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Controlled Cross-linking Strategy for Formation of Hydrogels, Microgels and Nanogels

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Abstract Hydrogels are a kind of unique cross-linking polymeric materials with three-dimensional networks. Various efforts have been devoted to manipulate the formation of functional hydrogels *in situ* and enrich the production of hydrogels, microgels and nanogels with improved modulation capacity. However, these methods always fail to tune the gel properties because of the difficulty in achieving the precise control of cross-linking extents once the gel formation is initiated. Therefore, the preparation of tailor-made hydrogels remains a great challenge. Herein, we summarize a controlled cross-linking strategy towards not only fabrication of hydrogels at nano-, micro- and macro-scales, but also achievement of controlled assembly of nanoparticles into multifunctional materials in macroscopic and microscopic scales. The strategy is conducted by controllably activating and terminating the disulfide reshuffling reactions of disulfide-linked core/shell materials with selective core/shell separation using system pH or UV triggers. So it provides a facile approach to producing hydrogels, hydrogel particles and nanoparticle aggregates with tunable structures and properties, opening up the design possibility, flexibility and complexity of hydrogels, microgels/nanogels and nanoparticle aggregates from nanoscopic components to macroscopic objects.

Keywords Thiol-disulfide exchange; Controlled cross-linking; Hybrid hydrogels; Hydrogel particles; Biodegradation

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INTRODUCTION

Hydrogels are three-dimensional and water-swollen networks of hydrophilic polymers with physical or chemical crosslinks. Due to the soft consistency and good biocompatibility, hydrogels are extremely suitable in applications of drug delivery[1, 2], bioimaging[3, 4], chemical separation[5] and catalysis[6, 7]. To produce hydrogels with tunable properties, stimuli-responsive hydrogels have been greatly developed for handily and readily manipulating their properties under various external stimuli, such as pH[8, 9], temperature[10, 11], electricity[12], redox[13, 14], light[15, 16], *etc*. In most common method, chemically cross-linked hydrogels are prepared from the solution of monomers together with a small and pre-calculated amount of cross-linkers. However, the conventional gelation methods fail to tune the gel structures and properties *in situ* as a result of the difficulty in achieving the precise control on the pre-set amount of multifunctional cross-linkers and degree of cross-linking once the gel formation is initiated[17–19]. To circumvent this problem, it is greatly desirable to design a 'living' controlled *in situ* gelling system with the explicit criteria that the gelation should have capacities to be activated, controllably terminated and interrupted, and reinitiated by the external stimuli whenever needed.

The authors' research group has recently proposed a controlled cross-linking strategy to dynamically optimize hydrogel properties[20]: from the solution to loose hydrogels with low crosslinking degree to compact hydrogels with high crosslinking degree (Fig. 1). The strategy can *in situ* tune the gelation process with the selective core/shell separation: dissociation of the shells and cross-linking of the cores. The fulfillment of controlled gelation is based on the precise manipulation of thiol-disulfide exchange reaction in aqueous solutions. As shown in the Fig. 2, the thiol-disulfide exchange reaction can be reversibly activated or terminated by deprotonating the free thiols or protonating the thiolates under different pH conditions with a rapid response; the dynamic disulfide bonds show redox responsiveness under the presence of glutathione (GSH) or dithiothreitol (DTT)[21]. By means of this congenital advantage, we report a facile method to create the 'living' *in situ* gelling system for the controlled hydrogels from a disulfide-linked core/shell structure. The gelation process can be triggered, dynamically interrupted and reinitiated by manipulating the pH-responsive gelling system, allowing the facile fabrication of hydrogels with the tailor-made structures and properties and opening up the novel

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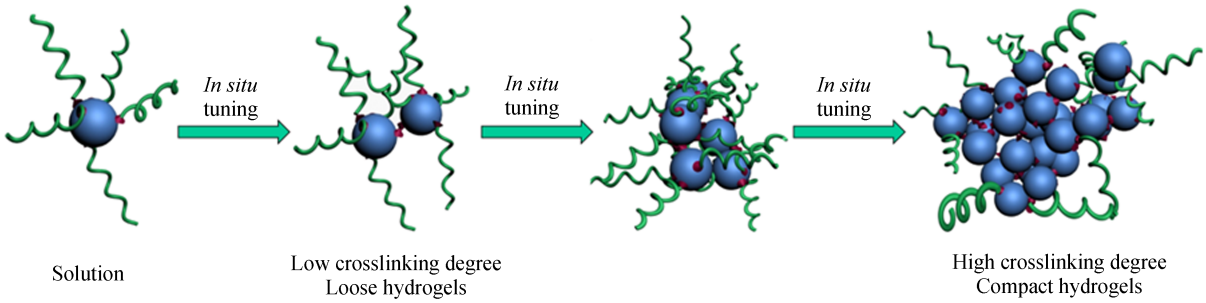


Fig. 1 Schematic illustration of controlled cross-linking strategy with core/shell separation process

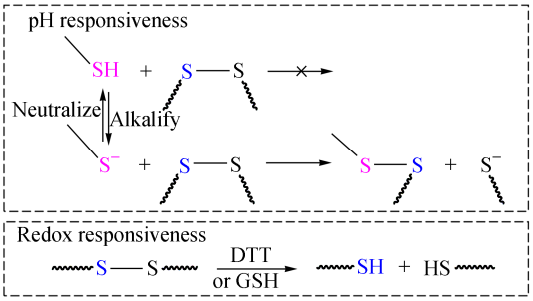


Fig. 2 Schematic illustration of pH and redox responsiveness in regard to the thiol-disulfide exchange reaction

design possibilities for the *in situ* hydrogels under mild reaction conditions.

