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A Novel Rapidly Self-healing Host-guest Supramolecular Hydrogel with High Mechanical Strength and Excellent Biocompatibility

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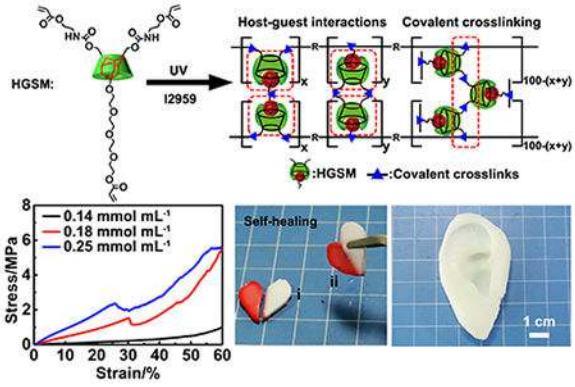
Abstract

It is still a challenge to achieve both excellent mechanical strength and biocompatibility in hydrogels. Here we exploited two interactions to form a novel biocompatible, slicing-resistant and self-healing hydrogel. One is the molecular host-guest recognition between a host (isocyanatoethyl acrylate-modified β-cyclodextrin, β-CD-AOI$_2$) and a guest (2-(2-(2-(2-(Adamantyl-1-oxy)ethoxy)ethoxy) ethoxy)ethanol acrylate, A-TEG-Ad) to form novel "three-arm" host-guest supramolecules (HGSMs). Another is the covalent bonding between HGSMs (achieved by UV-initiated polymerization) to form a strong crosslinking in the hydrogel. The host-guest interaction enabled the hydrogel to become rapidly self-healing; cutting it formed fresh surfaces with dangling host and guest molecules (due to the breaking of host-guest recognition), which rapidly recognized each other again to recombine the cut surfaces and thus heal the hydrogel. The smart hydrogels hold promise for use as biomaterials in soft tissue repair.

Graphical Abstract

A novel "three-arm" host-guest supramolecular monomer is designed to avoid steric hindrance effect, enabling more efficient host-guest inclusion. The monomer is further polymerized into hydrogels that are not only strong, slicing resistant and capable of rapid self-healing, but also biocompatible. The hydrogels hold promise for potential use as biomedical materials in regenerative medicine.



Keywords

host-guest supramolecule; supramolecular hydrogel; high mechanical strength; self-healing; biocompatibility

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Hydrogels derived from natural polymers have drawn great attention by virtue of their distinct biocompatibility and biodegradability.[1] Unfortunately, hydrogels suffer from poor mechanical strength owing to their physical structure and high water content, limiting their clinical medical applications.[2] For example, they are easily broken due to the lack of energy dissipation mechanisms to slow fracture propagation.[3] For single network

hydrogels, the low density or irregular distribution of crosslinking points results in different lengths of polymer chains, which readily induce the initial crack. Many efforts have been made to improve the strength of hydrogels.[4] However, balancing the biocompatibility and robustness of hydrogels is still a daunting challenge.

Host-guest supramolecular interactions have been exploited in forming hydrogels. The current strategies mainly relied on the grafting of host and guest molecules to the polymer chains in order to take advantage of the host-guest interactions.[5] However, such strategies were deficient because the low grafting degree of functional groups and steric hindrance effect of polymer backbones significantly reduced the mechanical strength of the hydrogels.[6] Although other monomers and functional groups were added to the system in order to solve this problem, these procedures either increased the difficulty in preparing the hydrogels, still did not improve their mechanical properties, or even rendered them less biocompatible.[7] Hence, there is a pressing need in the development of a facile method for constructing a supramolecular hydrogel with a combination of both good biocompatibility and outstanding mechanical strength.

