Thermo-sensitive hydrogel and their biomedical applications

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Abstract. Thermo-sensitive hydrogel has attracted considerable attention as a kind of intelligent material in the field of biochemistry and biomedicine. They have many kinds of advantages such as easy formulation to deliver hydrophilic and hydrophobic drugs, high drug loading capacity, convenient administration, sustained drug release capacity, target specificity by using injections thereby avoiding the need of surgery. Besides, thermo-sensitive hydrogel has attracted an increasing concern for its sustained release behavior, biocompatibility, and biodegradability. The biomedical application of thermo-sensitive hydrogel in the fields of tissue engineering, mucosal drug delivery, and local injection in recent years were studied in this review according to the latest relevant literature.

1. Introduction

The intelligent hydrogel is a form of stimuli-sensitive hydrogel that can change from sol to gel under the irritation of pH [1], temperature, light [2], electrical field [3] and magnetic field [4] so on. A thermo-sensitive hydrogel can transform into gels from aqueous solutions over changes in environmental temperatures [5]. This hydrogel which is fabricated by thermo-sensitive polymers and commonly possesses sustained-release properties has been regarded as an emerging vehicle for drug delivery in recent years. Moreover, it has shown great potentiality in cartilage tissue engineering, due to its capability to encapsulate cells within biomimetic, 3-dimensional (3D) microenvironments.

Hydrogel can absorb a large amount of water and cause swelling without dissolving, and maintain the original structure after swelling [6]. A sol-to-gel drug delivery system has many advantages including ease of preparation, efficient drug encapsulation, sustained drug release and injectable formulation [7]. Additionally, drug delivery systems such as microspheres, inclusion complex and nanoparticles can be loaded into a hydrogel to extend the controlled-release profile of the formulation and avoid 'burst effect', which is one of the main drawbacks of particulate systems [8, 9]. The hydrogel is similar with living tissue, with weak adhesion ability to proteins and cells on the surface, and it has good biocompatibility when in contact with human tissues, blood and body fluids. Additionally, due to the 3D network structure of hydrogel, metabolites can be discharged from the body without affecting the metabolic process of living things [10]. Therefore, thermo-sensitive hydrogel has bright application prospects in tissue engineering, biomedicine (such as drug sustained-release carriers, tissue engineering scaffolds) and other fields [11]. pH and light-responsive abilities in combination with temperature sensitivity can play a vital role in drug delivery and tissue engineering [12]. Dual pH-thermo-sensitive hydrogel has already been studied in various fields of biomedical areas, such as thermo-sensitive membranes [13].
2. Polymer materials in thermo-sensitive hydrogel

Naturally derived polymers and synthetic polymers can be used as thermo-sensitive materials, respectively or combined together (Table 1). And naturally derived polymers are less versatile than synthetic polymers because they have less capability of chemical modification, most of them are composed of polypeptide or sugar rings, which limit it. In contrast, synthetic polymers are flexible in molecular design and chemical modification.

Some of them like chitosan, hyaluronic acid and cellulose do not have thermo-sensitive properties. They should be either physically blended with some thermo-sensitive materials or chemically modified. Here are some methods to tailor their drug release kinetics to meet various of application requirements under different conditions: (a. Modulating gelation temperature by balancing hydrophobic and hydrophilic interactions; (b. Manipulating drug release rate, such as cross linking hydrogels can lead to a sustained release while dissociation of a hydrogel yields a facilitated release profile; (c. Incorporating multiple triggers such as using both stimulations of pH and temperature to tailor the drug release profile [14].

3. Classifications of thermo-sensitive hydrogel

The thermo-sensitive hydrogel can be classified in terms of physical structure, response type, cross-linking, etc (Table 2) [29]. There are amorphous, semi-crystalline and crystalline thermo-sensitive hydrogel based on their physical structures.

In order to meet the desired drug release profile, some dual or even triple-sensitive thermo-sensitive hydrogel was designed. These stimulations including pH, light, magnetic field, electric field and enzyme so on. And according to the categories of these stimulations except temperature, we can classify these thermo-sensitive hydrogels as chemical responsive, physical response and biochemical responsive thermo-sensitive hydrogel respectively.

