The development of low-molecular weight hydrogels for applications in cancer therapy

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To improve the anti-cancer efficacy and to counteract the side effects of chemotherapy, a variety of drug delivery systems have been invented in past decades, but few of these systems have succeeded in clinical trials due to their respective inherent shortcomings. Recently, low-molecular weight hydrogels of peptides that self-assemble via non-covalent interactions have attracted considerable attention due to their good biocompatibility, low toxicity, inherent biodegradability as well as their convenience of design. Low-molecular weight hydrogels have already shown promise in biomedical applications as diverse as 3D-cell culture, enzyme immobilization, controllable MSC differentiation, wound healing, drug delivery etc. Here we review the recent development in the use of low-molecular weight hydrogels for cancer therapy, which may be helpful in the design of soft materials for drug delivery.

1. Introduction

Cancer is currently the second leading cause of death in the world.¹ Until now chemotherapy or radiotherapy after surgery are the best ways to relieve patient pain. However, chemotherapy causes adverse side effects in patients.² As we know from the literature, the reason for this is from the inherent shortcomings (low solubility, non-specific target, serious side effects of the adjuvant, fast serum clearance, etc.) of most anti-cancer drugs.³ It is to address these problems with anti-cancer drugs that most drug delivery systems, such as polymer hydrogels,⁴ liposomes,⁵ self-assembled polymer micelles,⁶ dendrimers,⁷ protein assisted nanoparticles⁸ etc., have been developed. Challenges, such as the low drug capacity and efficiency, and the biodegradability and biocompatibility of drug carriers remain to be resolved.⁹ Since the 1990s, low-molecular weight hydrogels having the advantages of being easily designed, with good biocompatibility and biodegradability,¹⁰ have been widely applied in tissue engineering,¹¹ enzyme immobilization,¹² wound healing,¹³ selective bacteria inhibition,¹⁴ cell culture¹⁵ and drug delivery.¹⁴ Here, we review recent progress in the use of low-molecular weight hydrogels for cancer therapy.

Low-molecular weight hydrogels are formed by the assembly of gelators via non-covalent interactions (such as hydrogen bonds, π–π interactions and charge interactions). These molecules form three-dimensional networks in water by these non-covalent interactions. Compared to normal drug delivery systems, low-molecular weight hydrogels have the following advantages as possible novel drug delivery systems. Primarily, low-molecular weight hydrogels usually have a higher drug loading content (more than 10%) than normal delivery systems; secondly, encapsulation of anti-cancer drugs into the hydrogel or formation of the hydrogel by a drug derivative itself can eliminate the unexpected adverse effects from excipients; however, the most important feature is that low-molecular weight hydrogels can be designed to undergo controllable and sustained release of drugs by incorporating enzyme cleavable sites in the gelators. These advantages suggest the huge potential of low-molecular weight hydrogels for the delivery of anti-cancer drugs.

To improve the efficacy and to reduce the side effects of anticancer drugs, the method commonly used with hydrogels is to encapsulate hydrophobic drugs in the self-assembled materials via non-covalent interactions. The encapsulated drugs can then be released upon degradation of the gels. Recently the groups of Xu and Yang designed and applied efficient hydrogelators to connect anti-cancer drugs via degradable covalent bonds. These novel methods not only enhance drug loading efficacy, but are also injectable at in situ sites with passive targets. Cui et al. attached the self-assembled TAT peptide to Taxol, so forming a nanofiber containing 41% Taxol which can exert effective cytotoxicity against a number of cell lines comparable to that of free paclitaxel;¹⁷ the groups of Stupp and Schneider reported that simply attaching a short self-assembling peptide domain to a C-terminus peptide-epitope can dramatically enhance the activity of the peptide,¹⁸ any known epitope with anti-cancer activity could be easily attached to a self-assembling peptide domain using conventional solid phase peptide synthesis. Xu et al. also used an intracellular hydrogelation strategy to induce cancer cell death.¹⁹ It is still...
necessary and challenging to design and synthesize a precursor of self-assembled peptides that connect therapeutic agents. Recently, the Yang group highlighted their work on hydrophobic drugs as the building blocks for hydrogel formation. In this paper we summarize recent progress with hydrogels in cancer therapy, which not only contains the work of Yang, but also includes all of the recently reported work on the hydrogels of hydrophobic drugs and the novel strategies for treating cancer by self-assembled nanofibers. We think that this review can give both information and inspiration for cancer therapy and regenerative medicine.