Herein we report a novel strategy for fabricating robust and biocompatible hydrogels comprising host-guest inclusion and covalent networks. In this strategy, host-guest supramolecules (HGSMs) serve as monomers, which are polymerized into hydrogels via a facile ultraviolet (UV) -initiated polymerization (Figure 1). The HGSMs are formed due to the host-guest recognition of β-CD-AOI_2 (the host molecule) and A-TEG-Ad (the guest molecules) in water. Because the HGSMs in our strategy are prepared from the host and guest molecules before polymerization, our strategy avoids the problem of the steric hindrance effect of polymer backbones, enabling more efficient host-guest inclusion in the HGSM. The host-guest inclusion complex was broken into dangling free host and guest molecules when the hydrogels were damaged. Immediately, the dangling host and guest molecules could recognize each other again to recombine damaged surfaces, leading to the rapid healing of the hydrogels. Furthermore, our strategy employs covalent bonds to integrate all the HGSMs into a strong network, making the resultant hydrogels robust, fatigue-resistant and slicing-resistant. Moreover, the raw materials used in our strategy are biocompatible[8] and the overall fabrication process is mild. Thus, the host-guest supramolecular hydrogels (HGSMGels) developed by our strategy (Figure 1) bear both good mechanical strength and good biocompatibility for potential applications as biomedical materials.

Briefly, A-TEG-Ad was prepared through a two-step chemical modification[9] as shown in Figure 1A. The ^{1}H NMR spectrum indicated that it was successfully synthesized with a high purity (Figure S1). β-cyclodextrin (β-CD) was modified with 2-isocyanatoethyl acrylate (AOI) via a nucleophilic addition reaction (Figure 1A) to form the host molecule (β-CD-AOI_2) under a strict control of the reactant ratio and extent of reaction. The Fourier transform infrared (FT-IR) spectrum of β-CD-AOI_2 (Figure S2) showed IR bands at 1534 cm^{-1} and 1635 cm^{-1} representing the stretching vibrations of the secondary amides. This result, along with ^{1}H NMR spectrum (Figure S3) and MALDI-TOF MS spectrum (Figure S4), indicated the successful formation of β-CD-AOI_2. Furthermore, the signal integral area ratio between C_1-H of β-CD (d, 7H, 4.9 ppm) and the double bond (-CH=CH_2, m, 6H,

5.8~6.4 ppm) was 7: 6 in the ^{1}H NMR spectrum (Figure S3), suggesting the presence of approximately two double bonds in each β-CD molecule.

Subsequently, mixing oil-like A-TEG-Ad into an aqueous solution of β-CD-AOI_2 at an equimolar ratio resulted in the formation of the inclusion complex (HGSM) because the hydrophobic interior cavity of β-CD-AOI_2 could either partially or entirely accommodate the lipophilic A-TEG-Ad.[5b,6] There was a layer of yellow oil floating on the aqueous solution of β-CD-AOI_2 at the beginning of the mixing process (Figure 1B). However, after stirring for 24 h, the oil-water interface vanished, and the mixture became transparent again and remained so even after 12 h, further confirming the successful inclusion of the guest molecule into the host molecule to form a three-arm HGSM with three active functional groups (-C=CH_2) (Figure 1A). Furthermore, its 2D ROESY NMR spectrum (Figure S5) shows that the resonance signal between the protons in the cavity of β-CD ($C_{2\text{-}6}$-H) and the α-, β-, and γ-H protons of adamantane (Ad) exhibited the Nuclear Overhauser Effect (NOE), indicating the successful inclusion of A-TEG-Ad into the cavity of β-CD-AOI_2. These results demonstrated that constructing the HGSMs based on small molecules (forming the host-guest inclusion before polymerization) significantly improved the efficiency of supramolecular assembly, when compared to that by directly modifying polymers with these functional moieties (which in turn enhanced the steric hindrance effect of polymer backbones).

HGSMxGel hydrogels were then prepared by a simple UV-initiated polymerization after adding I2959 (a photoinitiator) into HGSM solutions with different concentration gradients (0.071 mmol mL^{-1}~0.25 mmol mL^{-1}, the "x" represent the concentration of HGSM). In this polymerization method, each HGSM can form covalent bonds with the surrounding HGSM molecules, resulting in the formation of a stable gel with a high-density three-dimensional (3D) network structure (Figure 1A). HGSMGels could be injected into different molds to fabricate various shapes, such as ear-shape, pentagram, triangle and heart, suggesting that hydrogels have good processability and formability (Figure 1C and S6). Scanning electron microscopy (SEM) images reveal that the microstructure of the lyophilized gels was composed of a high density of pores and macroparticles (Figure S7). Such microstructure was expected because β-CD molecules could easily combine to form molecular aggregates during polymerization. Similarly, the particle size of the hydrogels shown in the SEM image coincides with the size of the HGSMs in the solution prior to polymerization (Figure S8). The swelling kinetic curves (Figure S9) showed that all hydrogel samples exhibited a similar swelling trend with an equilibrium reached within 300 min, and their swelling ratio decreased with the reduction in the HGSM concentration. The swelling ratio increased from 265% to 520% as the concentration changed from high to low, which was ascribed to the increase in the intermolecular strength of the hydrogel network because of the higher crosslinking density. This result was consistent with the trend in the surface morphology of the hydrogels (Figure S7).