To the best of our knowledge, there is no review that summarizes the development of thiol-disulfide exchange reaction for the controlled gelling system. Herein, we intend to introduce a controlled cross-linking strategy for fabricating various kinds of macro-, micro- and nano-scale hydrogels by two stimuli-triggered cross-linking methods in detail. Conclusions and perspectives are also presented and discussed.

THE CONCEPT OF CONTROLLED CROSS-LINKING STRATEGY

Controlled Gelling Process and Mechanism

For a conventional hydrogel preparation, the compactness of cross-linked networks mainly relies on the pre-set amount of cross-linking agents in the initial system[18, 19] . But once the cross-linking reaction is activated, the crosslinking degree and network structure are usually difficult to be further adjusted. To overcome this difficulty, we performed the controlled cross-linking strategy to tailor the gelling process, which is enabled to be dynamically terminated and re-opened *via* controlling the thiol-disulfide exchange reaction under mild conditions. The gelling product, namely the 'controlled gel', can be fabricated with customized crosslinking degree and performance from rational design of the disulfide-containing gel precursors[20].

The unique chemical structure of gel precursors plays an important role in the controlled cross-linking process. We first synthesized a class of hyperbranched polymers (poly(*N*,*N*′-bis(acryloyl)cystamine-1-(2-aminoethy)piperazine-poly(ethylene glycol), BAP) with a disulfide-linked core-shell structure as a gel precursor, wherein the poly(amido amine) was the hydrophobic core[22, 23] and the poly(ethylene glycol) (PEG) acted as the easy-leaving shell. In this system, BAP aqueous solution had good stability under low pH but could be cross-linked to form the 'living' gel due to the trigger of thiol-disulfide exchange reaction under alkaline conditions. When the cross-linking time was prolonged, the 'living' gel network evolved from the loose to compact (Fig. 3A), generating a smaller pore size and denser network in Fig. 3(B). Notably, the cross-linking process could be interrupted at any time by neutralizing the pH of 'living' gel system. At this point, the 'living' gel was converted to 'stable' gel that could be inert for a long period of time in a neutral solution. Once the system was re-basified, the 'stable' gel could quickly revert to the 'living' gel state as well as the restart of size-shrinkage evolution. Therefore, hydrogels with customized structures and properties are easily fabricated through controlling the pH value at a predetermined time.

Biodegradable hydrogels are widely used in biomedical applications of drug delivery and tissue engineering[24–26]. The disulfide bonds, as dynamic covalent linkages, can be easily cleaved in response to the redox stimuli from GSH or DTT. So the controlled cross-linking strategy is used to develop the redox-responsive biodegradable hydrogels. These disulfide-containing hydrogels had a good long-term stability and basically maintained their shapes and sizes in phosphate buffer saline (PBS) solutions. However, these hydrogels were quickly disintegrated after addition of 10 mmol/L DTT, presenting a redox-responsive degradation behavior in Fig. 3(C). Since the hydrogels were formed through disulfide shuffling of the polymer cores, the resulting neutralized hydrogels, possessing the low cytotoxicity, were biocompatible as shown in Fig. 3(D). So these hydrogels were suitable for *in situ* encapsulation of active molecular drugs that can tolerate a relatively high pH environment. For example, two model drugs, hydrophilic doxorubicin (DOX) and hydrophobic paclitaxel (PTX), were used to yield the drug-loaded hydrogels. These results illustrated that tailor-made hydrogels allowed the fine modulation of drug delivery system to facilitate the preparation of smart drug vectors that were stable in an extracellular medium and decomposable in an intracellular environment to trigger the drug release.

The thiolate-mediated thiol-disulfide exchange mechanism can interpret the formation of loose and compact hydrogels and demonstrate this 'living' controlled process in Fig. 4. In detail, BAP system had a small number of thiols that could initially generate thiolates in the presence of alkali solution. The active thiolates could immediately attack the disulfide

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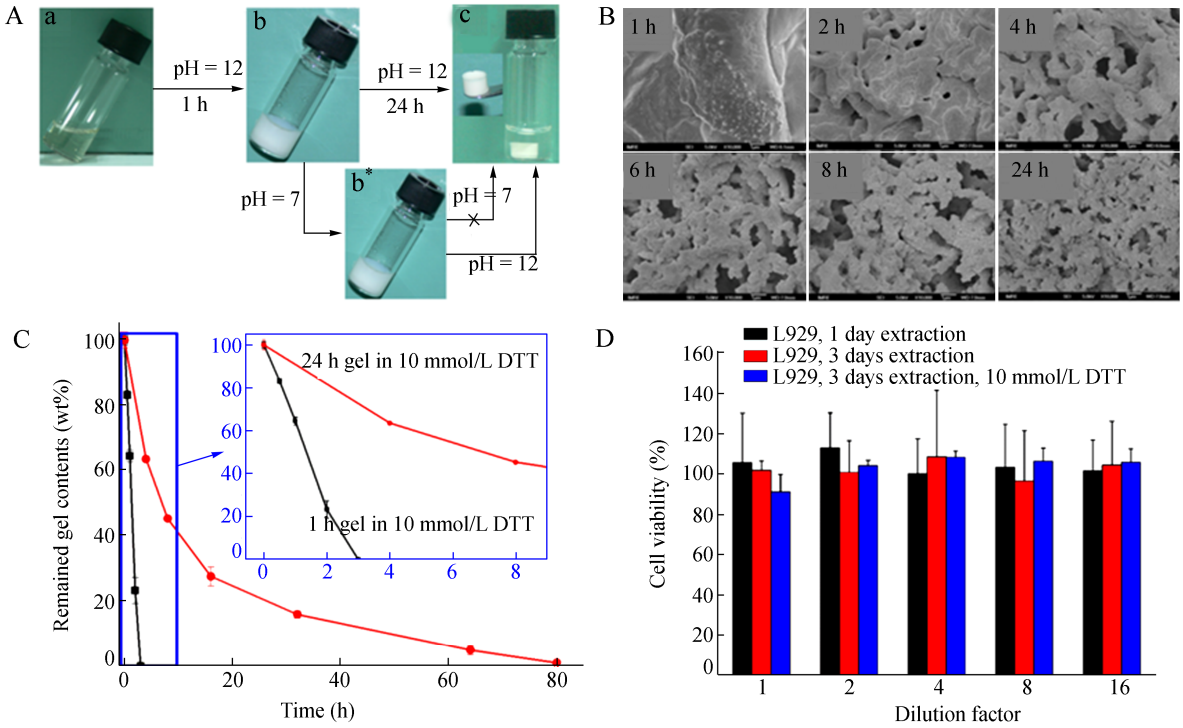