2. Physical encapsulation of hydrophobic drugs by low-molecular weight hydrogels

One application of low-molecular weight hydrogels is as a controlled drug release system. Like most drug carriers, embedding the drug in a vesicle through physical interaction is the normal method to design materials. However, controlling the rate of release of the drug and the degradation of the materials is still a challenge. Recently the John group designed a novel hydrogel system, which was formed through non-covalent interactions by renewable resources. The hydrogelator is based on amygdalin derivatives which can form nanoaggregates via self-assembly and are therefore capable of encapsulating a hydrophobic drug. The release rate of the drug can be controlled by manipulating the concentration of hydrolase enzyme and/or temperature. Fig. 1A is a schematic representation of curcumin encapsulation in the hydrogel; the curcumin can be released by lipolase-mediated enzyme degradation of the hydrogel at physiological temperature. Fig. 1B shows chemical structures and synthetic route of the hydrogelators which are degradable by lipolase. We can see that after 12 h visible changes have occurred (Fig. 1C), 100% of the gel had degraded, and the top solution became yellow in color which indicates the release of curcumin due to the enzyme-mediated gel. The release rate can be controlled both by enzyme concentration and temperature. By thin-layer chromatography it can be clearly demonstrated that the products are amygdalin, curcumin, and enzyme with little or no toxicity. This is a possible model system for drug encapsulation and enzyme-mediated delivery for in vivo formulations and may have potential applications in pharmaceutical research and the molecular design of value-added products from bio-based materials, which are otherwise underutilized.

Self-assembled peptides which form a 3D-network of nanofibers were applied in various fields: tissue engineering, mineralization of organic and inorganic materials, drug delivery, 3D-cell culture, etc. The Pochan and Schneider groups have conducted pioneering work with β-hairpin peptide hydrogels. Recently, it was reported that self-assembly of the MAX8 peptide can serve as an injectable drug delivery vehicle for curcumin. With the sequence VKVKVKV-K-PPTKVEKVKV-NH2 MAX8 can self-assemble in

Fig. 1 (A) Schematic representation of drug encapsulation in a supralow-molecular weight hydrogel and the subsequent release of the drug by enzyme-mediated degradation of the hydrogel at physiological temperature; (B) Synthetic scheme of amygdalin-based amphiphiles; (C) Real images of the hydrogels of R = 3 with (i–iv) and without (v and vi) curcumin (after complete gel degradation, the remaining white, fluffy powder which had settled at the bottom was characterized, i.e., water-insoluble fatty acids which had formed after the gel was degraded by the enzyme). Reprinted with permission from ref. 22. Copyright (2006) American Chemical Society.

Fig. 2 (a) Schematic of self-assembly of the MAX8 peptide. (b) Transmission electron microscopy image of negatively stained MAX8 fibrils. (c) DAOY cells seeded on 0.5 wt% MAX8 hydrogels with 0 mM curcumin and tissue culture plastic. (d) DAOY cells seeded on 0.5 wt% MAX8 hydrogels with 2 mM curcumin and tissue culture plastic. The white boxes in the insets of Fig. 2(c and d) represent the area where the optical microscopy images were taken. Scale bars represent 200 μm. (e) Curcumin concentration released into PBS supernatants from 0.5 wt% (□), 1 wt% (○) and 2 wt% (△) MAX8 hydrogels prepared at a fixed curcumin concentration (4 mM). (f) The cytotoxic effect of curcumin as determined by the levels of lactate dehydrogenase released. Reprinted with permission from ref. 29. Copyright (2011) Elsevier.
salt solution buffered to pH 7.4 or cell growth medium (pH 7.4). The interaction of the intermolecular hydrophobic side chains, contacts, and lateral hydrogen bonding lead to fibril formation (Fig. 2b). By culturing on the gels, it is easy to monitor cell propagation in vitro between the self-supporting nature of the hydrogels with or without curcumin. After being cultured for 24 h without curcumin or with 4 mM curcumin (Fig. 2c and d), DAOY cells were not capable of growing on gels with curcumin. Compared to the MAX8 hydrogel, no cells with normal morphology, excluding dead cells, were found in the gel with curcumin (Fig. 2d). It was also demonstrated that the peptide prevents curcumin molecules from moving freely throughout the porous hydrogel network, a higher peptide concentration leads to slower drug release (Fig. 2e). As shown in Fig. 2f, curcumin released from a hydrogel prepared with 4 mM of curcumin induced a higher amount of cytotoxicity compared to a hydrogel prepared with 2 mM curcumin. The results demonstrated that a minimally invasive curcumin delivery strategy can encapsulate and deliver sustained concentrations of curcumin locally to a target site.29