As for the classification basis of cross linking type, the physically cross linking hydrogel that is crosslinked by the non-covalent bond (such as hydrogen bond, hydrophobic effect, electrostatic effect, van der Waals force, etc.) has weak mechanical properties and network structure. This can be disrupted with the change of environmental parameters such as pH, temperature or ionic strength [40]. Generally, such hydrogel is reversible and can be transformed into flowing solutions by certain operations (such as heating, adjusting pH, etc.). The physically crosslinked hydrogel can form a three-dimensional network through non-covalent bonds which avoiding the addition of chemical cross linking agents and is more suitable for biomedical applications compared with the chemical crosslinked hydrogel [41]. In contrast, the chemically cross linking hydrogel is strong but the cross linking agents used for their preparation are usually toxic [42].

Besides, it’s reported that thermo-sensitive hydrogel can also be classified as naturally derived polymer-based thermo-sensitive hydrogel and synthetic thermo-sensitive hydrogel according to their basic composition material [43]. Naturally derived polymer-based thermo-sensitive hydrogel can be further classified as chitosan-based thermo-sensitive hydrogel, hyaluronic acid-based thermo-sensitive hydrogel, cellulose-based thermo-sensitive hydrogel and other naturally derived polymers; And synthetic thermo-sensitive hydrogel includes Pluronic hydrogel, PEG-polyester thermo-sensitive hydrogel, polyacrylamide derivatives-based thermo-sensitive hydrogel, poly(oligo(ethylene glycol) methacrylate)-based thermo-sensitive hydrogel and polyphosphazene-based thermo-sensitive hydrogel [15-28].

4. Biomedical applications of thermo-sensitive hydrogel

The biomedical applications of thermo-sensitive hydrogel include mucosal drug delivery, transdermal drug delivery and local injection delivery.
4.1. Mucosal drug delivery

**Table 1.** commonly used polymers in thermo-sensitive hydrogel.

| Sources                     | Name                  | Characteristics                                                                 | Reference |
|-----------------------------|-----------------------|--------------------------------------------------------------------------------|-----------|
| Naturally derived polymers   | Chitosan              | The second most abundant naturally derived Polymer, has no thermo-sensitive properties | [15-17]   |
|                             | Cellulose             | The most abundant naturally derived Polymer, has no thermo-sensitive properties   | [18]      |
|                             | Hyaluronic acid       | Has no thermo-sensitive properties                                                | [19]      |
|                             | Matrigel              | Has a positive thermo-sensitivity                                                | [20]      |
|                             | Collagen              |                                                                                  | [21]      |
|                             | Agarose               | A negative thermo-sensitivity                                                    | [22]      |
|                             | Gelatin               |                                                                                  | [23]      |
| Synthetic polymers          | Pluronic              | Thermo-sensitive, biocompatible, has a fast dissolution rate, low mechanical strength and non-degradability, sensitize tumor | [24]      |
|                             | Poly(N-isopropylacrylamide) | Non-degradable and thermo-sensitive with a gelation temperature of ~32°C          | [25]      |
|                             | PNIPAAM               |                                                                                  |           |
|                             | Polylactide-polyethylene glycol-Polylactide (PEG-PLA-PEG) | Thermo-sensitive and biodegradable, but its acidic degradation products may cause severe inflammation response in vivo | [26]      |
|                             | Poly (oligo (ethylene glycol) methacrylate) (POEGMA) | Thermo-sensitive, biocompatible and low protein adsorption properties          | [27]      |
|                             | Polyphosphazene       | Biocompatible, biodegradable with non-toxicity degradation products, are emerging candidates for anti-cancer drug delivery materials | [28]      |

