, the unfolding of hairpin-like structure requires extra energy input to break the "sacrificial" thin fibers that improve the toughness of printed gel structures. Also, the release of "hidden length" to the main structure endows the integrated structures with high stretchability.

To investigate the contributions of hidden length and sacrificial bonds in a more systematic way, we fabricated various hairpin-*m*-*n* structures where *m* and *n* denote the number of hairpins and the number of sacrificial thin fibers within each domain (Figure 3a). The force−displacement curve of hairpin-1-0 (folded thick fiber without constraining thin fibers) shows that negligible force is detected until the folded length is straightened (Figure 3b). In contrast, the force−displacement curve of hairpin-1-3 clearly exhibits three drops of loading force before the thick fiber is completely unfolded, indicating the role of thin fibers in dissipating energy and enhancing the toughness of the structure. The summation of energy accompanied by the breaking of thin fibers should account for the improved toughness. Under large extensions, the tensile curve of hairpin-1-3 becomes similar to that of hairpin-1-0. This result indicates that the presence of thin fibers to constrain the folded domain endows the printed structure with high initial stiffness and toughness, despite its similar breaking strength.

The printed hairpin structure can be further improved by involving stackable domains, mimicking the modular array in natural fibers.[8,14] We fabricated hairpin-3-3 gel structure with three folded domains, each of which is constrained by three microconnections (Figure 3a). This multidomain structure also exhibits sawtooth tensile curve in Figure 3b, and the force peaks are superimposed to form three main groups, corresponding to the domain unfolding, each of which consists of several daughter peaks caused by the failure of thin fibers. This hierarchical phenomenon stems from the continuous breaking of thin gel fibers within individual folded domains. During the stretching process, the thin fibers of each domain first sequentially break and release a fraction of the total hidden length, concomitant with the drop of force by a small

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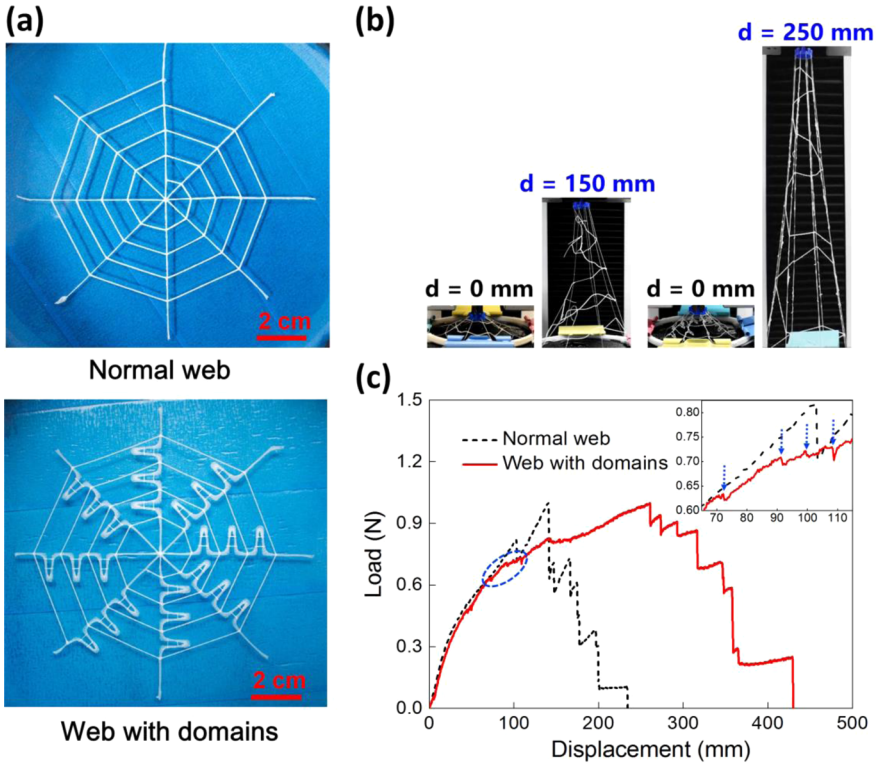

Figure 4. (a) Photos of printed network structures mimicking spider-webs: a normal web versus a web with folded domains. (b, c) Snapshots at certain displacement, *d*, (b) and force−displacement curves (c) of the two structures in a. The arrows in the inset of c indicate the breaking events of sacrificial bonds that lead to the unfolding of individual domains.

amplitude. When one domain is fully unfolded, a relatively large hidden length is released to the structure, and a large drop of force is observed. This hairpin-3−3 structure did not break even at the stretching limit of the tensile machine in use (Figure 3c), implying a breaking strain more than 50 times of its original length. It turns out that the combination of multiple folded domains with sufficient sublevel connections is a more effective recipe to enhance both extensibility and toughness of the gel structures.

The architecture of spider-webs has been proven to provide excellent mechanical performance under different loading conditions with an efficient distribution of materials.[29,30] To further demonstrate the potential application of our biomimetically designed structure, we fabricated a spider-web-like construct by incorporating multiple folded domains to the radial threads. Our goal is to explore the opportunity of further enhancing the toughness of spider webs and other network structures by including macroscopic folded domains. The structures of printed webs with and without folded domains are shown in Figure 4a; three folded domains with two sacrificial thin fibers in each domain are placed within each of the eight radial threads, which sustain the main part of applied load and dominate the energy absorption.[29] The tensile behaviors and force−displacement curves of the webs with and without folded domains have been shown in Figures 4b, c, when the force was applied at the web center and pulled perpendicular to the web plane. With the incorporation of folded domains, an evident drop of loading force appeared at a displacement of ∼260 mm because of the breakage of radial threads, which was twice that of the normal web, indicating that the catastrophic failure was effectively delayed by the incorporation of folded domains. Moreover, at the initial stage of stretching, the tensile curve of the normal web was smooth, similar to that of straight gel fibers. However, in the presence of folded domains, the tensile curve showed subtle sawtooth peaks at the initial loading phase (the inset in Figure 4c), indicating the breakage events of weak gel fibers within the folded domains. As the result, the release of programmed hidden length and subconnections gave rise to twice of the extensibility and toughness in the normal web (Figure 4c). It is easy to envision that the mechanical performance of the weblike structures can be further enhanced by employing more sophisticated distribution of constrained and folded domains in macroscopic-level topological design.

In conclusion, we have demonstrated the design of biomimetic gel structures with folded domains that release hidden length in response to force at macroscopic scale. Via 3D printing with multiple nozzles, heterogeneous gel structures with relatively weak fibers that constrain and enforce the folded structure were fabricated and tested. Under pulling, the weak portion sacrificially breaks to postpone the failure of the main structure. The force−displacement curves of these printed structures exhibit a sawtooth pattern, resembling the forcible response of titin despite of the vastly different scales. The programmed release of hidden length from stacking domains has effectively improved the extensibility and toughness of the gel structures. Such strategy was used in designing a spider-web-like gel structure with folded domains, which showed remarkably enhanced toughness compared to that of a normal web. This work may provide a new avenue for the design of artificial materials with desired mechanical properties by sophisticated material distribution within multilevel or heterogeneous structures.

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■ ASSOCIATED CONTENT

Supporting Information

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Experimental details, materials and printing parameters, geometric details of printed structures, stress−strain curves of printed fibers with different diameters (PDF)

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Notes

The authors declare no competing financial interest.

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