Additionally self-assembled peptides contain water to form hydrogels for cancer therapy. Physical encapsulation via hydrophobic collapse is another strategy for introducing therapeutic molecules noncovalently.29 Recently the Stupp group used self-assembled peptide amphiphilic (PA) molecules to encapsulate camptothecin (CPT) for cancer therapy.30 Stupp conducted pioneering work in the area of using alkyl tails as the head group to connect to the β-sheet-forming region of the peptide.31 Self-assembled PAs have potential application in bone mineralization,25a,32 spinal cord regeneration,33 angiogenesis, cartilage regeneration,12d and cancer therapy. Fig. 3A showed that CPT in the PA nanostructures had high encapsulation efficiency when solvent evaporation methods were used. Although CPT was encapsulated in the PAs, the fiber diameters of the resulting nanofibers did not appear to shift significantly in the presence of CPT, and the packing of the PA molecules into the cylinder morphology was not hindered by the presence of the CPT molecule (Fig. 3B). Accordingly as reported, the E3 PA alone had some toxicity, in vitro experiments showed that E3 PA CPT was more cytotoxic than excipient CPT (a CPT control dissolved in a 7.5% excipient mixture in PBS v/v) and was reflected by the lower IC_{50} values across all cell lines. In vivo testing also showed that CPT encapsulated in PA nanofibers exhibited a strongly significant antitumor effect compared to the PBS vehicle and PA alone (Fig. 3D), which is similar to CPT in the excipient mixture. These results demonstrated that the peptide amphiphile nanofibers can be applied to encapsulate the hydrophobic drug CPT as well as its biologically active lactone form, so other hydrophobic drugs as well as CPT could also be used for drug delivery by this method. This method of enriching nanofibers and other materials containing a hydrophobic core could be used for applications in cancer therapy, protein delivery and as a light-harvesting system.

3. Low-molecular weight hydrogels based on hydrophobic drugs

3.1 Low-molecular weight hydrogels based on a single hydrophobic drug

3.1.1 Low-molecular weight hydrogels based on Taxol. Recently the Xu and Yang group found that not only PAs, but hydrophobic compounds can self-assemble through non-covalent interactions to form nanofibers with jelly-like properties.24 Hydrophobic drug molecules might serve as a component of the self-assembly system for designing novel delivery systems for long term or local delivery of anti-cancer drugs for chemotherapy. Xu’s group reported the first example of a low-molecular weight hydrogel with a hydrophobic drug component.25 A Nap-FFKpY phosphorylated peptide was used to connect to Taxol via a hydrolysable ester bond. The dipeptide FF has been demonstrated to self-assemble into straight nanotubes,24 and Nap-FF has also been demonstrated to serve as a general motif to construct hydrogels.25 Compound 1a could be converted to 1b by phosphatase, and in this process, a hydrogel of Taxol with nanofiber morphology would be formed (Fig. 4A and B). It was also demonstrated that Taxol nanofibers exhibited similar activity, compared with Taxol, against cancer cells, and the Nap-FFKpY peptide is nontoxic to normal cells (Fig. 4C). Fig. 4D shows the release profiles of the hydrogels. After being treated with alkaline phosphatase (ALP), a solution of 4a (0.8%) could form a hydrogel, which could release 1b at a rate of 0.13% per hour. The release rate of 1b could be controlled by simply mixing 1a with 1b. For example, a mixed gel formed by treating a solution containing 0.6 wt% of 1b, 0.6 wt% of NapFFKpY and phosphatase, released 1b at a constant rate of 0.046% per hour.