**Table 2.** classifications of thermo-sensitive hydrogel.

| Classification basis | Category     | Example                     | Reference |
|----------------------|--------------|-----------------------------|-----------|
| Size                 | Microgels    |                             | [30]      |
|                      | Nanogels     |                             | [31]      |
|                      | Macrogels    |                             | [32]      |
| Response type        | Chemical responsive | pH                           | [33]      |
|                      |               | Light                       | [34]      |
|                      | Physical responsive | Electric field             | [3]       |
|                      |               | Magnetic field              | [35]      |
|                      | Biochemical responsive | Antigen                   | [36]      |
|                      |               | Enzyme                      | [37]      |
| Cross-linking type   | Chemically crosslinking | Covalent bond              | [38]      |
|                      | Physically crosslinking | Non covalent bond          | [39]      |
4.1.1. Ophthalmic drug delivery. General eye drops could not reach the position of the lesions due to the complicated and special physiological barriers of the eye. And they are deficient in the sustained release properties. Repeated administration of the drug may lead to poor patient compliance in the treatment of chronic ophthalmic disease [44]. Yu, YB et al [45] formulated a dual pH-thermo-responsive hydrogel based on nanostructures lipid carriers loaded with baicalin. The hydrogel has a higher apparent permeability coefficient and longer precorneal residence time compared with baicalin eye drops. Thermo-sensitive hydrogels based on PNIPAAM with ketoconazole was loaded (KCL PA-HA hydrogel) and hydrogel-based on hydroxypropyl trimethyl ammonium chloride chitosan and β-glycerophosphate encapsulated with dexamethasone nanoliposomes were prepared for the treatment of ophthalmic disorders via ophthalmic local drug delivery. KCLPA-HA hydrogel had sustained-release properties, and it showed no macroscopic signs of irritation in rabbit eyes [46, 47].

4.1.2. Oral drug delivery. A major problem in the oral drug delivery route is poor bioavailability resulting from the first-pass effect of the liver. The thermo-sensitive hydrogel can be served as a promising vehicle to prevent drugs from being digested. Insulin, a peptide drug, is usually administered via the parenteral route, owing to its easy deactivation by proteolytic enzymes in the gastrointestinal tract. Chaturvedi, K et al [48] reported an insulin-loaded vesicle-chitosan hydrogel for the treatment of type II diabetes. Insulin-loaded chitosan hydrogel vesicle was prepared based on sodium dodecyl sulfate and β-cyclodextrin. And then it was crosslinked with chitosan/β-glycerol phosphate solution. The hydrogel was characterized to have good dilution stability, sustained release and pH-depending release properties, and it provided an excellent method in dealing with the treatment of diabetes.

4.1.3. Buccal drug delivery. Buccal drug delivery is a promising route to for the treatment of pediatric patients and elderly people and patients with deglutition obstacle, it possesses many advantages such as high patient compliance and ease of administration, and it is also a promising alternative vehicle for drugs with relatively lower oral bioavailability. Zeng et al developed a thermo-sensitive in situ hydrogel based on Pluronic F127, Pluronic F68 and xanthan gum for buccal drug delivery [49]. This may be a progress with great promise for the medication of pediatric and the elderly.

4.1.4. Intranasal drug delivery. Intranasal drug delivery system commonly be used in the treatment of neurodegenerative diseases because of its brain targeting characteristics, with the existence of the nose-brain pathway which can transport drugs bypass the Blood Brain Barrier (BBB) directly along with olfactory and trigeminal nerves [50]. Thermo-sensitive hydrogel is formed upon the administration by sensing the physiological temperature of the nasal cavity. But one obstacle in this administration route is low retention time because of nasal rapid mucociliary clearance [51]. An intranasal formulation was prepared by using polymers with mucoadhesive properties. They were particularly helpful in providing intimate contact between the formulation and the nasal mucosa, hence maximizing the likelihood of the drug absorption. Dalia et al [52] formulated rivastigmine tartrate mucoadhesive in situ hydrogel with Plutonic F127 and Pluronic F68 as a thermo-sensitive hydrogel. And different mucoadhesive polymers (Hydroxypropyl methylcellulose, Chitosan, Carbopol 934, sodium carboxymethylcellulose) were chosen to improve its retention time in order to enhance drug bioavailability and increasing drug concentration of brain. The results showed good brain-targeted properties. And a considerable fraction of rivastigmine tartrate could be transported directly to the brain after nasal delivery in comparison to intranasal and intravenous injection, increasing the bioavailability of drugs in the brain. Besides, Plutonic F127-based thermo-sensitive hydrogel also has been studied as feasible non-needle alternative of intranasal vaccine delivery [53, 54].

