



Injectable Hydrogels for Localized Cancer Therapy

Dao-yang Fan, Yun Tian* and Zhong-jun Liu*

Department of Orthopedics, Peking University Third Hospital, Beijing, China

Traditional intravenous chemotherapy is relative to many systemic side effects, including myelosuppression, liver or kidney dysfunction, and neurotoxicity. As an alternative method, the injectable hydrogel can efficiently avoid these problems by releasing drugs topically at the tumor site. With advantages of localized drug toxicity in the tumor site, proper injectable hydrogel as the drug delivery system has become a research hotspot. Based on different types and stages of cancer, a variety of hydrogel drug delivery systems were developed, including thermosensitive, pH-sensitive, photosensitive, and dual-sensitive hydrogel. In this review, the latest developments of these hydrogels and related drug delivery systems were summarized. In summary, our increasing knowledge of injectable hydrogel for localized cancer therapy ensures us that it is a more durable and effective approach than traditional chemotherapy. Smart release system reacting to different stimuli at different time according to the micro-environment changes in the tumor site is a promising tendency for further studies.

Keywords: smart hydrogels, injectable, localized chemotherapy, stimuli responsive, drug delivery

OPEN ACCESS

Edited by:

Xing Wang,
Institute of Chemistry (CAS), China

Reviewed by:

Daifeng Li,
Stanford Bio-X, Stanford University,
United States
Xiangjie Su,
Utrecht University, Netherlands
Sophie Sun,
Chinese Academy of Sciences, China

*Correspondence:

Yun Tian
tiany@bjmu.edu.cn
Zhong-jun Liu
zjliu@bjmu.edu.cn

Specialty section:

This article was submitted to
Polymer Chemistry,
a section of the journal
Frontiers in Chemistry

Received: 19 August 2019

Accepted: 25 September 2019

Published: 11 October 2019

Citation:

Fan D, Tian Y and Liu Z (2019)
Injectable Hydrogels for Localized
Cancer Therapy. *Front. Chem.* 7:675.
doi: 10.3389/fchem.2019.00675

INTRODUCTION

With the deterioration of the environment, the incidence of cancer is increasing year by year. In 2018, there were 18.1 million new cancer cases worldwide (9.5 million males and 8.6 million females), and the death toll was 9.6 million (5.4 million males and 4.2 million females) (Bray et al., 2018). The global cancer burden is further aggravated. One in five men and one in six women worldwide will develop cancer, and 1 in 8 men and 1 in 11 women will die for cancer (Bray et al., 2018; Sivaram et al., 2018).

Different strategies and methods are applied for cancer patients according to their different diagnose and stage. Possible therapies include chemotherapy, radiation, surgery, immunotherapy, targeted therapy, and gene therapy (Wang et al., 2013; Roy and Trinchieri, 2017; Bykov et al., 2018; Senft et al., 2018). Among those options, chemotherapy plays a pivotal role in tumor control and prevention of recurrence. The anti-tumor activity of chemotherapeutic drugs kill tumor cells with its drug toxicity (Zhou et al., 2017). Without selective or targeted killing, normal tissues could be harmed by chemical drugs along with tumor cells (Andreyev et al., 2014).

With the development of hydrogel, traditional chemotherapy drugs are booming a new life. Drugs are injected within the hydrogel directly into the tumor or adjacent to it (Elias et al., 2015; Ma et al., 2015; Pan et al., 2018). Drugs could be localized in a crosslinked 3D network of hydrophilic polymer chains (Wang et al., 2013, 2017; Bu et al., 2017). In this way, drug toxicity is limited within a localized area where tumor cells lie. Meanwhile, localized hydrogel reveals the ability for continuous efficient drug delivery in the tumor site (Yang Y. Y. et al., 2016; Yang et al., 2018). To enhance this character, a variety of systems have been developed with different composition, including polyphosphazene (PPZ), polyethylene glycol (PEG), and Polylactate glycolic acid PLGA (Bu et al., 2017; Ahmad et al., 2018; Cheng et al., 2018; Gajendiran et al., 2019).

Most of the hydrogel is insoluble in water but excellent in water absorption capacity (from 10% to thousands of times their dry weight) (Bin Imran et al., 2014; Murgia et al., 2018) (**Figure 1**). The soft, moist surface, and affinity with the tissue greatly reduce the stimulation of the human body. Most materials that make hydrogels are non-toxic (Zhang et al., 2015; Batista et al., 2019; Gajendiran et al., 2019). According to the response to external stimuli, hydrogels can be divided into ordinary hydrogels and smart hydrogels (Gu et al., 2017). Original hydrogels are not sensitive to environmental changes (Castelletto et al., 2019; Kerdsirichairat et al., 2019; Luo et al., 2019). While smart hydrogels could be affected by pH, temperature, and photoelectricity. It results in changeable gel volume and traits for smart hydrogels (Deepa et al., 2012; Chen and Liu, 2016; Chen X. et al., 2016; Milcovich et al., 2017; Chen et al., 2019). These hydrogels are currently widely used in tissue engineering, drug delivery, and other fields (Bae et al., 2013; Del Bufalo et al., 2016; Casey et al., 2017; Castelletto et al., 2019).

THERMOSENSITIVE HYDROGELS

Temperature-sensitive hydrogels are hydrogels that change in volume as the ambient temperature changes (Klouda, 2015; Chen et al., 2018). The gel always has a certain proportion of hydrophobic and hydrophilic groups. Temperature changes can affect the hydrophobic interaction of these groups. The

hydrogen bonding between the macromolecular chains causes the gel structure to change, and volume changes occur (Fan et al., 2015). The temperature at which the volume changes is referred as lower critical solution temperature (LCST) (Sapino et al., 2019). Under this temperature, the gel swells in aqueous solution. Once the temperature rises to LCST, the gel shrinks (Lei et al., 2012; Wang et al., 2015). Its unique properties can be used as a drug carrier, which is injected into the body after being combined with a drug at a low temperature (Le et al., 2018). Forming a colloidal state with the help of body temperature makes it a drug sustained-release system, which simplifies not only medical treatment but also patients' suffering (Wang et al., 2015, 2016; Yang Y. et al., 2016). Main thermosensitive injectable hydrogels include chitosan/glycerophosphate (C/GP), hyaluronic acid (HA), PLGA based hydrogel, PEG-based hydrogel, PECE, and PECT (Guo et al., 2012; Klouda, 2015; Huang et al., 2016; Le et al., 2018; Sapino et al., 2019). Their characters and drug delivery systems were summarized in **Table 1**.

In one study (Huang et al., 2016), injectable thermosensitive doxorubicin (DOX) delivery system was developed with PECT hydrogel. Instead of hydrogel based on free DOX diffusion, which suffered from rapid drug clearance and poor drug penetration in tumor tissue, self-polymerized drug-loaded nanoparticles were encapsulated into PECT hydrogel (Huang et al., 2016). After *in vivo* injection, PECT gel exhibited a transition phase between sol and gel. The viscosity increased abruptly once the