Fig. 3 (A) Plot of E3 PA-encapsulated CPT prepared by the solvent evaporation method versus the initial PA concentration used. The initial concentration of CPT was held constant at 0.5 mM (the inner picture is the chemical structure of the model “E3 PA” peptide amphiphile used in these experiments with the sequence palmitoyl-A4G3E3 and camptothecin in the active lactone form). (B) Transmission electron microscopy image of E3 PA-encapsulated CPT using the solvent evaporation method and reconstituted in PBS. (C) E3 PA-encapsulated CPT is cytotoxic to breast cancer cells (BT-474) in vitro. (D) E3 PA-encapsulated CPT inhibits breast cancer xenograft growth in vivo. Reprinted with permission from ref. 30. Copyright (2011) American Chemical Society.
This nice work suggests that drug molecules are excellent candidates for engineering functional hydrogels or soft materials for various biomedical applications, including sustained or controlled drug delivery. Using this method other hydrophobic drugs can be applied for cancer therapy. Instead of ALP other enzymes which are overexpressed by abnormal cells, can by in situ hydrogelation, induce cell death in a moderate way.

3.1.2 Folic acid and Taxol as one building block to construct a low-molecular weight hydrogel. Inspired by Xu’s work, Yang et al.38 rationally designed and synthesized a folic acid–Taxol conjugate (FA-GpYK–Taxol shown in Fig. 5A). In this novel design, FA not only has a strong tendency to form a stable tetramer, but offers the FA–Taxol conjugate the ability to target cancer cells. After being treated with phosphatase, FA–GpYK–Taxol was converted to FA–GYK–Taxol which could self-assemble to form a low-molecular weight hydrogel. The morphology of this gel is not the same as that reported by Xu, or other hydrogel systems based on peptides. It exhibits a nanosphere structure of uniform size of about 50 nm (Fig. 5B). Comparing it to other drug delivery systems, it has the highest drug loading percentage at 49.4%. Due to the enhanced permeability and retention (EPR) effect of the nanospheres, the nanosphere would be enriched in the tumor cells, and the side effects would therefore be decreased. Few nanospheres which have been reported to encapsulate hydrophobic drugs have satisfactory efficacy due to the dilution effect, but the nanospheres reported by the Yang group were stable in different types of aqueous solutions including PBS buffer and cell culture DMEM medium with 10% FBS at 37 °C. The reported IC50 value of this nanosphere suggested that it has a comparable anticancer activity with respect to Taxol® (Fig. 5C). Not only is this FA–Taxol system novel for Taxol, it could also serve as a general type of novel drug delivery system for other hydrophobic drugs to treat different diseases. Because of the viscous nature of the hydrogel, most are difficult to inject by i.v.; this system may be an ideal method to construct a hydrogel suspension for i.v. injection.

3.1.3 The inhibition of tumor growth and metastasis by self-assembled nanofibers of Taxol. The above reported low-molecular weight hydrogels are either with a carrier or formed by a gelator–drug conjugate. These systems all show promising potential for effective delivery of anti-cancer drugs, however, practical applications of this drug delivery system also require long development time and the cost of evaluating the compatibility of carriers or efficacy of drug derivatives is relatively high. Actually, there are only three FDA approved drug delivery systems of low-molecular weight hydrogels, and these are those formed by the therapeutic agents themselves without any chemical modifications. Recently the Yang group reported a low-molecular weight hydrogel consisting of Taxol itself undergoing a self-hydrolysis process. As Fig. 6A shows the precursor of the hydrogel is mainly formed by the therapeutic agents: Taxol, oxidized glutathione (GSSG), and succinic acid. This precursor could form the hydrogel by self-hydrolysis of the ester-bond (indicated by the arrow in Fig. 6A) at 25 °C or 37 °C in PBS buffer (pH = 7.4). Uniform nanofibers entangled with each other to form the self-supporting hydrogel. It has been demonstrated that this hydrogel not only hinders tumor growth efficiently, in a dose dependent way, but that it could also prevent metastasis in breast cancers (Fig. 6D–F). The fatal dose...
of Taxol hydrogels was higher than 300 mg kg\(^{-1}\), 7.5 times higher than Taxol\(^\circ\). This carrier free drug delivery system may be a novel strategy worth exploring for clinical application of hydrophobic drugs. Most non-steroidal anti-inflammatory drugs are also hydrophobic drugs; simply attaching a hydrophilic drug may be an ideal method to form hydrogels for disease therapy.