To further investigate the UV-initiated polymerization and the effect of the host-guest crosslinking on the hydrogels, rheological measurements were performed before and after the polymerization during the formation of the hydrogels. First, oscillatory frequency sweeps during the polymerization process showed that the polymerization started within 25 s

and displayed the capability of rapid curing (Figure S10). After 200 s, the modulus of the HGSMGel remained stable, indicating the completion of polymerization. The hydrogel samples at various concentrations were compared using strain sweeps (0.1%~2000%). The results shown in Figure S11A provided important information about the relationship between the polymer concentration and the strength and deformability of the hydrogels. It was observed that the hydrogel strength increased with the increase of the polymer concentration, whereas the deformability of the hydrogels decreased slightly because the covalent network was further enhanced. As shown in Figure S11B, the frequency sweep curves of the HGSMGels also illustrated that the G' and G'' increased with the HGSM concentration, which is consistent with the results of the amplitude sweeps. Specifically, compared with the G' value of $HGSM_{0.07}Gel$, the G' value of $HGSM_{0.14}Gel$ increases from 0.941 kPa to 141.64 kPa at a 10 rad s^{-1} angular frequency owing to the increase in network strength with the increased HGSM concentration. Moreover, G' and G" were dependent on the frequency at 0.1-100 rad s^{-1}. This result could be attributed to the existence of a large amount of loose physical crosslinks in the network of the HGSMGels, although covalent crosslinking was present as well. In summary, these results indicated that there are two kinds of crosslinks forming a hybrid network in the HGSMGel structure: a covalent bonding crosslink due to the polymerization between HGSM and a noncovalent crosslink due to the host-guest molecular recognition.

The mechanical properties of HGSMGels were determined using compression, tensile and cyclic compression tests as well as an anti-slicing experiment. The stress-strain curves (Figure 2A, and Figure S12A) displayed that all HGSMGels exhibited not only high strength but also over 80% strain recovery. The hydrogels did not have a yield point or breaking point during the compression process, indicating their non-fragile nature. The HGSMGel exhibited a larger modulus with the increasing concentration as a result of the higher crosslinking density (Figure 2B and Figure S12B). Thus, the Young's modulus could be adjusted to a value between 0.1~9.13 MPa through the control of the concentrations. The hydrogels remained in their original condition without any damage on the surface after 10 loading-unloading cycles (Figure 2C and 2H), while the traditional PEGDA hydrogels were easily disrupted under same deformation (Figure S13). Although the recovery curves of the HGSMGels presented a large hysteresis, a common characteristic of weak-bond-reinforced (e.g., host-guest inclusion) hydrogels, the strength of the hydrogels was nearly identical at maximum deformation. The notable hysteresis loops in the loading-unloading cycle indicates that HGSMGel dissipated energy effectively. Interestingly, the squeezed HGSMGels could recover to the original shape within 8 seconds (Figure 2I), even after being squeezed for multiple times (Video S1). Moreover, the stretching length of HGSMGels could reach 48% (Figure 2D), which also displayed fairish elasticity. These results demonstrate that the HGSMGels are fatigue resistant, elastic, anti-compressing and can recover their shapes due to the reversibility of the existing host-guest interactions. Moreover, their mechanical strength has excellent comparability with human soft tissue.

Simultaneously, the crosslinked networks offered an energy dissipation mechanism to buffer the applied internal stresses and then delayed or averted the fractures. Additionally, most traditional hydrogels with a single covalent network, such as PEGDA gels and CDGels, were easily broken by either pressing them with a finger or slicing them with a cutter (Figure

2E and F). However, surprisingly, HGSMGels resisted against the slicing with an iron ruler cutter despite the largest cutting scale had been reached (Figure 2G and Video S2). Therefore, the high-strength HGSMGels showed fatigue-resistant and slicing-resistant properties because of the combination of host-guest interactions and covalent bonds in the hydrogel networks.