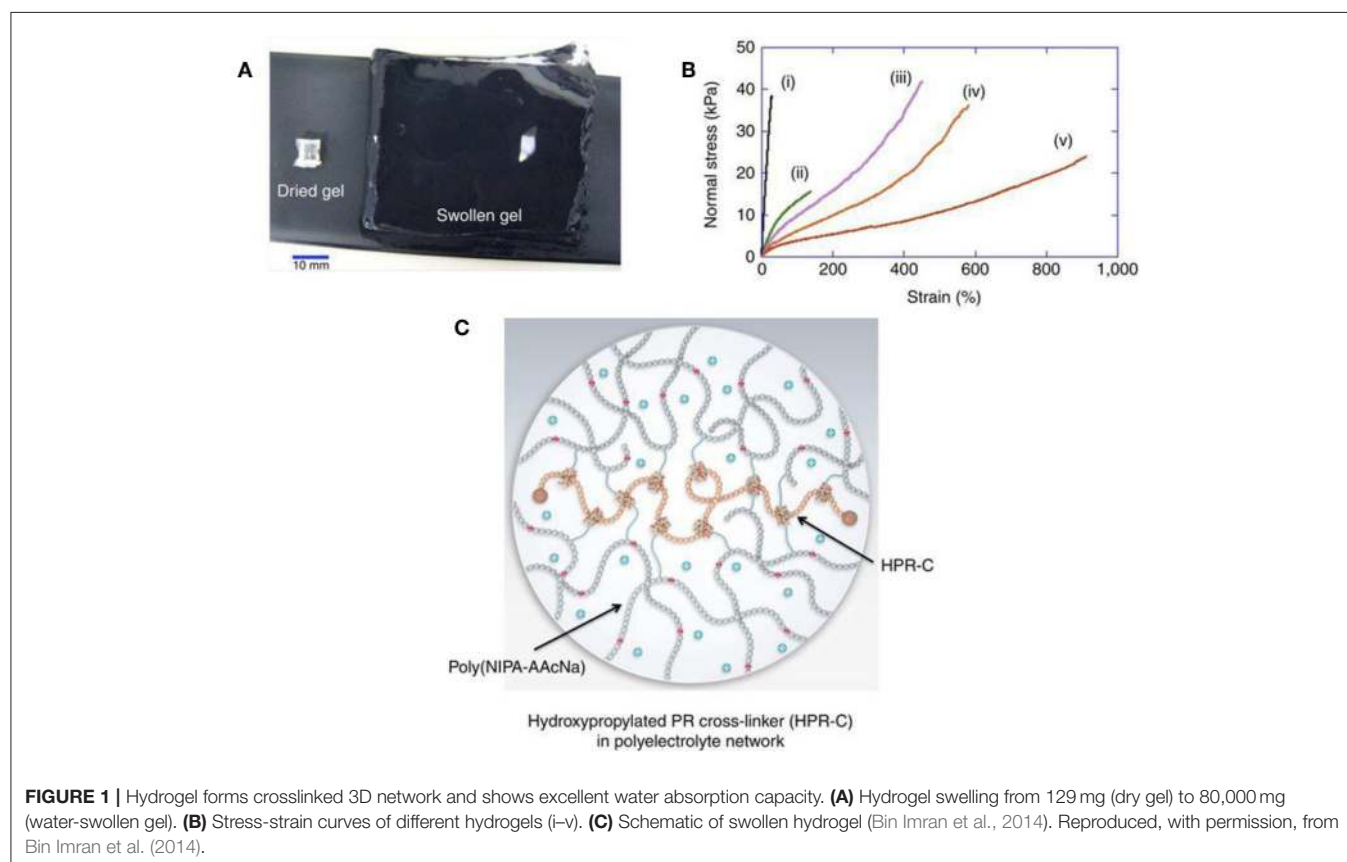
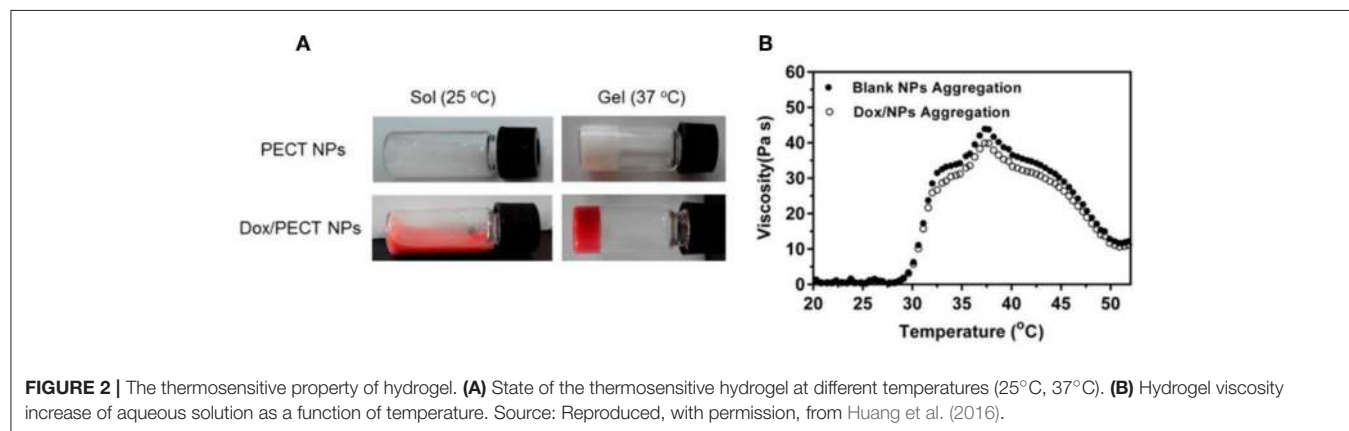


TABLE 1 | Main thermosensitive injectable hydrogel and drug delivery system.

Hydrogel	Drug	Cell line (<i>in vitro</i>)	Cancer (<i>in vivo</i>)	Characteristics and applications	References
Chitosan/ glycerophosphate (CS/GP)	Indocyanine green (ICG)	Hepatocellular carcinoma (HCC)	–	Hydrogel loaded with ICG is a feasible agent for fluorescence imaging and drug delivery. It forms <i>in situ</i> compact gel and has a good ability for filling vessels	Salis et al., 2015
CS/GP	DOXorubicin (DOX)	H22 and SMMC 7721 (hepatoma)	Hepatoma	This <i>in situ</i> gelling thermosensitive hydrogel is capable of drug delivery to tumor tissue constantly and efficiently	Ren et al., 2016
Hyaluronic acid (HA) and Pluronic F127 (PF127)	DOXorubicin (DOX) and Docetaxel (DOC)	CT-26 (colorectal carcinoma)	Colorectal carcinoma	Thermosensitive hydrogels loaded with DOX and DOX has a good potential for dual drug delivery, which efficiently enhanced cancer management with minimal side effects	Sheu et al., 2016
PLGA-PEG- PLGA	PLK1shRNA/PEI- Lys and DOX	Saos-2 and MG63 (osteosarcoma)	Osteosarcoma	Localized hydrogel with RNA and DOX co-loaded is promising for efficient clinical management of osteosarcoma	Ma et al., 2014
PLGA	Paclitaxel (PTX)	M234-p (mammary tumor)	mammary tumor	Four times the efficacy of existing commercial drugs	Pesoa et al., 2018
POR-PEG-PCL	fluorescence tag	HepG2 (hepatoma)	hepatoma	Good safety and biocompatibility <i>in vitro</i> and <i>in vivo</i>	Dong et al., 2016
PCL-PTSUO- PEG	TNP/DOX/ZnPC	5637 (bladder cancer cells)	Bladder carcinoma	Double insurance from TNP/ZnPC and TNP/DOX loaded in hydrogel for the management of bladder carcinoma	Huang et al., 2018
PEG-PCL- PEG, PECE	PTX	4T1 (breast cancer)	Breast cancer	Great antitumor effect and wound healing promotion.	Lei et al., 2012
PECT	PTX	MCF-7	Breast cancer	High concentration in tumor tissue for 21 days	Lin et al., 2016



temperature is higher than 28°C. With the highest viscosity at 37°C, the hydrogel turned to gel from sol (**Figure 2**). Loaded nanoparticles were dissociated from hydrogel and diffused within tumor tissue by EPR effect. Intracellular chemical drug release limited its toxic effects and enhanced its anti-tumor effectiveness. Contrasted with intravenous drug injections (*I.V.*), a thermosensitive hydrogel with nanomedicine loaded is an efficient drug delivery system, which enabled continuous drug release around tumor tissues (Huang et al., 2016).

Two or more elements loaded in one thermosensitive hydrogel has emerged as a promising drug delivery system for its superior

anti-tumor efficacy. Polo-like kinase 1 (PLK1) gene is recognized as a key regulator of tumor cell meiosis and mitosis (Ma et al., 2014). RNA interference-based on PLK1shRNA can specifically reduce the function of the target gene in the tumor. A strategy of DOX and PLK1shRNA/PEI-Lys co-delivery hydrogel was developed for the treatment of osteosarcoma (Ma et al., 2014). In this method, PLK1shRNA/PEI-Lys in the hydrogel can greatly enhance the anti-tumor effect of DOX. With the synergistic effect from PLK1shRNA/PEI-Lys and DOX, significant osteosarcoma apoptosis was caused by the co-loaded hydrogel (**Figure 3**) (Ma et al., 2014).