### 3.2 Nanostructure of a peptide with two or more cooperative hydrophobic drugs

Two or multiple components of anti-cancer drugs are often used together to improve the efficiency, reduce side effects, and overcome drug resistance during chemotherapy. For example, dexamethasone (Dex) is often used twelve hours before Taxol is administered. Therefore, it is necessary and beneficial to develop a self-assembling system of multiple anti-cancer drugs for their effective delivery. Yang et al. recently reported the first example of a low-molecular weight hydrogel system composed of two complementary anti-cancer drugs (Dex and Taxol/10-hydroxycamptothecin (HCPT)).

Dex is an anti-inflammatory and immunosuppressant, which is usually given alone or in combination with other anti-cancer drugs during chemotherapy, and has a chemical structure similar to cholic acid which is frequently used to promote self-assembly. Fig. 7A illustrates the chemical structure of the designed compound. Dex-FFFK-ss-EE was used as a building block to connect Taxol or HCPT via a hydrolysable ester bond. After 1.0 equiv. of DTT was added to clear solutions containing 1.0 wt% of Dex-FFFK(Taxol/HCPT)-ss-EE, a self-supporting hydrogel of nanofibrous morphology is formed (Fig. 7B and C). At physiological temperature (37°C), the Tgel (the hydrogel formed by the precursor of Dex-FFFK(Taxol)-ss-EE) released Taxol at a rate of about 4.16 mg mL\(^{-1}\) per hour for the first 12 hours, followed by a lower rate of about 1.87 mg mL\(^{-1}\) per hour for the next 12 hours. Hgel (the hydrogel formed by the precursor of Dex-FFFK(10-hydroxycamptothecin)-ss-EE) released HCPT at a high rate of about 106 mg mL\(^{-1}\) per hour for the first 4 hours due to the burst effect; and then at a lower rate of 5.4 mg mL\(^{-1}\) from 8 to 24 hours. Approximately 2.2% and 35.9% of Taxol and HCPT were released from Tgel and Hgel at 37°C for 24 hours, respectively. A mixture of Tgel and Hgel in different ratios would obtain different rates of release profiles. These preliminary results...
imply the good potential of hydrogel systems for dual delivery of both types of anti-cancer drugs from mixed gels at controlled release rates. It was also demonstrated that HCPT is very stable in its lactone form under physiological conditions and no non-carboxylate form of HCPT or its derivatives was found in the Hgel that could lead to toxicity. This novel co-delivery hydrogel system composed of two complementary anti-cancer drugs could be applied for the long-term release of anti-cancer drugs after surgical removal of tumors. This opens up the possibility of the simultaneous delivery of two drugs to the same location and offers the potential for a great degree of control.