The self-healing capability was also investigated with HGSMGels when swelling equilibrium was reached (Figure 3). Two heart-shaped HGSMGels (one of them was colored by rhodamine B) were cut into two pieces. Then, the fresh cut surfaces with different colors were kept in close contact with a clamp at room temperature to form group (i) and (ii) (Figure 3A). In group (i), the two cut surfaces were coated with a large amount of free "adamantane" solution (actually, 1-bromoadamantane were added in DMSO solvent) in advance to inhibit the recombination of the dangling host and guest molecules between the two pieces. Group (ii) was not subjected to any treatment. After approximately 60 min, to validate the self-healing property, tweezers were used to clamp one of the pieces arbitrarily. We found that the two pieces in group (ii) were tightly integrated into the original hydrogel to achieve the healing and that the recovered hydrogel could further withstand repeated shaking without breakage (Figure 3A and Video S3). However, the cut surface of group (i) was pretreated with adamantane solution, and thus the two pieces could not be "healed" into the integrated hydrogel again. Figure 3B shows the microscopy images recorded during the self-healing process on the hydrogel. The results indicated that the notch on HGSMGels could autonomously self-heal within 60 min without any healing agent.

The mechanism of rapid self-healing was presented in Figure 3D. When the hydrogel was cut into two pieces, the host molecules (β-CD) and guest molecules (Ad) of the network at the cut surface were "free" due to the breaking of the host-guest inclusion at the interface. However, when the two cut surfaces were in close contact, the "free" host and guest molecules recognized each other and reformed a new host-guest inclusion network, enabling the hydrogels to rapidly self-heal (Figure 3D(ii)). When excess free guest molecules (Ad) were applied to the cut surfaces before they contacted each other (Figure 3D(i)), the hydrogels would lose their self-healing ability because there are no free host-guest recognition sites available for reforming the inclusion complex. To study the healing time scale of hydrogels with strain-induced damage, a maximum of 2000% amplitude strain sweep was performed to break the whole HGSMGel. Then, continuous cyclic deformation (1% strain→1000% strain→1% strain) under constant angular frequency (10 rad s^{-1}) was applied at 37 °C (Figure 3C). The high shear strain of 1000% was used to induce structural destruction for 60 s, which resulted in the decrease in the G' of the self-healing HGSMGel from ≈ 61 kPa to ≈ 30 Pa. Then, a low strain of 1% was employed to allow the hydrogel to heal for 300 s. During the time under the lower strain (1%), the G' of the self-healing HGSMGel recovered quickly to the initial value, and the hydrogel underwent a rapid sol-to-gel transition. Even after the hydrogel underwent several cycles, the value of G' remained consistent with the initial value.

The healing efficiency of HGSMGels was further measured using tensile testing. The stress-strain curves (Figure 3E) shows that the self-healed HGSMGels and the normal samples shared similar profiles except the former had a smaller elongation at break. These results

indicated that HGSMGels could rapidly self-heal when damaged at a high shear strain owing to the reversibility of host-guest recognitions. Namely, the host and guest molecules could immediately recognize each other to reform the host-guest inclusion complex to achieve self-healing after they are separated due to the hydrogel damage.

To evaluate the biocompatibility of HGSMGel, we cultured mBMSCs and MDSCs for 1, 3, 5 and 7 days in the presence of HGSMGel extracts by a CCK-8 assay (Figure 4A and B). When cultured with different concentrations of hydrogel, both MDSCs and mBMSCs have a proliferation rate consistent with that of the positive control group (a normal complete medium without hydrogel). This result shows that the HGSMGels have good cell compatibility. For another demonstration, the Live/Dead Staining Kit was employed to assess the influence of the hydrogel extracts on MDSCs cultured for 1, 3, 5 and 7 days (Figure 4C). The live cells were stained green, and the dead cells were stained red. The MDSCs cultured with the HGSMGel extracts exhibited similar cell proliferation densities as the control group. Hence, these results suggest that the HGSMGel extracts did not significantly influence the viability of cells and that the novel HGSMGels are promising candidate biomaterials.