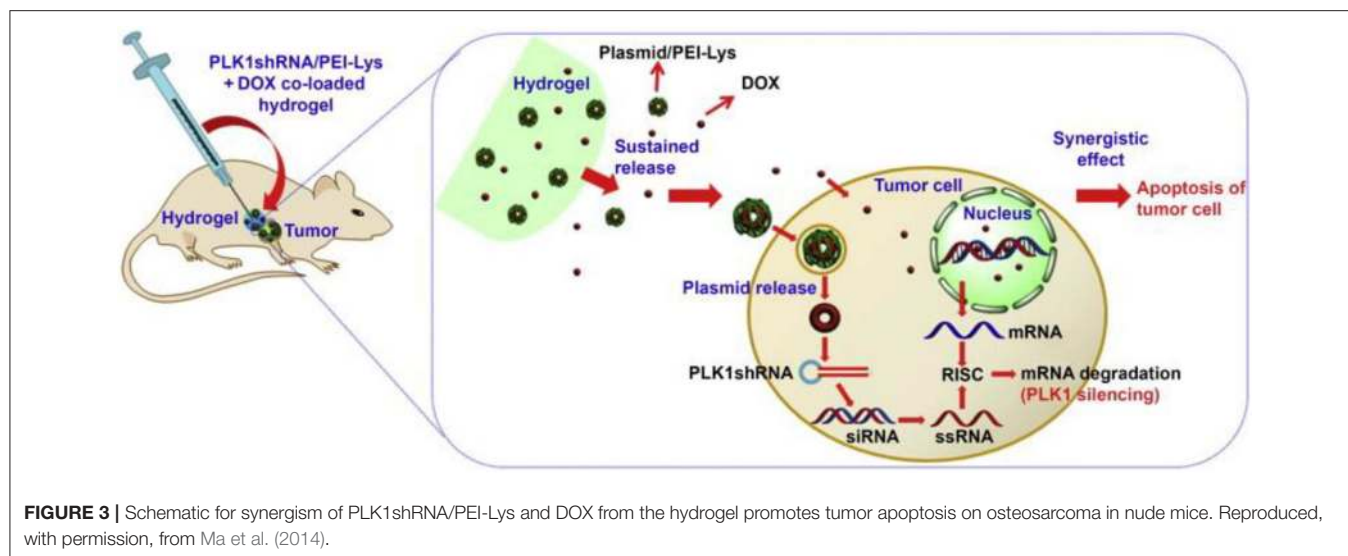


TABLE 2 | pH-sensitive hydrogels and drug delivery system.

Hydrogel	Drug	Cell line (<i>in vitro</i>)	Cancer (<i>in vivo</i>)	Characteristics and applications	References
Acrylic Acid and PEGDA	Salicylic Acid (SA)	3t3 fibroblast cells	–	Sa based pH sensitive hydrogel reveals good pH sensibility and great biocompatibility	Demirdirek and Urich, 2017
CS and GP	DOX	Mcf-7 cells (breast cancer)	Breast cancer	With an LCST of 39°C, the gel reveals DOX when pH =5.5	Fathi et al., 2019
Chitosan Dihydrocaffeic Acid (CS-DA) and Oxidized Pullulan (OP)	DOX and Amoxicillin	Hct116 cells (colon tumor cells) and e. Coli	Colon tumor and infection	DOX and amoxicillin co-loaded hydrogel response to pH decrease. The hydrogel is an ideal system for mucosa-localized tumor and infection management	Liang et al., 2019
GC-PF127	DOX	H22 (breast cancer)	Breast cancer	Response to small pH change, hydrogel release DOX-loaded micelles with tumor-targeted	Liu et al., 2017
CEC-PEGDA	DOX	Hepg2 (liver cancer) and I929	Liver carcinoma	Good cytocompatibility and anti-tumor effect. The pH-responsive hydrogel is a promising delivery system for liver cancer treatment	Qu et al., 2017
PEGMA and AAC	5-FU	Hepg2 (liver cancer) and LO2	Liver carcinoma	Controlled and targeted drug delivery for liver carcinoma	Yue et al., 2019
CS/HA/GP	DOX	Hela (cervical cancer)	Cervical cancer	Good pH sensitivity for the localized management of cervical cancer	Zhang et al., 2018

PH-SENSITIVE HYDROGELS

Glycolysis of tumor cells can cause acidification in the environment next to tumor tissues, resulting in lower pH value in the extracellular matrix than normal tissues (Kenney et al., 2018; Hu et al., 2019). A pH-sensitive hydrogel is a polymer gel in which the volume of the hydrogel changes depending on the pH of the external environment and the ionic strength (Liao et al., 2017; Liu et al., 2019). Such gels contain a large number of readily hydrolyzable or protonated acids, base groups such as carboxyl groups and amino groups (Lym et al., 2016). The dissociation of these groups is affected by the external pH. When the external pH changes, the degree of dissociation of these groups changes correspondingly, causing the internal and

external ion concentration to change (Norouzi et al., 2016). In addition, the dissociation of these groups will destroy the corresponding hydrogen in the gel. The bond reduces the cross-linking point of the gel network, causing a change in the structure of the gel network and the degree of swelling of the hydrogel (Qu et al., 2017; Oroojalian et al., 2018). With this property, the rate of diffusion and release of the drug in the gel can be conveniently adjusted and controlled (Samanta et al., 2015).

A variety of elements for pH-sensitive hydrogel were explored in the past decades. Their characters and drug delivery systems were summarized in **Table 2**. One of the choices is based on chitosan-grafted-dihydrocaffeic acid (CS-DA) and oxidized pullulan (OP) (Liang et al., 2019). With classical drug for anti-tumor therapy, DOX-loaded hydrogel was tested to explore its

reactions for the pH changes in the tumor environment. With glycolysis in the tumor site, a decrease of pH triggered the drug release (Liang et al., 2019). Compared with the morphology of hydrogel at pH 7.4, significant disintegration of hydrogel resulted in larger pore size at pH 5.5 (**Figure 4**) (Liang et al., 2019). After 60 h at pH 5.5, more than 80% of DOX was released from the hydrogel. The hydrogel was co-cultured with Hct116 cells (colon tumor cells) to test its anti-tumor effect (Liang et al., 2019). DOX is continuously stable released from hydrogel at pH 5.5 and 7.4. In both conditions, DOX can be effectively released for more than 3 days (Liang et al., 2019).

In recent years, aspirin has been found to inhibit carbon monoxide synthase, inhibit nitrite-mediated DNA damage, reduce surviving, inhibit nuclear transcription factors, proteasomes, and calcium-activated neutral protease genes by inhibiting cyclooxygenase (Lu et al., 2008; Choi et al., 2013). The expression and other mechanisms play an anti-tumor effect. After loading aspirin into the hemicellulose hydrogel, it was found that 85% of the drug could be released continuously for 5–6 h under pH 7.4 (Choi et al., 2013). Sun et al. successfully prepared a series of acrylic acid and acrylamide copolymer grafted hemicellulose hydrogels by free radical polymerization (Sun et al., 2013). The combination of aspirin and the drug showed that the release rate of the drug in the simulated gastric juice was slower, and the release rate in the simulated intestinal fluid was significantly faster than that of the simulated gastric juice. When the release time was 12 h, the cumulative release rate reached 90%, which shows excellent sustained release properties (Sun et al., 2013).

In addition, Wang et al. first inserted dexamethasone phosphate into molecularly imprinted polymer nanospheres and loaded the polymer onto a pH-sensitive hydrogel, making it a biosensor that inhibits inflammation (Wang et al., 2010). Since the inflammatory reaction can lead to an acidic environment, this pH-sensitive hydrogel can rapidly release the drug at pH 6.0~7.4 to inhibit the inflammation (Wang et al., 2010). This different method is resistant to pH-sensitive hydrogels. The application of oncology drugs has brought new ideas.