4. Integrated bioactive epitope within self-assembled peptides

4.1 Induction of cancer cell death by self-assembled nanostructures incorporating a cytotoxic peptide

The utility of peptides designed to activate cell death in cancer cells is a promising strategy for cancer therapy. Recently the Stupp group integrated a cationic α-helical (KLAKLAK)₃ peptide into a peptide-amphiphile (Fig. 8), that can self-assemble into bioactive, cylindrical nanofibers, that therefore has the ability to induce cancer cell death by membrane disruption. KLAK PA monomers self-assemble into nanostructures of 100 to 900 nm in length and 8 to 10 nm in diameter (Fig. 8B). KLAK peptide induced cancer cell death is dependent on the α-helical conformation. Fig. 8C shows that KLAK PA has a helical structure, whereas the KLAK peptide was predominantly formed as a random coil. Cellular uptake experiments also demonstrated that KLAK PA, but not the peptide, was internalized. Hence, KLAK PA not only stabilizes its helical conformation but has the ability to deliver intracellularly. Fig. 8E shows the mechanism of KLAK PA induced cell death. The plasma and mitochondrial membrane potentials were reduced within minutes of adding KLAK PA to cells which suggested membrane disruption. After treatment with PBS, KLAK peptide, or KLAK PA, MDA-MB-231 cells exhibited different ultrastructures. Cells treated with KLAK PA exhibited distorted plasma membranes, abundant fiber-like structures (arrows), and lysosome-like structures (arrowheads), and did not exhibit apoptotic morphology, characteristics which were not observed in cells treated with KLAK peptide (Fig. 8G) or PBS (Fig. 8H). These results clearly demonstrated that KLAK PA nanostructures induce cell death by disrupting cell membranes, and for the first time demonstrated that PAs can be rationally designed to self assemble into nanostructures that deliver cytotoxic peptides to cancer cells, representing a promising nanotechnology platform for cancer therapy.

4.2 Membrane-induced folding triggers activity

The membrane of most cancer cells has more negative charges on its surface. To take advantage of this property, several cationic antimicrobial peptides were used and found to show anticancer activity. Most of these peptides are unstructured in aqueous solution, but adopt a bioactive, helical conformation at the cell surface, whereas others adopt a β-sheet structure. Recently Schneider reported a small anticancer peptide which can fold at the surface of cancer cells, adopting an amphiphilic β-hairpin structure capable of membrane disruption (Fig. 9A). SVS-1 could form a β-hairpin structure at the surface of the cell, the α-isomer of the peptide (DSVS-1) is used to confirm that the mechanism of the observed anticancer activity was membrane lysis not receptor-mediated. SVS-2 however could not form a β-hairpin structure. Four different cancer cell lines: A549 (lung carcinoma), KB (epidermal carcinoma), MCF-7 (breast carcinoma) and MDA-MB-436 (breast carcinoma) were used to test the in vitro activity of the peptide. Fig. 9B show that the cytotoxic activity of the peptide is dose-dependent in all the
cancer cell lines tested. Comparing with the anticancer activity of DSVS-1, SVS-1 acts under a lytic mechanism and not a mechanism involving specific receptor binding. SVS-2 shows negligible toxicity toward both cancer cell lines, which verifies the hypothesis that hairpin formation is necessary for SVS-1 activity. Liposome leakage assays (Fig. 9C) were performed to demonstrate that a negatively charged surface is necessary to invoke the membrane-disruption action of SVS-1 and DSVS-1. TEM and SEM were utilized to directly visualize the cell membrane integrity as a function of added SVS-1. Fig. 9D–I showed that, untreated cells have an intact cell membrane and clearly visible intact organelles. In contrast, disrupted membranes and leakage of both intracellular and cytoplasmic content can be seen from cells which had been treated with SVS-1. Disrupted membranes with pore formation could be seen after SVS-1 treatment. It should be pointed out that noncancerous cells could also be killed with higher concentrations of peptides (Fig. 9F and I). This work, firstly, elicits the mechanism of action of anticancer peptides that adopt a bioactive β-structure at the cell surface while remaining unstructured in solution, which is widely instructive to scientists who are interested in peptides and self-assembly.

5. Intracellular enzymatic formation of nanofibers results in hydrogelation and regulated cell death

Xu and Ulijn developed enzymatic triggered formation of the hydrogel\(^{16,41}\) which has been applied in cell culture, drug delivery, wound healing, enzyme immobilization, inhibitor screening et al.\(^{41}\) Since most tumor cells expressed enzyme more abundantly than normal cells, the Xu group first proposed the idea of intracellular hydrogelation to induce cancer cell death.\(^{41}\) Fig. 10A illustrates the process of this idea. A rationally designed precursor, that does not self-assemble extracellularly, is converted into a hydrogel with the aid of an endogenous enzyme in the cell. The formed nanofibers then exert stresses on the cell and cause cell death. To validate this idea, precursors 4a and 4b were designed, 4a forms a hydrogel after esterase is added in vitro or in culture with HeLa cells, while 4b does not form nanofibers (Fig. 10C). These results confirmed that cell death is associated with intracellular formation of the nanofibers and the subsequent hydrogelation. It was also demonstrated that normal mammalian cell lines which do not highly express esterase cannot form nanofibers, therefore, the cell does not die for days after administration. This novel approach offers a new method to trigger cell death specifically, which is potentially important and more useful for cancer therapy.\(^{44}\)