Fig. 3 (A) Graphical representation of basification of BAP solution and derived methodology for producing loose and compact hydrogels: (a) 10 wt% BAP solution, (b) loose hydrogel after basification for 1 h, (b*) inert neutralized loose hydrogel after 1 h of cross-linking, and (c) compact hydrogel after basification for 24 h; (B) SEM images of the hydrogels obtained after basification for various time; (C) Remained amounts of the hydrogels with different gelling time in 1 × PBS containing 10 mmol/L of DTT, at 37 °C as a function of time (n = 3); (D) Cytotoxicity of the loose hydrogel after 4 h of cross-linking in L929 cells after incubation for 1 day and 3 days (n = 4) (Reproduced with permission from Ref. [20]; Copyright (2010) American Chemical Society) (The online version is colorful.)

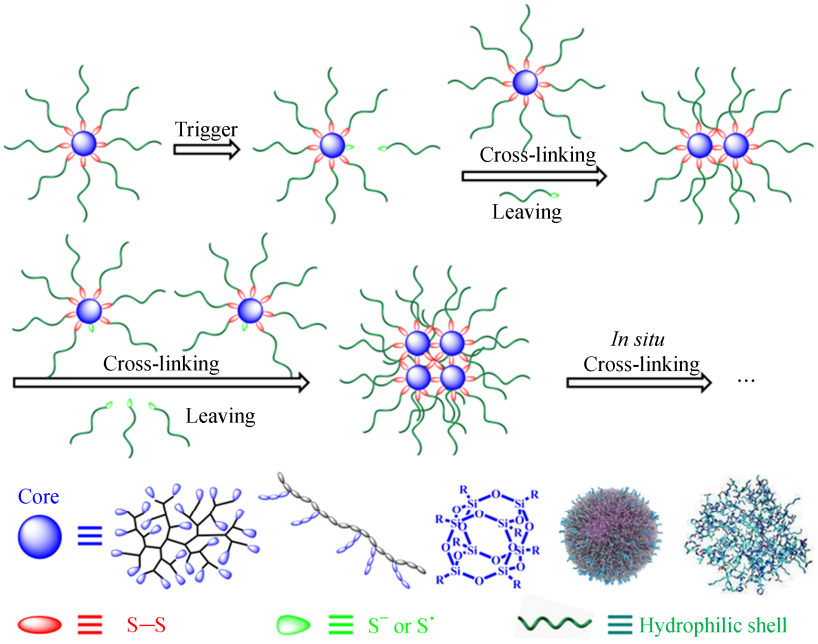


Fig. 4 Schematic illustration of gelation mechanism of cross-linked cores and release of shells

linkages between the core and shell of BAP, resulting in the newly-formed disulfides linkages between the hydrophobic cores and released PEG shells because of the shifting of equilibrium toward the stable cross-linked products. Based on the above-mentioned process, the initially clear solution could quickly transform into a white hydrogel with the loosely cross-linked network within a short time, and then the continuous thiol-disulfide exchange reactions further increased the crosslinking degree of hydrogel networks along with the shape transformation from the loose to compact

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structures. After removal of the alkali stimulus, the thiol groups could be regenerated in order that the termination of thiol-disulfide exchange reaction immobilized the shape and size of hydrogels for a long time under mild environments. However, once the gelling system rebasified again, the reactive thiolates restarted a new round of cross-linking reaction and the stable hydrogel network further evolved into the dense and tight structures.

Controlled Formation of Micro- and Nano-gels in a Confined Space

The controlled cross-linking strategy, as a versatile platform, also provides a new approach towards the well-defined tailor-made hydrogel particles[27]. Combined with an inverse emulsion technique, the strategy is further developed to produce various micro- and nano-gels in a confined space as depicted in Fig. 5(A). By manipulating gelation of micro/nanosized droplets of aqueous BAP solutions, the controlled formation of micro/nanogels is *in situ* facilely fabricated with flexibly designable structures and properties. In detail, the BAP solution was firstly well-dispersed into the micro- or nano-droplets using the inverse emulsion method, then the initial diameter of droplets was adjusted *via* controlling the emulsion conditions, including the organic continuous phase, surfactant, stirring rate and so on. Upon basification of the droplets, gelation caused the formation of loose hydrogel particles with the similar diameter of initial droplets. When gelling time was extended, the dissociation of hydrophilic shells resulted in the shrinkage from the loose to small and compact hydrogel particles. Therefore, adjusting the initial diameters of droplets from nano- to micro-scales and tailoring the gelation time, the controlled formation of macro/nanogels was facilely achieved in the confined space. Meanwhile, after neutralization of gelation system to trap the intermediate particles at a predetermined time, various stable hydrogel particles could be produced with customized chemical structure, particle size and stability, because the transition process from loose to compact hydrogel particles was arbitrarily terminated and immobilized.