4.1.5. Intravesical drug delivery. Intravesical instillation is the main therapy for bladder disorders such as bladder cancer and interstitial cystitis. But drugs are usually diluted by continuous urine formation and most drugs are eliminated after the first urination. Zhu, GC et al [55] prepared vaporized perfluoropentane-encapsulated floating hydrogel. Lin, TS et al [56] prepared floating hydrogel with self-
generating micro-bubbles using the foamability of Pluronic F127 instead of a traditional gas-producing method. They all overcome the demerits of low drug retention time in bladder and this drug delivery vehicle will not be eliminated from the body by urination. The floating hydrogels also had a well-sustained release characteristic. Besides, they would not cause severe urinary obstruction and bladder irritation like others might.

4.1.6. Intravaginal drug delivery. The locally acting drugs could be absorbed through the vaginal mucosa that has a large surface and abundant vessels. Besides, systematical drug delivery and uterine targeting can also be put into effect through vaginal delivery [57]. The intravaginal delivery route is widely used in many kinds of formulations related to HIV and gynecological diseases [58-60].

In the study of pre-exposure prophylaxis of HIV, nanosized layered double hydroxides and Pluronic F127 were used to formulate a thermo-sensitive hydrogel loaded with both hydrophobic drug model Nile red and hydrophilic drug theaflavine. Its release behavior through intravaginal administration was investigated. And the results showed sustained-release properties for both hydrophobic and hydrophilic drugs with hardly any mucosal irritations [61]. Date et al [62] developed a thermo-sensitive vaginal hydrogel containing poly (lactic-co-glycolic acid) nanoparticles loaded with raltegravir and efavirenz. And the hydrogel was prepared based on Pluronic F127 (20% w/v) and Pluronic F68 (1% w/v).

In the study about gynecological disorders, vaginitis is a very common gynecological problem among women in all age groups. Deshkar, SS and Palve VK [63] developed a voriconazole/HP-β-CD inclusion complex loaded vaginal in situ hydrogel with Pluronic F127 and Pluronic F68 for the treatment of vaginitis. There are three types of infectious vaginitis: candidiasis, trichomoniasis, and bacterial vaginosis. Vaginal candidiasis is a very common disease affecting women in reproductive age. Zhou S et al [64] developed a thermo-sensitive hydrogel based on chitosan and poloxamer loaded with the extracts of an empirical traditional Chinese prescription (which consist of viz Cortex, Phellodendri, RhizomaCoptidis, Olibanum, Myrrha, Borneol and Catechu) for the treatment of cervical erosion via intravaginal administration. The results investigated in a rat model showed that is a promising formulation for cervical erosion. Mirza et al [65] developed a synergistic thermo-sensitive hydrogel loaded with itraconazole and tea tree oil for the same purpose of treating vaginal candidiasis.

4.2. Transdermal drug delivery
The thermo-sensitive hydrogel is superior to the conventional formulation of treating superficial disorders such as fungal infections and atopic dermatitis. It is characterized by good stability, convenient of use for patients, improved local bioavailability, and efficacy at low doses [66]. Huang et al [67] developed a thermo-sensitive hydrogel based on chitosan, β-glycerophosphate, and glycerol. It could change color rapidly and obviously under higher temperature so that it could be used to prevent a side effect of overheating in the focused ultrasound therapy. The optimized prescription was 0.5% CS, 5%β-GP, and 25% glycerol. Ibuprofen loaded thermo-sensitive hydrogel based on liquid lecithin and Pluronic F127was formulated. It provided a delivery system for sustained release of hydrophobic drugs through percutaneous delivery [68]. Parhi R [69] developed a thermo-sensitive hydrogel loaded with metoprolol succinate based on hydroxypropyl methylcellulose and PluronicF127 for anti-hypertension. The optimal prescription was 0.92% of hydroxypropyl methylcellulose and 15% of PluronicF127. And the results indicated that the hydrogel exhibited strong potential against hypertension and could become parable to the oral formulation. Arslan A et al [64] developed a thermo-sensitive hydrogel encapsulated with oxiconazole nitrate based on PluronicF127 and PEG400 for the treatment of superficial fungal infections caused by candida species.