PHOTOSENSITIVE HYDROGELS

The mechanism of a light-sensitive hydrogel is divided into two types according to the properties of the photosensitive material (Chang et al., 2019). One is to directly add the photosensitive molecular material to the temperature-sensitive gel, and convert the light energy into heat energy to make the temperature inside the gel reach the phase transition temperature. In this way, hydrogel produces photosensitivity. Another kind of chromophore is introduced into the gel structure (Norouzi et al., 2016). The physicochemical properties of the chromophore are changed when subjected to light stimulation. By changing the network structure, hydrogel macroscopically exhibits photosensitivity. Usually, a structure such as azobenzene, spiropyran, o-naphthoquinone, anthracene, nitrophenyl, and coumarin is introduced into the gel (Tam et al., 2017). Among them, ruthenium, nitrophenyl, and coumarin compounds mainly

take advantage of photo-cleavage photosensitive groups, which are bonded to the hydrophobic end through an aryl methyl bond (Norouzi et al., 2016; Tam et al., 2017). Under ultraviolet light or near-infrared light, the ester group is broken. The photosensitive reaction is caused. The hydrophobic end is converted to a hydrophilic end, causing the gel to dissociate. The azobenzene compound is controlled by the conversion of the cis-trans structure. Their characters and drug delivery systems were summarized in **Table 3**.

One of the applications for photosensitive hydrogels is the platform for cell culture and 3D tumor micro-environment studies. With advantages of its photosensitive character, cell detachment on the surface of hydrogel was done layer-by-layer to form a 3D cell culture medium (**Figure 5**) (Wang et al., 2014). Photoinitiated copolymerization of P (OEGMA-co-VDT-co-SPAA) (POVSP) hydrogels happened with UV irradiation. The compressive strengths of hydrogels were up to 5.1 MPa, which is strong enough for cell culture (Wang et al., 2014). It is revealed that photosensitive hydrogel is suitable for 3D cell culture model, which is vital for the study of the mechanism for tumor development.

To achieve the same purpose, a photocleavable terpolymer hydrogel was developed as the basic technique for 3D bio-printing. This hydrogel is capable of self-shaping directly to the UV irradiation. It is designable by using selective illumination to UV light with the specific area covered with darkness (**Figure 6**) (Liao et al., 2015). The printable hydrogel is an inspiring design for controlled drug delivery with district distribution. It is a key technique for the realization of 4D drug delivery with both dimensions of time and space. With drugs loaded in the 3D space of hydrogels, dynamic drug release can be realized. In this process, different drug could be controlled to be released in different time with purpose (Xu et al., 2015; Chen Y. et al., 2016; Kim et al., 2017; Guo et al., 2018). This is the typical way of 4D drug delivery with additional dimensions of time.

DUAL-SENSITIVE HYDROGELS

With the increasing requirements for the precision of controlled release of drugs, multi-sensitive hydrogels have received more and more attention (Bardajee et al., 2017). In particular, co-sensitive hydrogels for temperature and pH is widely researched. Temperature and pH are two important factors in physiological, biological, and chemical systems (Bardajee et al., 2017; Fathi et al., 2019). The temperature-pH double-sensitive hydrogel consists of a temperature-sensitive and pH-sensitive two-part hydrophilic polymer network (Lym et al., 2016). Usually formed with two or more monomers or polymers, which respond to temperature and pH, respectively.

The combination of temperature and pH sensitivity is crucial for the management of locoregional tumor recurrence (Mackiewicz et al., 2019). A novel pH-sensitive thermosensitive hydrogel loaded with modified doxorubicin-based prodrug nanoparticles (PDNPs), which is more efficient for tumor management than free DOX (Liu et al., 2019). Good biocompatibility and anti-tumor activity were verified by

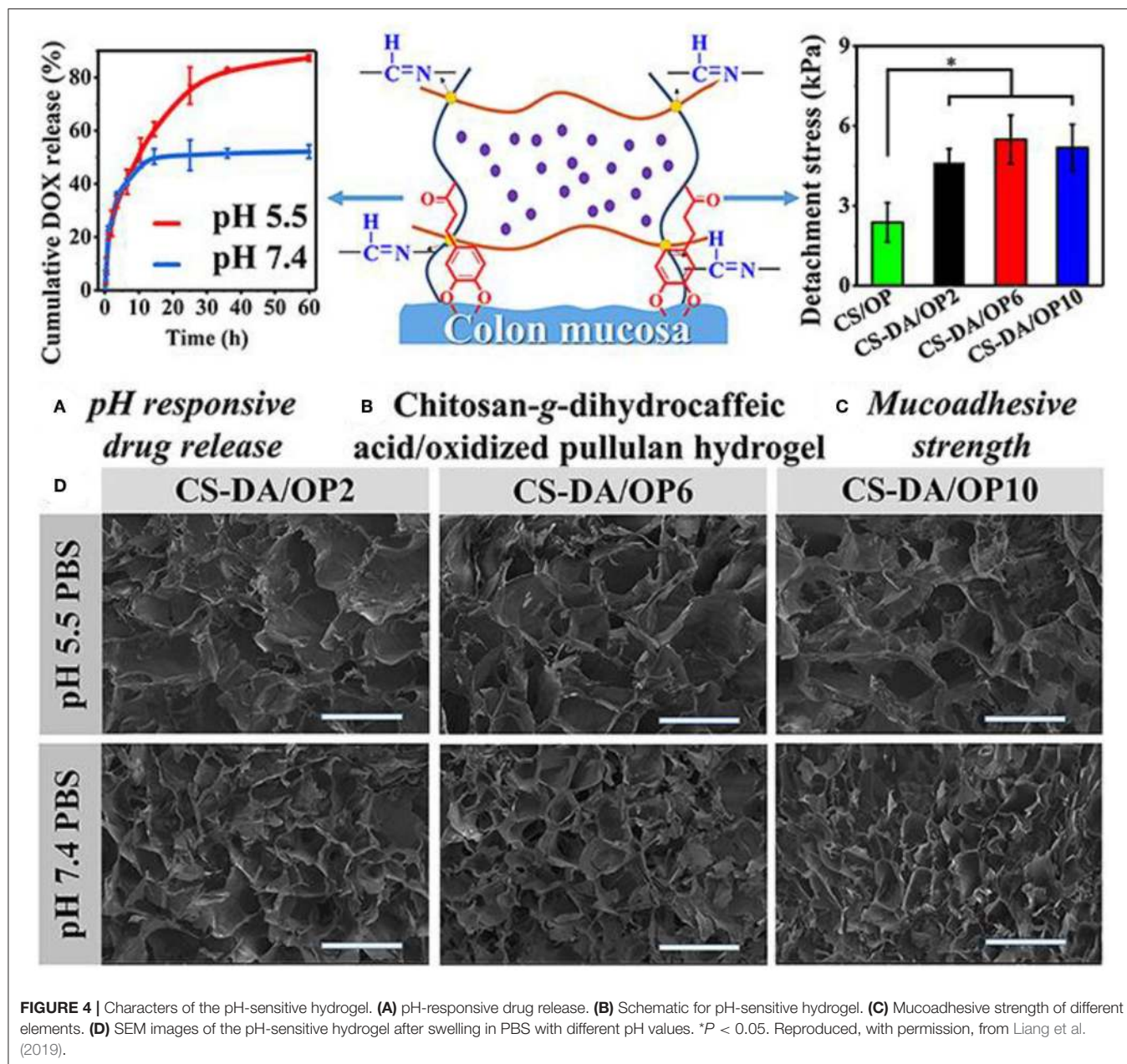
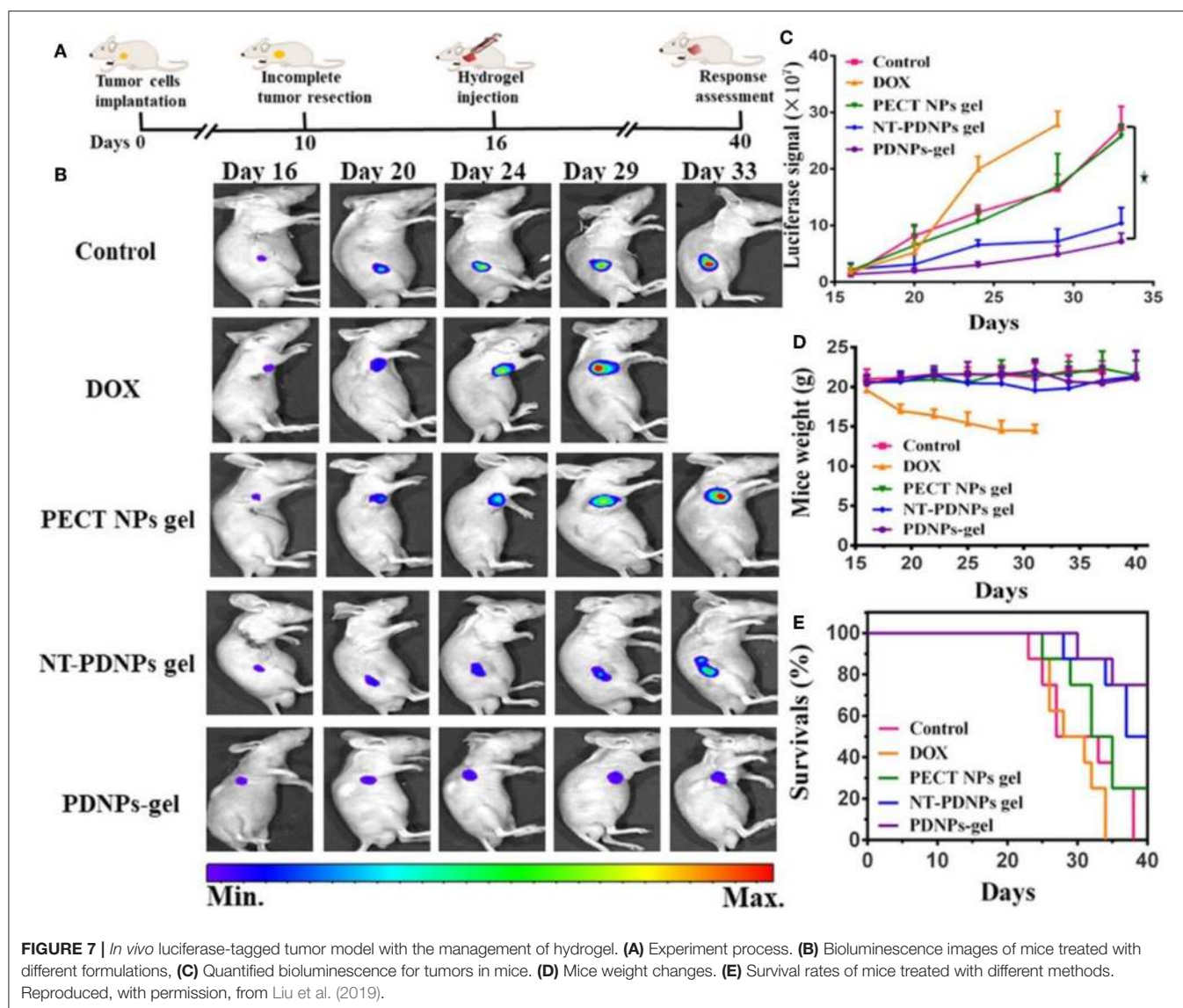