This process is also imaged in a recent report.\(^{45}\)

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**Fig. 9** (A) Illustration: SVS-1 exists in a random coil conformation in solution. On engaging the negatively charged membrane surface, SVS-1 folds into a bioactive, lytic β-hairpin conformation capable of membrane disruption. Inner: peptide sequences of SVS-1 and its controls. Underlined amino acids are D-isomers, and all others are L-isomers; the stereochemistry of the turn region is specified for clarity; (B) in vitro cytotoxicity of SVS-1 toward A549 lung carcinoma, KB epidermal carcinoma, MCF-7 breast adenocarcinoma, MDA-MB-436 breast adenocarcinoma, and human erythrocytes (hRBCs); (C) Tb/DPA release from model POPC/POPS (1:1) liposomes monitored as a function of time following the addition of peptide and subsequent detergent. Tb/DPA release was monitored via luminescence. At t = 0, peptides were added, and Tb/DPA release was measured as a function of time. The final peptide/lipid ratio was 1:25. After equilibrium was reached, 1% OG detergent was added to obtain 100% release (arrows). The effect of SVS-1 on cell membrane integrity observed by TEM (D and G) and SEM (E, F, H and I). The membranes of the untreated A549 cancer cells (D and E) and noncancerous HUVEC (F) appeared intact. A549 cells (G and H) were incubated with 8 μM SVS-1 in serum-free media. HUVEC cells (I) incubated with a large excess (80 μM) of SVS-1 for 4 h at 37°C and 5% CO₂. Cells display leakage of cellular contents (dashed arrow) and pore formation (solid arrow). Scale bar: 10 μm for SEM, 2 μm for TEM. Reprinted with permission from ref. 18b Copyright (2012) American Chemical Society.

**Fig. 10** (A) Schematic intracellular formation of nanofibers that leads to hydrogelation and cell death; (B) Chemical structures and graphic representations of the precursor (4a), the control molecule (4b), and the hydrogelator (5a); (C) TEM of the hydrogels formed by the dead HeLa cells after culturing with 4a for three days (arrows indicate the nanofibers formed by 5a), inset: optical image of the hydrogel. Reprinted with permission from ref. 19. Copyright (2007) Wiley-VCH.
6. Conclusions and outlook

Low-molecular weight hydrogels have attracted considerable attention due to their inherent advantages. Prodrugs as precursors of low-molecular weight hydrogels has become the emerging area in recent years. For cancer therapy, the recently reported work is only in the first stages. Some of the issues that should be considered when designing low-molecular weight hydrogel anti-cancer drugs, are: (1) How to control the drug release rate remains a challenge. Although most drug carriers can improve the solubility of hydrophobic drugs, this also suffers from burst release of the cargo. Smart low-molecular weight hydrogels with tuned release rates in time and space is still a challenge; (2) low-molecular weight hydrogels also have relatively weak mechanical properties, which limit the use of these materials in structural tissue engineering. In some cases, this means that it can be easily washed away in hydrogel-derived medications. Using a hybrid hydrogel or by diluting the hydrogel for i.v. injection is a feasible way to overcome this; (3) most hydrogels are subcutaneously injected into the tumor or beside the tumor due to the viscous nature of the hydrogel, which can cause serious inflammation. New methods for generating hydrogels are also a challenge. Photo- or enzymatic triggered hydrogelation may be a better prospect; (4) last but not least is the in vivo activity of hydrogels which are formed by hydrophobic drugs. Most references only studied in vitro activity of the hydrogel, and seldom tested in vivo activity. With the future development of hydrogels, an ‘ideal’ hydrogel for the safe, effective treatment of patients will become a reality.

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Notes and references