Multilayered hydrogel particles, originating from their fascinating multilayer structures that can offer the attractive integration of core-based carrier properties and shell-mediated biological interactions, receive the steady interest and widespread attention. Unfortunately, the reported preparation methods are basically cumbersome for independently tailoring the architectures and properties of the respective layer[28–30]. To address the challenge, we fabricate a multilayered hydrogel particle using the controlled *in situ* gelation method in association with the seed emulsion technology[27]. By freestanding tailor of each on-demand layer, the multilayered hydrogel particles were easily gained

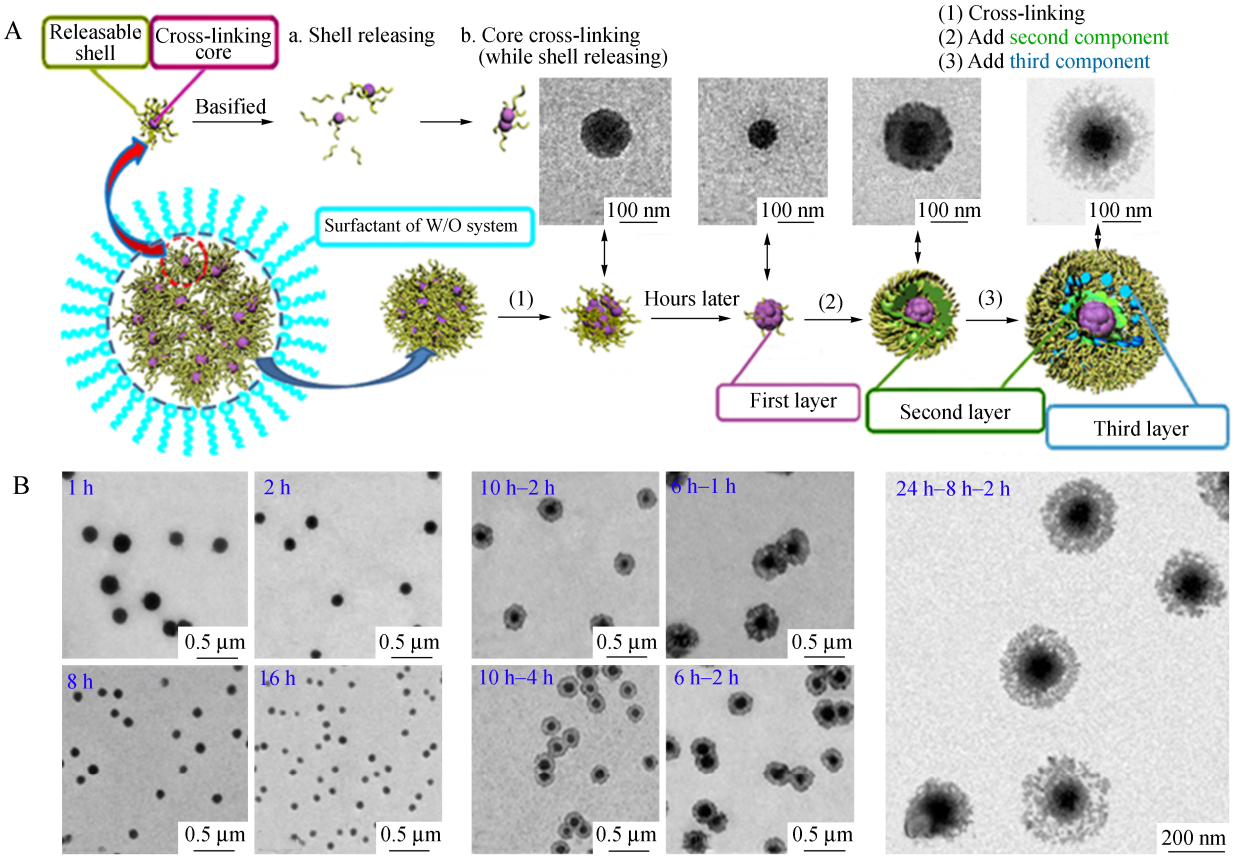


Fig. 5 (A) Schematic illustration of the core/shell separation process: dissociation of the shells and cross-linking of the cores and depiction of the synthetic approach to controlled formation of (multilayered) hydrogel particles; (B) TEM images of the mono-/double-/triple-layered nanogels obtained by cross-linking for various time (Reproduced with permission from Ref. [27]; Copyright (2012) American Chemical Society) (The online version is colorful.)

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with adjustable structural performance for every layer. Specifically, the pre-prepared hydrogel particles were utilized as the seed cores for the heterogeneous growth of subsequent deposition and cross-linking of the shell layer, generating the double-layered core/shell hydrogel particles. After an appointed time, the other fresh BAP solution was added into the core/shell hydrogel particles for the continuously heterogeneous growth of subsequent deposition and cross-liking of the secondary shell layer, producing the smart triple-layered (core/double shells) hydrogel particles as clearly observed in Fig. 5(B). Notably, the structures and properties of multilayered hydrogels can be selectively designed and simultaneously tailored by optimizing the suitable crosslinking time and/or adopting various functional materials with similar disulfide-linked core/shell structures, *e.g.*, inorganic nanoparticle or stimulus-responsive polymer cores and hydrophilic polymer shells. The formation of hydrogel particles was ascribed to the cross-linking of polymer cores linked by a number of built-up disulfide bonds; as a result, the hydrogel particles were biodegradable since disulfide linkages can be effectively cleaved by GSH or DTT agents. Therefore, this general method can be utilized to build up the high performance and intelligent layers of drug carriers, paving a facile approach toward customized multilayered hydrogel particles in applications of drug diagnostics and therapeutic delivery system.