In summary, “three-arm” host-guest supramolecular monomers with highly efficient host-guest interactions were successfully constructed, resulting in novel high-strength biocompatible supramolecular hydrogels (HGSMGels) based on two types of interactions, the host-guest interaction to form inclusion complex and the covalent bonding between the inclusion complexes. Mechanical tests demonstrated that these HGSMGels are strong, fatigue-/slicing-resistant and capable of rapid self-healing due to the reversibility and energy-dissipation mechanism of the host-guest interaction in the hydrogels. Such HGSMGels with both outstanding mechanical properties and biocompatibility hold promise for potential use as biomedical materials in regenerative medicine (e.g. cartilage repair).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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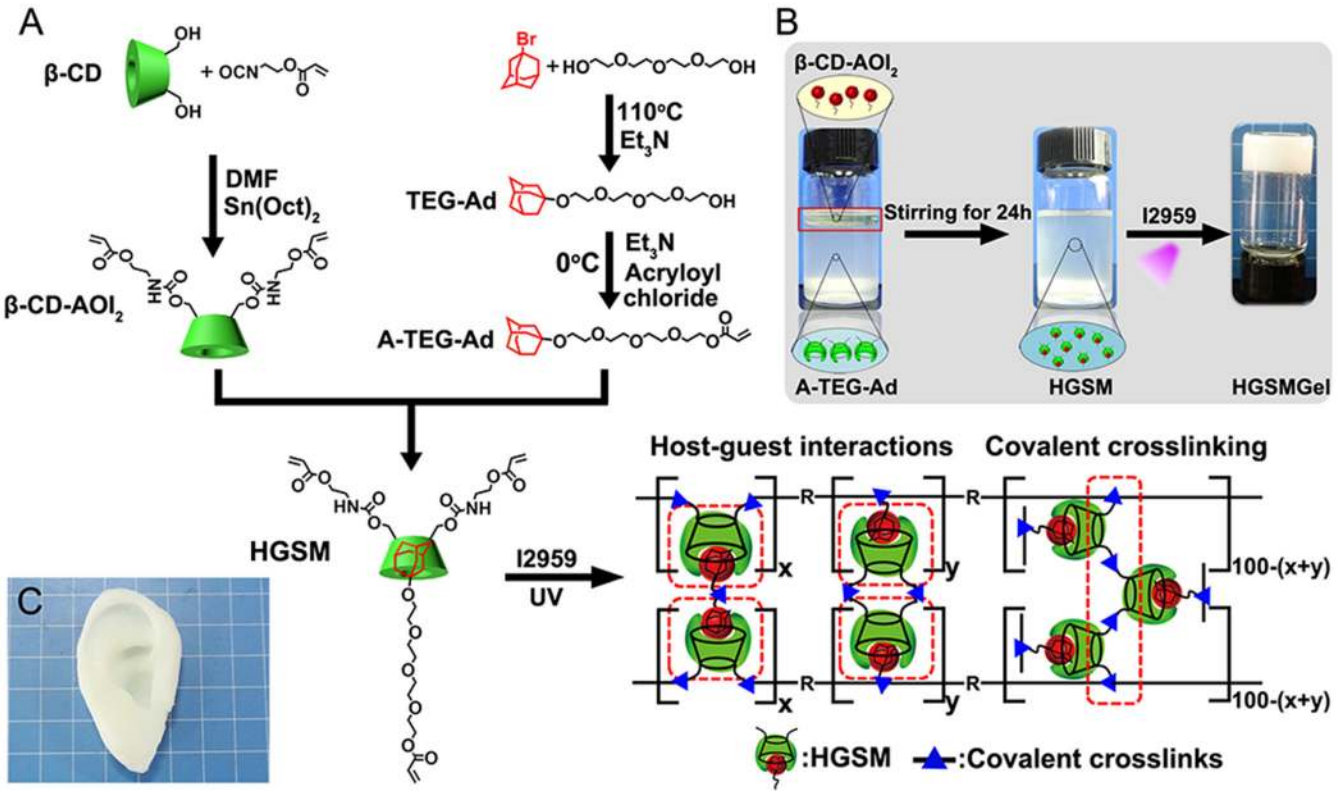

Figure 1.

Construction of the novel host-guest supramolecules (HGSMs) hydrogel. (A) Schematic showing the synthesis of HGSMs through the inclusion of the guest molecule (A-TEG-Ad) into the host molecule (β-CD-AOI_2) and the polymerization of HGSMs into injectable hydrogels (HGSMGels) under UV. The red dashed frame highlights the host-guest interactions and covalent crosslinking as indicated. (B) Photographs of the host-guest inclusion process and hydrogel's preparation process. (C) The photograph of ear-shape hydrogel prepared with the injectable HGSMGels.