TABLE 3 | Photosensitive hydrogels and drug delivery system.

Hydrogel	Drug	Cell line (<i>in vitro</i>)	Cancer (<i>in vivo</i>)	Characteristics and applications	References
Hyaluronic Acid (HA)	Matrix metalloproteinase (MMP)	MDA-MB-231 cells (breast cancer)	–	Biomimetic 3D cell culture model for cancer researches.	Tam et al., 2017
DNA Polyacrylamide Conjugate (DPC)	DNA and DOX	CEM (lymphocytic leukemia)	Lymphocytic leukemia	Controlled release with DNA crosslinked inside the photo-responsive hydrogel	Kang et al., 2011
Hyperbranched polyprodrug (PPM)	PPM	A549 cells (lung cancer)	Lung Cancer	DOX and amoxicillin co-loaded hydrogel response to pH decrease. The hydrogel is an ideal system for mucosa-localized tumor and infection management	Guo et al., 2018



CONCLUSIONS

The unique character of hydrogel makes it an efficient functional medium for drug delivery (Zhang et al., 2018; Fathi et al., 2019; Mackiewicz et al., 2019). Given the limits from chemical drug toxicity for normal tissue and organs, localized drug delivery system by hydrogel has been a crucial method for cancer management. Related studies mainly focused on the delivery function and the methods of stimuli-response (Lym et al., 2016; Bardajee et al., 2017; Wei et al., 2017; Fathi et al., 2019). The smart hydrogel was developed with accurate responses to tiny changes in temperature, pH, and light. For now, drugs can be easily delivered to cancer tissue at the right time point. In the future, co-loaded drugs, including DNA, RNA, protein, and related products, would be a key point. The constantly accurate drug

delivery system can realize anti-tumor drugs release followed by tissue repair factors. In this way, demission of time and space for drug delivery would be mixed in one hydrogel, making it a 4D functional hydrogel. It can make hydrogel a perfect choice for local chemotherapy and cancer management.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

The financial support from Ministry of Science and Technology of China (2016YFB1101501) is gratefully acknowledged.