Multilayered Composite Nanoparticles from a Controlled Nanogel Template for Bone Generation

Multilayered composite nanoparticles (MC-NPs) receive significant attention in the pharmaceutical applications of controlled drug delivery and diagnostic bioimaging[31, 32]. Typically, the fabrication process involves layer-by-layer deposition of biologically functional surfaces onto the silica (Si), gold (Au) or polystyrene (PS) templates, but the traditional fabrication methods, including chemical coating, condensation from vapor and solid-state process, offer little control over the morphology, elasticity, particle size and property of individual layers.

Inspired from the design flexibility and complexity of customized multilayered nanogels, we applied the general controlled *in situ* method towards the production of multi-responsive MC-NPs with fine-tunable functional layers[33] as shown in Fig. 6. With the facile control on the tailor-made structures and biodegradable properties, a smart disulfide-linked nanogel, possessing the varying size, was utilized as an ideal template for fabricating the MC-NPs, which exhibited excellent advantages on the biofunctional surfaces, tunable drug release kinetics and multi-responsive functions for targeted drug/growth factor releases towards the bone tissue formation *in vivo*. Therefore, this layer-by-layer technique is scalable and highly controllable, providing a powerful tool for considerable therapeutic potential in clinical fields of oncology and orthopedics[34].

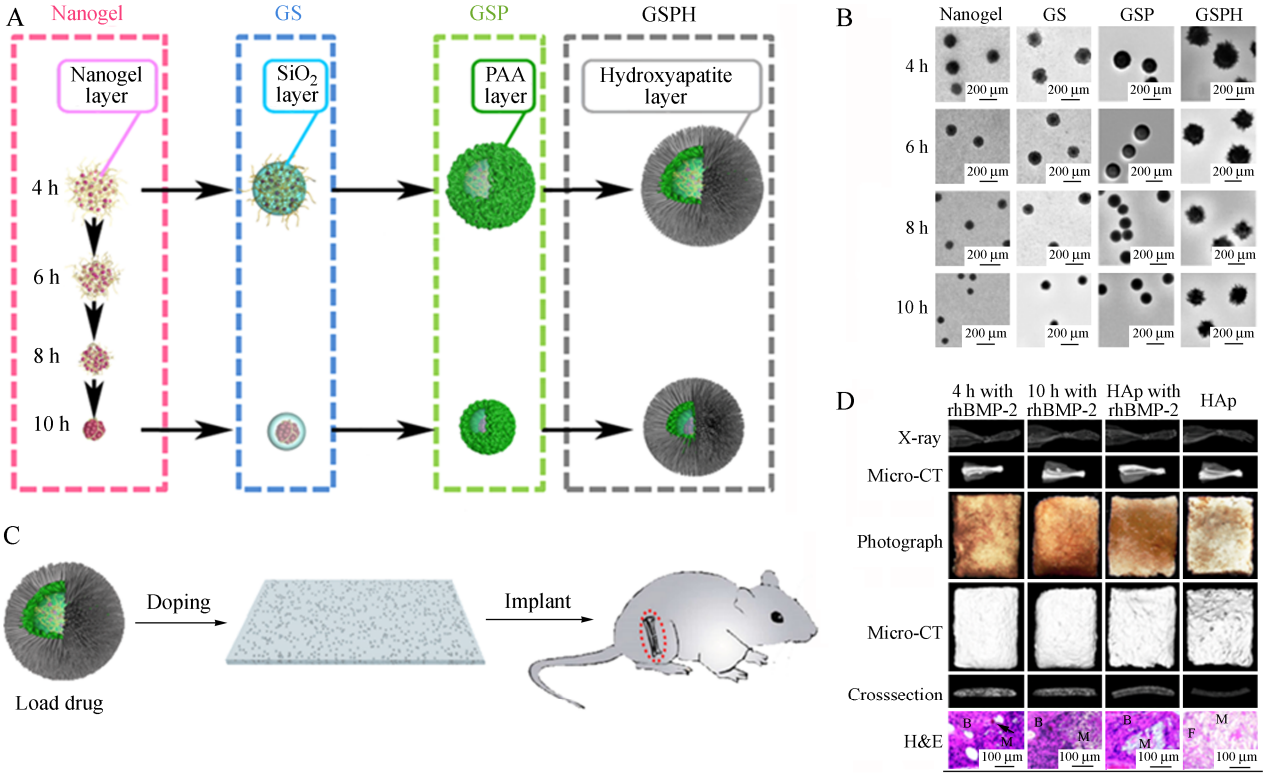


Fig. 6 (A) Controlled formation of the nanogel core, silica shell-nanogel core NPs (GS), pH-responsive poly(acrylic acid) (PAA)-silica-nanogel NPs (GSP), hydroxyapatite (HA) coated PAA-silica-nanogel NPs (GSPH) and doped biomimic films; (B) TEM images of various multilayered composite nanoparticles; (C) *In vivo* translation of multilayered composite nanoparticles toward periosteum-mimetic biomaterials for bone repair; (D) X-ray, micro-CT, photograph, and H&E characterizations of ectopic bone formation in the mouse gastrocnemius pocket model after 4 weeks of film implantation (Reproduced with permisison from from Ref. [33]; Copyright (2017) Elsevier) (The online version is colorful.)