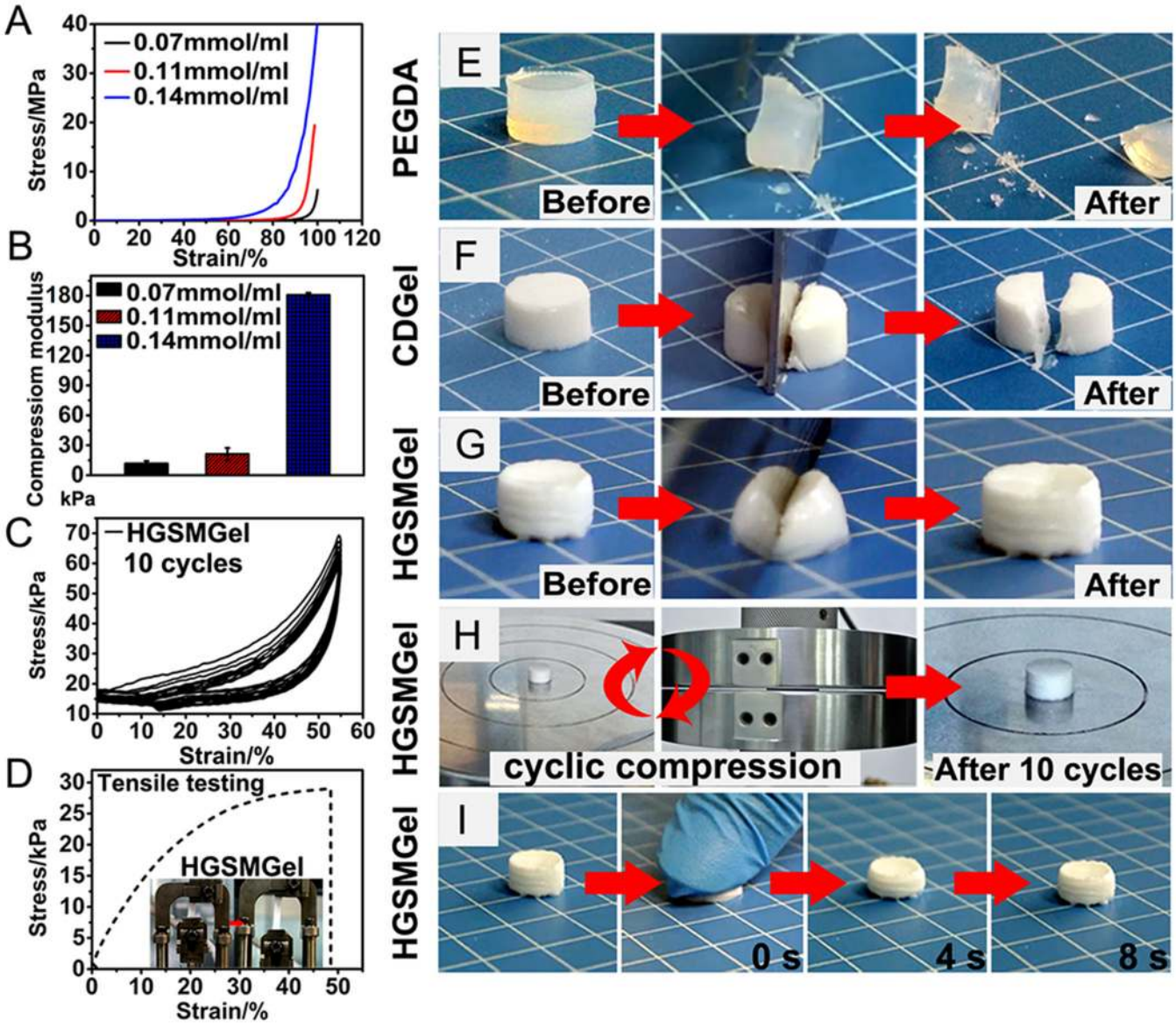

Figure 2.