REFERENCES

- Ahmad, R., Kaus, N. H. M., and Hamid, S. (2018). Synthesis and characterization of PLGA-PEG thymoquinone nanoparticles and its cytotoxicity effects in tamoxifen-resistant breast cancer cells. *Adv. Exp. Med. Biol.* 1, 1–18. doi: 10.1007/5584_2018_302
- Andreyev, J., Ross, P., Donnellan, C., Lennan, E., Leonard, P., Waters, C., et al. (2014). Guidance on the management of diarrhoea during cancer chemotherapy. *Lancet Oncol.* 15, e447–e460. doi: 10.1016/S1470-2045(14)70006-3
- Bae, W. K., Park, M. S., Lee, J. H., Hwang, J. E., Shim, H. J., Cho, S. H., et al. (2013). Docetaxel-loaded thermoresponsive conjugated linoleic acid-incorporated poloxamer hydrogel for the suppression of peritoneal metastasis of gastric cancer. *Biomaterials* 34, 1433–1441. doi: 10.1016/j.biomaterials.2012.10.077
- Bardajee, G. R., Hooshyar, Z., Farsi, M., Mobini, A., and Sang, G. (2017). Synthesis of a novel thermo/pH sensitive nanogel based on saleg modified graphene oxide for drug release. *Mater. Sci. Eng. C Mater. Biol. Appl.* 72, 558–565. doi: 10.1016/j.msec.2016.11.109
- Batista, R. A., Espitia, P. J. P., Quintans, J. S. S., Freitas, M. M., Cerqueira, M. A., Teixeira, J. A., et al. (2019). Hydrogel as an alternative structure for food packaging systems. *Carbohydr. Polym.* 205, 106–116. doi: 10.1016/j.carbpol.2018.10.006
- Bin Imran, A., Esaki, K., Gotoh, H., Seki, T., Ito, K., Sakai, Y., et al. (2014). Extremely stretchable thermosensitive hydrogels by introducing slide-ring polyrotaxane cross-linkers and ionic groups into the polymer network. *Nat. Commun.* 5:5124. doi: 10.1038/ncomms6124
- Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 68, 394–424. doi: 10.3322/caac.21492
- Bu, Y., Shen, H., Yang, F., Yang, Y., Wang, X., and Wu, D. (2017). Construction of tough, *in situ* forming double-network hydrogels with good biocompatibility. *ACS Appl. Mater. Interfaces* 9, 2205–2212. doi: 10.1021/acsami.6b15364
- Bykov, V. J. N., Eriksson, S. E., Bianchi, J., and Wiman, K. G. (2018). Targeting mutant p53 for efficient cancer therapy. *Nat. Rev. Cancer* 18, 89–102. doi: 10.1038/nrc.2017.109
- Casey, J., Yue, X., Nguyen, T. D., Acun, A., Zellmer, V. R., Zhang, S., et al. (2017). 3D hydrogel-based microwell arrays as a tumor microenvironment model to study breast cancer growth. *Biomed. Mater.* 12:025009. doi: 10.1088/1748-605X/aa5d5c
- Castelletto, V., Edwards-Gayle, C. J. C., Greco, F., Hamley, I. W., Seitsonen, J., and Ruokolainen, J. (2019). Self-assembly, tunable hydrogel properties and selective anti-cancer activity of a carnosine-derived lipidated peptide. *ACS Appl. Mater. Interfaces* 11, 33573–33580. doi: 10.1021/acsami.9b09065
- Chang, G., Zhang, H., Li, S., Huang, F., Shen, Y., and Xie, A. (2019). Effective photodynamic therapy of polymer hydrogel on tumor cells prepared using methylene blue sensitized mesoporous titania nanocrystal. *Mater. Sci. Eng. C Mater. Biol. Appl.* 99, 1392–1398. doi: 10.1016/j.msec.2019.02.056
- Chen, C. H., Kuo, C. Y., Chen, S. H., Mao, S. H., Chang, C. Y., Shalumon, K. T., et al. (2018). Thermosensitive injectable hydrogel for simultaneous intraperitoneal delivery of doxorubicin and prevention of peritoneal adhesion. *Int. J. Mol. Sci.* 19:E1373. doi: 10.3390/ijms19051373
- Chen, X., and Liu, Z. (2016). A pH-responsive hydrogel based on a tumor-targeting mesoporous silica nanocomposite for sustained cancer labeling and therapy. *Macromol. Rapid Commun.* 37, 1533–1539. doi: 10.1002/marc.201600261
- Chen, X., Liu, Z., Parker, S. G., Zhang, X., Gooding, J. J., Ru, Y., et al. (2016). Light-induced hydrogel based on tumor-targeting mesoporous silica nanoparticles as a theranostic platform for sustained cancer treatment. *ACS Appl. Mater. Interfaces* 8, 15857–15863. doi: 10.1021/acsami.6b02562
- Chen, Y., Hao, Y., Huang, Y., Wu, W., Liu, X., Li, Y., et al. (2019). An injectable, near-infrared light-responsive click cross-linked azobenzene hydrogel for breast cancer chemotherapy. *J. Biomed. Nanotechnol.* 15, 1923–1936. doi: 10.1166/jbn.2019.2821
- Chen, Y., Zhang, F., Fu, Q., Liu, Y., Wang, Z., and Qi, N. (2016). *In vitro* proliferation and osteogenic differentiation of human dental pulp stem cells in injectable thermo-sensitive chitosan/beta-glycerophosphate/hydroxyapatite hydrogel. *J. Biomater. Appl.* 31, 317–327. doi: 10.1177/0885328216661566
- Cheng, C., Meng, Y., Zhang, Z., Li, Y., and Zhang, Q. (2018). Tumoral acidic pH-responsive cis-diaminodichloroplatinum-incorporated Cy5.5-PEG-g-A-HA nanoparticles for targeting delivery of CDDP against cervical cancer. *ACS Appl. Mater. Interfaces* 10, 26882–26892. doi: 10.1021/acsami.8b07425
- Choi, B. H., Chakraborty, G., Baek, K., and Yoon, H. S. (2013). Aspirin-induced Bcl-2 translocation and its phosphorylation in the nucleus trigger apoptosis in breast cancer cells. *Exp. Mol. Med.* 45:e47. doi: 10.1038/emmm.2013.91
- Deepa, G., Thulasidasan, A. K., Anto, R. J., Pillai, J. J., and Kumar, G. S. (2012). Cross-linked acrylic hydrogel for the controlled delivery of hydrophobic drugs in cancer therapy. *Int. J. Nanomed.* 7, 4077–4088. doi: 10.2147/IJN.S30149
- Del Bufalo, F., Manzo, T., Hoyos, V., Yagyu, S., Caruana, I., Jacot, J., et al. (2016). 3D modeling of human cancer: a PEG-fibrin hydrogel system to study the role of tumor microenvironment and recapitulate the *in vivo* effect of oncology adenovirus. *Biomaterials* 84, 76–85. doi: 10.1016/j.biomaterials.2016.01.030
- Demirdirek, B., and Uhrich, K. E. (2017). Novel salicylic acid-based chemically crosslinked pH-sensitive hydrogels as potential drug delivery systems. *Int. J. Pharm.* 528, 406–415. doi: 10.1016/j.ijpharm.2017.05.047
- Dong, X., Wei, C., Lu, L., Liu, T., and Lv, F. (2016). Fluorescent nanogel based on four-arm PEG-PCL copolymer with porphyrin core for bioimaging. *Mater. Sci. Eng. C Mater. Biol. Appl.* 61, 214–219. doi: 10.1016/j.msec.2015.12.037
- Elias, P. Z., Liu, G. W., Wei, H., Jensen, M. C., Horner, P. J., and Pun, S. H. (2015). A functionalized, injectable hydrogel for localized drug delivery with tunable thermosensitivity: synthesis and characterization of physical and toxicological properties. *J. Control. Release* 208, 76–84. doi: 10.1016/j.jconrel.2015.03.003
- Fan, R., Tong, A., Li, X., Gao, X., Mei, L., Zhou, L., et al. (2015). Enhanced antitumor effects by docetaxel/LL37-loaded thermosensitive hydrogel nanoparticles in peritoneal carcinomatosis of colorectal cancer. *Int. J. Nanomed.* 10, 7291–7305. doi: 10.2147/IJN.S89066
- Fathi, M., Alami-Milani, M., Geranmayeh, M. H., Barar, J., Erfan-Niya, H., and Omid, Y. (2019). Dual thermo- and pH-sensitive injectable hydrogels of chitosan/(poly(N-isopropylacrylamide-co-itaconic acid)) for doxorubicin delivery in breast cancer. *Int. J. Biol. Macromol.* 128, 957–964. doi: 10.1016/j.ijbiomac.2019.01.122
- Gajendiran, M., Jo, H., Kim, K., and Balasubramanian, S. (2019). Green synthesis of multifunctional PEG-carboxylate pi back-bonded gold nanoconjugates for breast cancer treatment. *Int. J. Nanomed.* 14, 819–834. doi: 10.2147/IJN.S190946
- Gu, D., O'Connor, A. J., G. H. Q., and Ladewig, K. (2017). Hydrogels with smart systems for delivery of hydrophobic drugs. *Expert Opin. Drug Deliv.* 14, 879–895. doi: 10.1080/17425247.2017.1245290
- Guo, D., Xu, S., Huang, Y., Jiang, H., Yasen, W., Wang, N., et al. (2018). Platinum(IV) complex-based two-in-one polyprodrug for a combinatorial chemo-photodynamic therapy. *Biomaterials* 177, 67–77. doi: 10.1016/j.biomaterials.2018.05.052
- Guo, D. D., Hong, S. H., Jiang, H. L., Kim, J. H., Minai-Tehrani, A., Kim, J. E., et al. (2012). Synergistic effects of Akt1 shRNA and paclitaxel-incorporated conjugated linoleic acid-coupled poloxamer thermosensitive hydrogel on breast cancer. *Biomaterials* 33, 2272–2281. doi: 10.1016/j.biomaterials.2011.12.011
- Hu, S. W., Wang, J., Zhang, T. T., Li, X. L., Chen, H. Y., and Xu, J. J. (2019). Targeted transmembrane delivery of Ca(2+) via FA-nanogel for synergistically enhanced chemotherapy. *ACS Appl. Mater. Interfaces* 11, 16412–16420. doi: 10.1021/acsami.9b04967
- Huang, P., Song, H., Zhang, Y., Liu, J., Zhang, J., Wang, W., et al. (2016). Bridging the gap between macroscale drug delivery systems and nanomedicines: a nanoparticle-assembled thermosensitive hydrogel for peritumoral chemotherapy. *ACS Appl. Mater. Interfaces* 8, 29323–29333. doi: 10.1021/acsami.6b10416
- Huang, Z., Xiao, H., Lu, X., Yan, W., and Ji, Z. (2018). Enhanced photo/chemo combination efficiency against bladder tumor by encapsulation of DOX and ZnPC into *in situ*-formed thermosensitive polymer hydrogel. *Int. J. Nanomed.* 13, 7623–7631. doi: 10.2147/IJN.S179226
- Kang, H., Liu, H., Zhang, X., Yan, J., Zhu, Z., Peng, L., et al. (2011). Photoresponsive DNA-cross-linked hydrogels for controllable release and cancer therapy. *Langmuir* 27, 399–408. doi: 10.1021/la1037553
- Kenney, R. M., Boyce, M. W., Whitman, N. A., Kromhout, B. P., and Lockett, M. R. (2018). A pH-sensing optode for mapping spatiotemporal