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THE DEVELOPMENT OF CONTROLLED CROSS-LINKING STRATEGY

Controlled Gels Functionalized by Functional Cores

The controlled cross-linking strategy is a general method because it can be also suitable for other materials with functional disulfide-linked core/shell structures, *e.g.*, silica (Si), metal, metal oxide, polyhedral oligomeric silsesquioxane (POSS), polyester-, dendritic-, comb-, capsule- and polysaccharide-like cores[35–41]. To validate this proposal, we produced an organic-inorganic hybrid hydrogel from a hybrid precursor of POSS-(SS-PEG)$_8$ polymer. On account of its well-defined cage structure (~0.53 nm) with an inorganic silica-like core[42–45], this star-shaped polymer was adopted to yield a kind of hybrid hydrogels and nanoparticle aggregates with the customized structures and properties on the basis of a pH-responsive 'on/off' reaction. Specially, the polymeric solution (10 wt%) could keep unchanged after basification at the initial stage (Fig. 7Aa) but turned into a loose hydrogel after addition of cysteamine for 1 h (Fig. 7Ab). Then the loose hydrogel started to shrink into a compact hydrogel in 24 h (Fig. 7Ac), which ultimately converted into a glass-like aggregate after standing for another 9 days (Fig. 7Ad). The transition from the polymeric solutions to the hybrid hydrogels then to the irregular aggregates can be facilely interrupted and reinitiated by neutralization and rebasification at any time. Besides, these neutralized hybrid hydrogels exhibited high stability during the long-term storage (Fig. 7Ab*). Therefore, rational pH control of gelling system can facilely obtain the customized hybrid hydrogels and nanoparticle aggregates.

Similarly, the 'living' controlled cross-linking mechanism is also based on the reversibly deprotonating thiols and protonating thiolates through the tailor of pH values in aqueous solutions, and thus switching on/off the exchange reaction can obtain the hybrid hydrogels and macroscopic nanoparticle aggregates with tunable structures and properties. Distinct from the existence of many sharp peaks for the POSS-(SS-PEG)$_8$ polymer in Fig. 7(B), XRD patterns displayed that the aggregates only had a broad glass-like peak, indicating the successful transitions from hybrid hydrogels to macroscopic aggregates. The rheological measurement gave

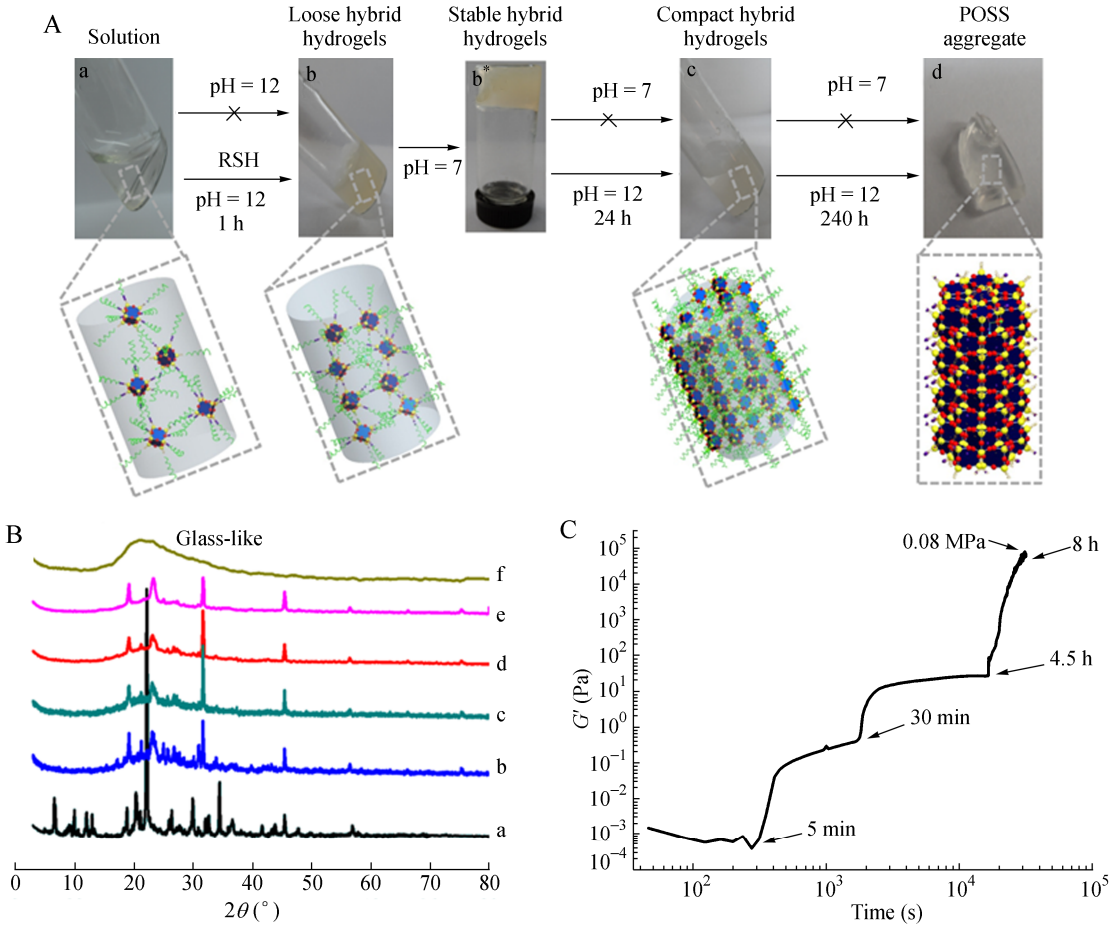


Fig. 7 (A) Schematic representation of the production of loose, compact hybrid hydrogels and the aggregate: 10 wt% POSS-(SS-PEG)$_8$ solution after basification for (a) 1 h without addition of cysteamine, (b) 1 h, (c) 24 h and (d) 240 h with addition of cysteamine, (b*) an inert neutralized loose hydrogel after 1 h of cross-linking; (B) XRD patterns of (a) POSS-(SH)$_8$, (b) POSS-(SS-PEG)$_8$ and the resulted hydrogels or aggregate after basification of solution for (c) 1 h, (d) 8 h, (e) 24 h and (f) 240 h with addition of cysteamine; (C) Storage modulus G' of POSS-(SS-PEG)$_8$ solution after basification to pH 12 as a function of time (Oscillatory frequency: 1 rad/s.) (Reproduced from Ref. [42] with permission from The Royal Society of Chemistry) (The online version is colorful.)