The marvelous mechanical properties of HGSMGels. (A) Mechanical compression curve HGSMGels with different concentrations. (B) Compression modulus of HGSMGels. (C) Cyclic compression test curves of HGSMGel (0.11 mmol/L). (D) Mechanical tensile curve of HGSMGels. (E, F) Conventional PEGDA gels and CDGels were easily cut into halves with an iron ruler cutter. (G) Photographs showing the strength of HGSMGels, which resisted against slicing with an iron ruler cutter and recovered their shapes after the cutter was removed. (H) Digital images of HGSMGel during the cyclic compression test and after 10 cycles of compression. (I) The rapidly recover process of HGSMGel after being squashed.

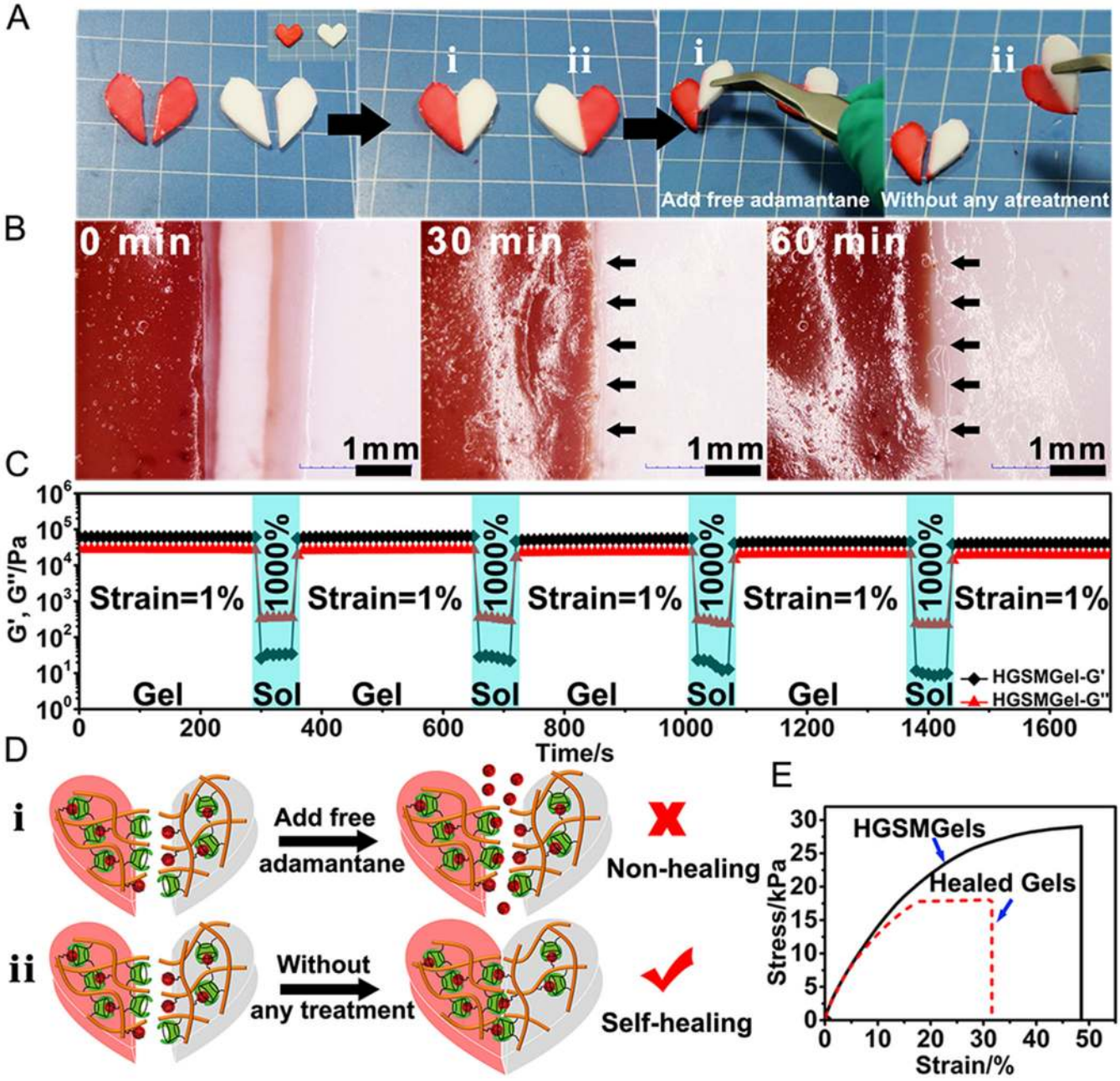

Figure 3.