- gradients in 3D paper-based cell cultures. *Anal. Chem.* 90, 2376–2383. doi: 10.1021/acs.analchem.7b05015
- Kerdsirichairat, T., Narang, A. K., Thompson, E., Kim, S. H., Rao, A., Ding, K., et al. (2019). Hydrogel spacer for borderline resectable and locally advanced pancreatic cancer: a feasibility human study. *Gastroenterology* 157, 933–935. doi: 10.1053/j.gastro.2019.07.012
- Kim, Y. M., Potta, T., Park, K. H., and Song, S. C. (2017). Temperature responsive chemical crosslinkable UV pretreated hydrogel for application to injectable tissue regeneration system via differentiations of encapsulated hMSCs. *Biomaterials* 112, 248–256. doi: 10.1016/j.biomaterials.2016.10.025
- Klouda, L. (2015). Thermoresponsive hydrogels in biomedical applications: a seven-year update. *Eur. J. Pharm. Biopharm.* 97(Pt B), 338–349. doi: 10.1016/j.ejpb.2015.05.017
- Le, P. N., Huynh, C. K., and Tran, N. Q. (2018). Advances in thermosensitive polymer-grafted platforms for biomedical applications. *Mater. Sci. Eng. C Mater. Biol. Appl.* 92, 1016–1030. doi: 10.1016/j.msec.2018.02.006
- Lei, N., Gong, C., Qian, Z., Luo, F., Wang, C., Wang, H., et al. (2012). Therapeutic application of injectable thermosensitive hydrogel in preventing local breast cancer recurrence and improving incision wound healing in a mouse model. *Nanoscale* 4, 5686–5693. doi: 10.1039/c2nr30731f
- Liang, Y., Zhao, X., Ma, P. X., Guo, B., Du, Y., and Han, X. (2019). pH-responsive injectable hydrogels with mucosal adhesiveness based on chitosan-grafted-dihydrocaffeic acid and oxidized pullulan for localized drug delivery. *J. Colloid Interface Sci.* 536, 224–234. doi: 10.1016/j.jcis.2018.10.056
- Liao, W. C., Lilienthal, S., Kahn, J. S., Riutin, M., Sohn, Y. S., Nechushtai, R., et al. (2017). pH- and ligand-induced release of loads from DNA-acrylamide hydrogel microcapsules. *Chem. Sci.* 8, 3362–3373. doi: 10.1039/C6SC04770J
- Liao, Y., An, N., Wang, N., Zhang, Y., Song, J., Zhou, J., et al. (2015). Photoactive self-shaping hydrogels as noncontact 3D macro/microscopic photoprinting platforms. *Macromol. Rapid Commun.* 36, 2129–2136. doi: 10.1002/marc.201500390
- Lin, Z., Xu, S., Gao, W., Hu, H., Chen, M., Wang, Y., et al. (2016). A comparative investigation between paclitaxel nanoparticle- and nanocrystal-loaded thermosensitive PECT hydrogels for peri-tumoural administration. *Nanoscale* 8, 18782–18791. doi: 10.1039/C6NR05498F
- Liu, H., Shi, X., Wu, D., Kahsay Khshen, F., Deng, L., Dong, A., et al. (2019). Injectable, biodegradable, thermosensitive nanoparticles-aggregated hydrogel with tumor-specific targeting, penetration, and release for efficient postsurgical prevention of tumor recurrence. *ACS Appl. Mater. Interfaces* 11, 19700–19711. doi: 10.1021/acsami.9b01987
- Liu, Z., Xu, G., Wang, C., Li, C., and Yao, P. (2017). Shear-responsive injectable supramolecular hydrogel releasing doxorubicin loaded micelles with pH-sensitivity for local tumor chemotherapy. *Int. J. Pharm.* 530, 53–62. doi: 10.1016/j.ijpharm.2017.07.063
- Lu, M., Strohecker, A., Chen, F., Kwan, T., Bosman, J., Jordan, V. C., et al. (2008). Aspirin sensitizes cancer cells to TRAIL-induced apoptosis by reducing survivin levels. *Clin. Cancer Res.* 14, 3168–3176. doi: 10.1158/1078-0432.CCR-07-4362
- Luo, Y., Wei, X., Wan, Y., Lin, X., Wang, Z., and Huang, P. (2019). 3D printing of hydrogel scaffolds for future application in photothermal therapy of breast cancer and tissue repair. *Acta Biomater.* 92, 37–47. doi: 10.1016/j.actbio.2019.05.039
- Lym, J. S., Nguyen, Q. V., Ahn da, W., Huynh, C. T., Jae, H. J., Kim, Y. I., et al. (2016). Sulfamethazine-based pH-sensitive hydrogels with potential application for transcatheter arterial chemoembolization therapy. *Acta Biomater.* 41, 253–263. doi: 10.1016/j.actbio.2016.05.018
- Ma, H., He, C., Cheng, Y., Li, D., Gong, Y., Liu, J., et al. (2014). PLK1shRNA and doxorubicin co-loaded thermosensitive PLGA-PEG-PLGA hydrogels for osteosarcoma treatment. *Biomaterials* 35, 8723–8734. doi: 10.1016/j.biomaterials.2014.06.045
- Ma, H., He, C., Cheng, Y., Yang, Z., Zang, J., Liu, J., et al. (2015). Localized co-delivery of doxorubicin, cisplatin, and methotrexate by thermosensitive hydrogels for enhanced osteosarcoma treatment. *ACS Appl. Mater. Interfaces* 7, 27040–27048. doi: 10.1021/acsami.5b09112
- Mackiewicz, M., Romanski, J., Drabczyk, K., Waleka, E., Stojek, Z., and Karbarz, M. (2019). Degradable, thermo-, pH- and redox-sensitive hydrogel microcapsules for burst and sustained release of drugs. *Int. J. Pharm.* 569:118589. doi: 10.1016/j.ijpharm.2019.118589
- Milcovich, G., Lettieri, S., Antunes, F. E., Medronho, B., Fonseca, A. C., Coelho, J. F. J., et al. (2017). Recent advances in smart biotechnology: hydrogels and nanocarriers for tailored bioactive molecules depot. *Adv. Colloid Interface Sci.* 249, 163–180. doi: 10.1016/j.cis.2017.05.009
- Murgia, X., Loretz, B., Hartwig, O., Hittinger, M., and Lehr, C. M. (2018). The role of mucus on drug transport and its potential to affect therapeutic outcomes. *Adv. Drug Deliv. Rev.* 124, 82–97. doi: 10.1016/j.addr.2017.10.009
- Norouzi, M., Nazari, B., and Miller, D. W. (2016). Injectable hydrogel-based drug delivery systems for local cancer therapy. *Drug Discov. Today* 21, 1835–1849. doi: 10.1016/j.drudis.2016.07.006
- Oroojalian, F., Babaei, M., Taghdisi, S. M., Abnous, K., Ramezani, M., and Alibolandi, M. (2018). Encapsulation of thermo-responsive gel in pH-sensitive polymeric microsomes as dual-responsive smart carriers for controlled release of doxorubicin. *J. Control. Release* 288, 45–61. doi: 10.1016/j.jconrel.2018.08.039
- Pan, A., Wang, Z., Chen, B., Dai, W., Zhang, H., He, B., et al. (2018). Localized co-delivery of collagenase and trastuzumab by thermosensitive hydrogels for enhanced antitumor efficacy in human breast xenograft. *Drug Deliv.* 25, 1495–1503. doi: 10.1080/10717544.2018.1474971
- Pesoa, J. I., Rico, M. J., Rozados, V. R., Scharovsky, O. G., Luna, J. A., and Mengatto, L. N. (2018). Paclitaxel delivery system based on poly(lactide-co-glycolide) microparticles and chitosan thermo-sensitive gel for mammary adenocarcinoma treatment. *J. Pharm. Pharmacol.* 70, 1494–1502. doi: 10.1111/jphp.13006
- Qu, J., Zhao, X., Ma, P. X., and Guo, B. (2017). pH-responsive self-healing injectable hydrogel based on N-carboxyethyl chitosan for hepatocellular carcinoma therapy. *Acta Biomater.* 58, 168–180. doi: 10.1016/j.actbio.2017.06.001
- Ren, S., Dai, Y., Li, C., Qiu, Z., Wang, X., Tian, F., et al. (2016). Pharmacokinetics and pharmacodynamics evaluation of a thermosensitive chitosan based hydrogel containing liposomal doxorubicin. *Eur. J. Pharm. Sci.* 92, 137–145. doi: 10.1016/j.ejps.2016.07.002
- Roy, S., and Trinchieri, G. (2017). Microbiota: a key orchestrator of cancer therapy. *Nat. Rev. Cancer* 17, 271–285. doi: 10.1038/nrc.2017.13
- Salis, A., Rassu, G., Budai-Szucs, M., Benzoni, I., Csányi, E., Berkó, S., et al. (2015). Development of thermosensitive chitosan/glycerophosphate injectable *in situ* gelling solutions for potential application in intraoperative fluorescence imaging and local therapy of hepatocellular carcinoma: a preliminary study. *Expert Opin. Drug Deliv.* 12, 1583–1596. doi: 10.1517/17425247.2015.1042452
- Samanta, D., Meiser, J. L., and Zare, R. N. (2015). Polypyrrole nanoparticles for tunable, pH-sensitive and sustained drug release. *Nanoscale* 7, 9497–9504. doi: 10.1039/C5NR02196K
- Sapino, S., Chirio, D., Peira, E., Abellán Rubio, E., Brunella, V., Jadhav, S. A., et al. (2019). Ocular drug delivery: a special focus on the thermosensitive approach. *Nanomaterials* 9:884. doi: 10.3390/nano9060884
- Senft, D., Qi, J., and Ronai, Z. A. (2018). Ubiquitin ligases in oncogenic transformation and cancer therapy. *Nat. Rev. Cancer* 18, 69–88. doi: 10.1038/nrc.2017.105
- Sheu, M. T., Jhan, H. J., Su, C. Y., Chen, L. C., Chang, C. E., Liu, D. Z., et al. (2016). Codelivery of doxorubicin-containing thermosensitive hydrogels incorporated with docetaxel-loaded mixed micelles enhances local cancer therapy. *Colloids Surf. B Biointerfaces* 143, 260–270. doi: 10.1016/j.colsurfb.2016.03.054
- Sivaram, S., Majumdar, G., Perin, D., Nessa, A., Broeders, M., Lyng, E., et al. (2018). Population-based cancer screening programmes in low-income and middle-income countries: regional consultation of the International Cancer Screening Network in India. *Lancet Oncol.* 19, e113–e122. doi: 10.1016/S1470-2045(18)30003-2
- Sun, X. F., Wang, H. H., Jing, Z. X., and Mohanathas, R. (2013). Hemicellulose-based pH-sensitive and biodegradable hydrogel for controlled drug delivery. *Carbohydr. Polym.* 92, 1357–1366. doi: 10.1016/j.carbpol.2012.10.032
- Tam, R. Y., Smith, L. J., and Shoichet, M. S. (2017). Engineering cellular microenvironments with photo- and enzymatically responsive hydrogels: toward biomimetic 3D cell culture models. *Acc. Chem. Res.* 50, 703–713. doi: 10.1021/acs.accounts.6b00543
- Wang, C., Javadi, A., Ghaffari, M., and Gong, S. (2010). A pH-sensitive molecularly imprinted nanospheres/hydrogel composite as a coating for implantable biosensors. *Biomaterials* 31, 4944–4951. doi: 10.1016/j.biomaterials.2010.02.073