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another intuitional evidence to observe this transformation process. Figure 7(C) showed a multistage abrupt increase of the storage modulus G' during the gelation process; in particular, the G' of hybrid hydrogel was more than 0.08 MPa after cross-linking for 8 h, manifesting the formation of stable hydrogel with high mechanical strength.

Currently, we are also utilizing another two kinds of functional cores, multi-site drugs and fluorescent agents[46, 47], to customize the functionalized hybrid hydrogels in applications of drug delivery and bioimaging. Therefore, the 'living' controlled strategy is not only beneficial for preparation of functionalized hydrogels but also applicable for assembling individualized nanoparticles into cluster aggregates with tailored features and customized functionalities, expanding a new pathway for the controlled assembly of nanoparticles.

Controlled Gelation Triggered by Ultraviolet Light

In contrast to the anion-mediated disulfide exchange mechanism, the dynamic disulfide bond can also undergo the thiol-disulfide exchange reactions with sulfhydryl radical *via* a radical-mediated mechanism under the photo-irradiation condition[48]. In consideration of the requirement of alkali environment to induce the controlled gelling process, many functional and intelligent hydrogel materials are not stable in the strong alkali conditions and thus not conducive to biomedical applications. Compared with the harsh pH environment to modulate the thiol-disulfide exchange reaction, UV switcher has unique advantages of remote manipulation, tunable intensity, spatiotemporal precision and "turn-off" effect, which enables to realize real-time control over the photochemical process. So we developed a photochemical method to prepare the controlled cross-linking hydrogels using a comb-like P(EMA-SS-PEG) polymer (poly(ethyl methacrylate)-dithio-poly(ethylene glycol)) based on the UV-triggered thiol-disulfide exchange reaction, which could not only avoid the strong alkali environments but also provide the precise spatiotemporal control on the structures and properties of hydrogels[49]. By varying the irradiation time, the crosslinking degrees and morphology of the hydrogels were also easily adjusted, exhibiting the concomitant transitions in the conformation, mechanical and swelling properties in Fig. 8(A).

For the thiolates-mediated exchange reaction, the driving force is determined by the shifting of equilibrium toward the stable disulfide-linked materials through release of PEG shells by the selective core/shell separations. In contrast, UV irradiation can break one disulfide into two freely thiyl radicals in the presence of photoinitiator (2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone, IR2959) and catalyst (mercaptoethylamine, MCA), which induced the random radical-centered disulfide exchange reaction to prepare the hydrogels in an aqueous solution. By attacking disulfide bonds of the nearby polymeric chains, the thiyl

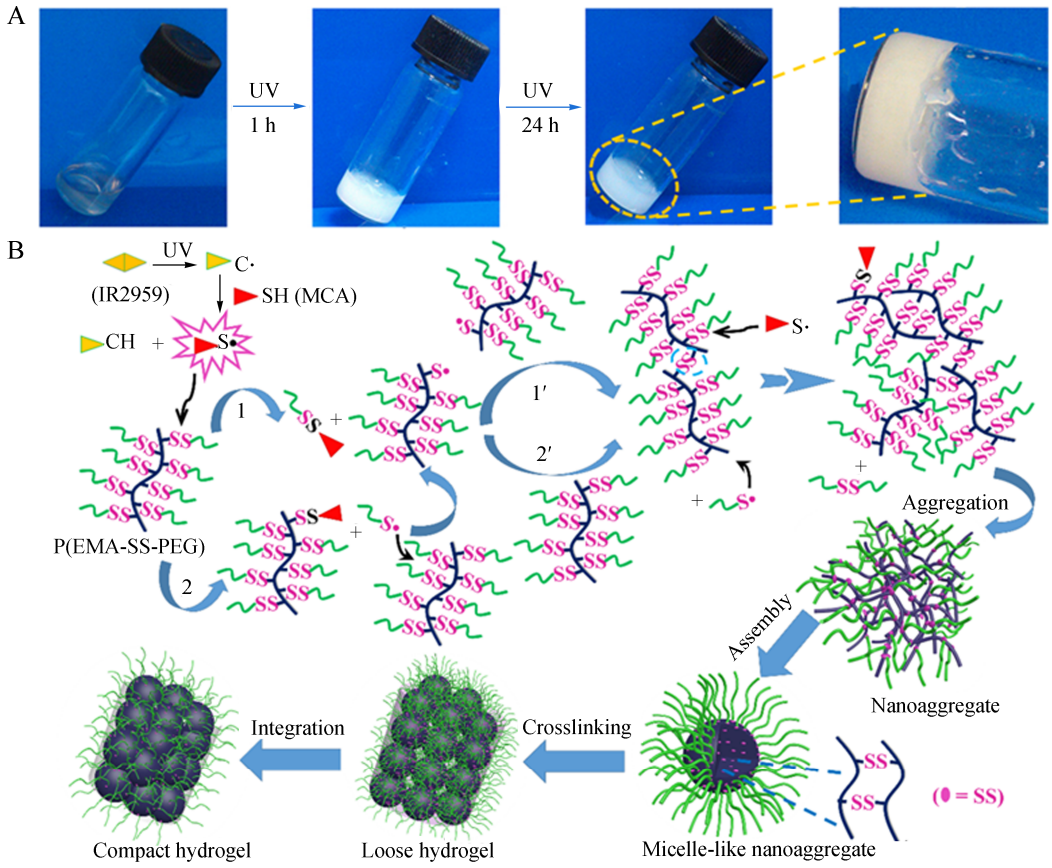


Fig. 8 (A) Schematic representation of the hydrogel formation under various UV irradiation time; (B) The mechanism of forming hydrogels *via* a UV-triggered thiol-disulfide exchange reaction (Reproduced from Ref. [49] with permission from The Royal Society of Chemistry) (The online version is colorful.)