The good self-healing capacity of HGSMGels. (A) Digital images showing the self-healing behavior of the heart-shaped HGSMGels (ii) and the non-healing behavior of HGSMGels (i) after added free adamantane. (B) 3D rotational microscopy images of the incision on the hydrogel over time. (C) Rapid self-healing property of HGSM hydrogels demonstrated by continuous step strain (1% strain→1000% strain→1% strain) measurements at 37 °C. (D) Schematic of the proposed self-healing mechanism of the normal HGSMGels (ii) whereas self-healing of HGSMGels was disabled after the addition of free adamantane at the incision (i). (E) Stress-strain curves of original (solid lines) and self-healed (dash lines) HGSMGels.

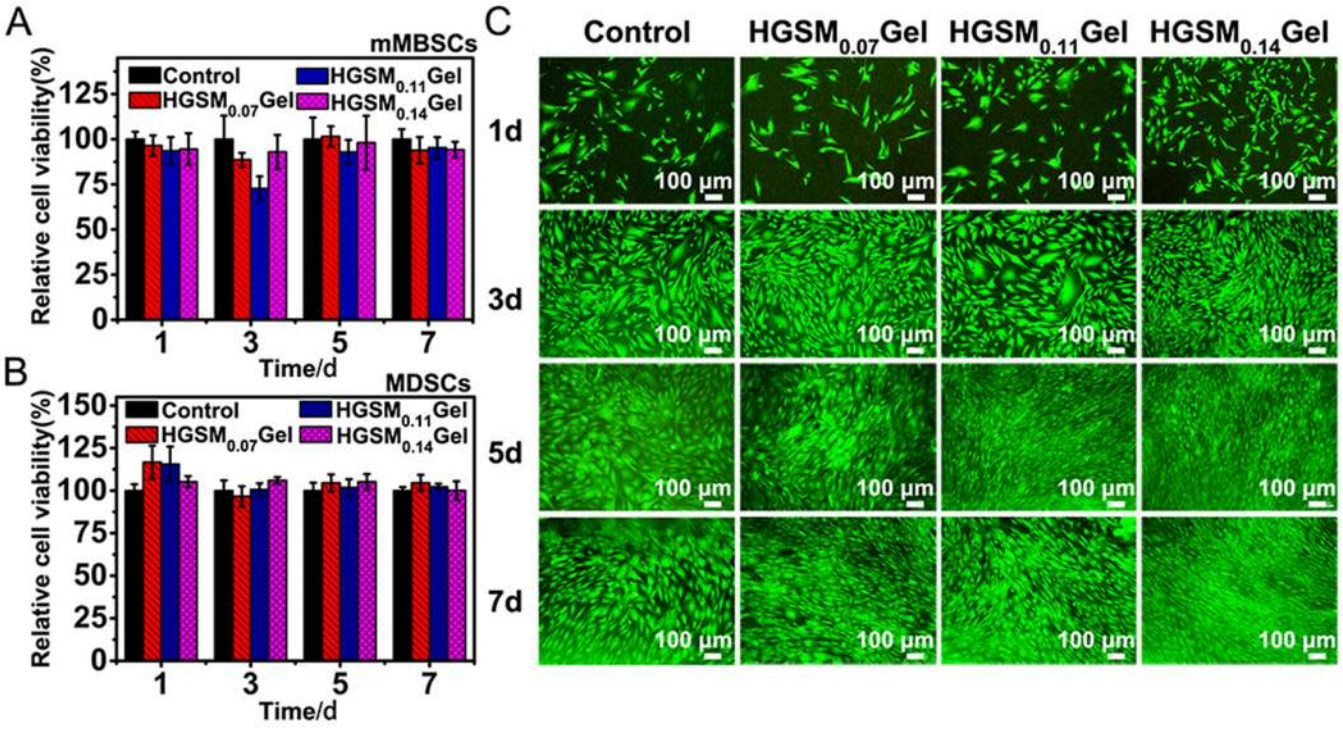

Figure 4.

The biocompatibility of HGSMGels. (A-B) Cell proliferation ability of mBMSCs and MDSCs after cultured in the presence of the HGSMGels extracts for 1, 3, 5 and 7 days (A normal complete medium was used as the positive control group). (C) Live/dead fluorescence images of MDSCs after cultured in the presence of the HGSMGels extracts for 1, 3, 5 and 7 days.