- Wang, N., Zhang, J., Sun, L., Wang, P., and Liu, W. (2014). Gene-modified cell detachment on photoresponsive hydrogels strengthened through hydrogen bonding. *Acta Biomater.* 10, 2529–2538. doi: 10.1016/j.actbio.2014.02.017
- Wang, W., Song, H., Zhang, J., Li, P., Li, C., Wang, C., et al. (2015). An injectable, thermosensitive and multicompartiment hydrogel for simultaneous encapsulation and independent release of a drug cocktail as an effective combination therapy platform. *J. Control. Release* 203, 57–66. doi: 10.1016/j.jconrel.2015.02.015
- Wang, X., Li, D., Yang, F., Shen, H., Li, Z., and Wu, D. (2013). Controlled cross-linking strategy: from hybrid hydrogels to nanoparticle macroscopic aggregates. *Polym. Chem.* 4, 4596–4600. doi: 10.1039/c3py00811h
- Wang, X., Wang, J., Wu, W., and Li, H. (2016). Vaginal delivery of carboplatin-loaded thermosensitive hydrogel to prevent local cervical cancer recurrence in mice. *Drug Deliv.* 23, 3544–3551. doi: 10.1080/10717544.2016.1205158
- Wang, X., Wang, J., Yang, Y., Yang, F., and Wu, D. (2017). Fabrication of multi-stimuli responsive supramolecular hydrogels based on host-guest inclusion complexation of a tadpole-shaped cyclodextrin derivative with the azobenzene dimer. *Polym. Chem.* 8, 3901–3909. doi: 10.1039/C7PY00698E
- Wei, L., Chen, J., Zhao, S., Ding, J., and Chen, X. (2017). Thermo-sensitive polypeptide hydrogel for locally sequential delivery of two-pronged antitumor drugs. *Acta Biomater.* 58, 44–53. doi: 10.1016/j.actbio.2017.05.053
- Xu, Y., Li, Z., Li, X., Fan, Z., Liu, Z., Xie, X., et al. (2015). Regulating myogenic differentiation of mesenchymal stem cells using thermosensitive hydrogels. *Acta Biomater.* 26, 23–33. doi: 10.1016/j.actbio.2015.08.010
- Yang, Y., Wang, X., Yang, F., Wang, L., and Wu, D. (2018). Highly elastic and ultratough hybrid ionic-covalent hydrogels with tunable structures and mechanics. *Adv. Mater. Weinheim.* 30:e1707071. doi: 10.1002/adma.201707071
- Yang, Y., Zhao, H., Jia, Y., Guo, Q., Qu, Y., Su, J., et al. (2016). A novel gene delivery composite system based on biodegradable folate-poly (ester amine) polymer and thermosensitive hydrogel for sustained gene release. *Sci. Rep.* 6:21402. doi: 10.1038/srep21402
- Yang, Y. Y., Wang, X., Yang, F., Shen, H., and Wu, D. (2016). A universal soaking strategy to convert composite hydrogels into extremely tough and rapidly recoverable double-network hydrogels. *Adv. Mater.* 28, 7178–7184. doi: 10.1002/adma.201601742
- Yue, Z., Che, Y., Jin, Z., Wang, S., Ma, Q., Zhang, Q., et al. (2019). A facile method to fabricate thermo- and pH-sensitive hydrogels with good mechanical performance based on poly(ethylene glycol) methyl ether methacrylate and acrylic acid as a potential drug carriers. *J. Biomater. Sci. Polym. Ed.* 30, 1375–1398. doi: 10.1080/09205063.2019.1634859
- Zhang, J., Wang, X., Wang, L., Yang, F., and Wu, D. (2015). Controlled cross-linking strategy for formation of hydrogels, microgels and nanogels. *J. Control. Release* 213:e25. doi: 10.1016/j.jconrel.2015.05.038
- Zhang, W., Jin, X., Li, H., Zhang, R. R., and Wu, C. W. (2018). Injectable and body temperature sensitive hydrogels based on chitosan and hyaluronic acid for pH sensitive drug release. *Carbohydr. Polym.* 186, 82–90. doi: 10.1016/j.carbpol.2018.01.008
- Zhao, Y., Shi, C., Yang, X., Shen, B., Sun, Y., Chen, Y., et al. (2016). pH- and temperature-sensitive hydrogel nanoparticles with dual photoluminescence for bioprobes. *ACS Nano* 10, 5856–5863. doi: 10.1021/acsnano.6b00770
- Zhou, J., Yu, G., and Huang, F. (2017). Supramolecular chemotherapy based on host-guest molecular recognition: a novel strategy in the battle against cancer with a bright future. *Chem. Soc. Rev.* 46, 7021–7053. doi: 10.1039/C6CS00898D

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Fan, Tian and Liu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.