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radicals could effectively trigger thiol-disulfide exchange reaction using two different ways, both of which resulted in the formation of thiyl radicals on the polymeric backbones (Fig. 8B). So that the extramolecular disulfide exchange or radical combination reaction could link the two isolated polymers together by detachment of PEG side chains. Prolongation of irradiation time further caused the continuous disulfide exchange reactions, thus leading to the crosslinking of more polymer backbones and rupturing of PEG chains to the solutions. In other words, rational control of UV irradiation time can easily tailor the hydrophobic/hydrophilic ratio and topological structures, which are two key factors for manipulating the self-assembly behaviors in aqueous solutions[50–54]. As the hydrophilic/hydrophobic ratio was decreased to a critical value, the hydrophobic polymer sections were aggregated together along with the hydrophilic PEG side chains extending into the water layer to reduce the free energy of system, ultimately resulting in the formation of self-assembled aggregates. Further reducing the hydrophilicity, the aggregates transferred into the micelle-like structures in aqueous solutions, in which the hydrophobic polymer areas acted as the core while the hydrophilic PEG chains modeled as the shell. Notably, the UV-triggered thiol-disulfide exchange reaction was simultaneously conducted inside and/or outside the micelle-like nanoaggregates; thereby the loose hydrogels were finally formed by a mass of disulfide-crosslinked nanoaggregates. If the irradiation time was further extended, the sustained disulfide shuffling and hydrophobic interactions drove the integration of micelle-like nanoaggregates to form the large hydrophobic ones as well as more reassemble PEG chains releasing into the aqueous solutions; in this case, these resultant hydrophobic cores expelled out more encapsulated water, thus inducing the loose hydrogel to further shrink to the compact and dense network structure. Based on this UV-triggered hydrogel preparation, we recently produce another visible light-triggered controlled formation of hydrogels with a rapid self-healing behavior, which will be discussed in details in our future work.

UV-triggered thiol-disulfide exchange method is also an efficient and universal strategy to prepare the bioreducible hydrogels with good temporal and spatial control, paving a promising way for the precise spatiotemporal control on the photochemical gelation process in applications of macro-/micro-patterned hydrogels, photolithography and controlled drug delivery.

CONCLUSIONS AND PERSPECTIVES

In summary, we have mainly demonstrated a controlled cross-linking strategy for the formation of hydrogels and hybrid hydrogels at macro-, micro- and nanoscales based on the precise manipulation of thiol-disulfide exchange reaction. The reversibly activated or terminated by deprotonating the free thiols or protonating the thiolates under different pH conditions furnished the disulfide-linked core/shell polymers with a rapid response, resulting in the dissociation of shells and cross-linking of cores and thus dynamically optimizing hydrogel structures: from the solution to loose and to compact hydrogels in macroscopic dimensions. Combined with the inverse emulsion technique, the cross-linking strategy can be utilized to produce various micro/nanogels in microscopic scales and fabricate the multilayered hydrogel particles in the confined space with flexibly designable structures and properties. It is worth noting that this controlled cross-linking strategy is a general method because it can be also suitable for other materials with functional disulfide-linked core/shell structures, by which the various multifunctional hybrid hydrogels are yielded with tunable networks and high-performance features. Meanwhile, UV-triggered thiol-disulfide exchange reaction has been developed to prepare the hydrogels with a radical-centered disulfide exchange mechanism, opening up another cross-linking method for the precise spatiotemporal control on the photochemical gelation process. More importantly, these hydrogel materials, possessing a large number of the disulfide linkers among the cross-linking cores, are biocompatible and biodegradable in medical applications. So this controlled cross-linking strategy has great potentials to be developed as a powerful tool for the design, synthesis and self-assembly of novel intelligent biomaterials and drug delivery systems.

Although remarkable advantages on the preparation of tailor-made hydrogels have been represented by this controlled cross-linking strategy, the wide application fields of controllable hydrogels materials remain a grand challenge. On one hand, the currently investigated hydrogels are mainly based on the hydrophobic cores; other hydrophilic or multifunctional cores have rarely been reported. On the other hand, although the crosslinking degree of hydrogel network can be well-fabricated by termination of the disulfide exchange reactions, the networks themselves are heterogeneous crosslinking during the gelation or transition process in order that the single chemically-cross-linked networks make these highly swollen hydrogels notoriously brittle, which greatly limits their applications in the antifouling materials, drug delivery, biological imaging and tissue engineering. To overcome this challenge, we currently manage to fabricate the controlled double-network (DN) hydrogel by merging the cross-linking strategy into the DN gel systems[55] or other robust hydrogel systems[56, 57]. Our groups are committed to design and develop a series of DN hydrogels with remarkable mechanical strengths[58–60]. Therefore, combining controlled cross-linked strategy with excellent properties of DN hydrogels, we aim to elaborately design a type of controlled DN hydrogels by precisely adjusting the network topologies and mechanical properties, hoping to elucidate the underlying mechanisms for further enhancement of gel properties.

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