4.3. Injection drug delivery
Injectable thermo-sensitive hydrogel has been researched in various biomedical applications, such as local drug delivery, cell-loaded carrier, and tissue repairing [29].
4.3.1. Local injection drug delivery. Thermo-sensitive hydrogel can be used for the treatment of various infections through local injection delivery. Zou MW et al [70] prepared an injectable thermo-sensitive hydrogel of indomethacin-conjugated polymer which was synthesized from N-isopropyl acrylamide, methacrylic acid, and 2-hydroxyethyl methacrylate-g-poly (trimethylene carbonate)-indomethacin. This hydrogel which had good biocompatibility and sustained release properties offered a promising treatment option for uveitis. Sarıgöl, E et al [71] developed an injectable thermo-sensitive hydrogel with 5% (w/v) enzyme of Serratiopeptidase, 0.8% (w/v) free Vancomycin HCl and 40% (w/v) Vancomycin HCl loaded microspheres based on Pluronic F127 to eradicate bone associated bacterial biofilm. It offered an alternative to the standard treatment available for periprosthetic joint infection. Daptomycin was encapsulated into a biodegradable thermo-sensitive hydrogel based on vinyl sulfonated triblock copolymer could be used for the treatment of bone and implant-associated infections which mainly caused by staphylococcus aureus and coagulase-negative staphylococci. And it was proved that the hydrogel has a significant biofilm bactericidal effect as a promising release scaffold against orthopedic infections especially to Staphylococcus aureus [72].

Thermo-sensitive hydrogel can also be used as anti-cancer therapy by being injected into the tumor. Zeng JF et al [10] prepared the photo-thermo responsive hydrogel based on chitosan, β-glycerophosphate and gold nanoparticles. The prepared hydrogels which had good biocompatibility, biodegradability, and photosensitivity, could achieve multiple treatments for killing the tumor cells effectively and completely without other side effects via injected into the tumor site. A chitosan based thermo-sensitive hydrogel modified by glutaraldehyde and polyvinyl alcohol, for intratumoral delivery of paclitaxel, showed similar anti-tumor properties [73].

Thermo-sensitive hydrogel can also be injected for other disorders. Zhou HY et al [6] encapsulated Aspirin into β-cyclodextrin to form Aspirin-β-cyclodextrin inclusion. Then chitosan-β-cyclodextrin based injectable in situ thermo-sensitive hydrogel was prepared by adding Aspirin-β-cyclodextrin inclusion solution into chitosan solution. It had controlled release properties and might be a promising formulation in reducing the risk of arterial vascular events through injection. Biodegradable thermo-sensitive PLGA–PEG–PLGA hydrogel loading Huperzine-A could be used hypodermic or intramuscular injection. And it proved that the hydrogel was a good drug delivery system for sustained release for Alzheimer's disease [3]. An injectable thermo-sensitive hydrogel based on poly (N-isopropyl acrylamide), chitosan and hyaluronic acid was prepared as an ideal barrier to prevent peritoneal adhesion after abdominal surgery [74]. It was easy to prepare and preserve and did not interfere with normal peritoneal tissue healing or elicit acute toxicity from blood analysis and tissue biopsy examination. Thermo-sensitive hydrogel can also be used as a marker-assisted precise hepatectomy. Methylene blue-loaded hydrogel based on Pluronic F127 and Pluronic F68 was expected to occlude the blood flow temporarily and dye the target resection region synchronously. It is an effective alternative for color-labeled reversible blood occlusion [75].

4.3.2. Cell-loaded carrier injection drug delivery. Thermo-sensitive hydrogel can also be designed as a cell-culturing matrix or cell-loaded carrier. The polyglycolic acid-PEG-polyglycolic acid triblock polymer-based thermo-sensitive hydrogel was prepared as a three-dimensional cell culture matrix for ovarian cancer cells [76]. Thermo-sensitive chitosan hydrogel with rat mesenchymal stem cells induced by sex-determining region Y-box 9 was injected into the nude mice. The results showed that the stem cell could differentiate into chondrocyte to form new chondroid tissue in vitro [77]. Niu H et al [78] developed an injectable cell carrier that has the potential to improve cell retention largely to regenerate ischemic cardiovascular tissues in stem cell therapy.

4.3.3. Tissue repairing injection drug delivery. The thermo-sensitive hydrogel is widely used in tissue repairing such as wound healing and cartilage repairing because it can mimic the three-dimensional microenvironment of cells. Zhang L et al [79] developed a thermo-sensitive hydrogel loaded with bone marrow-derived mesenchymal stem cells for the treatment of severe skin wound healing. It showed good biocompatibility and reduced the inflammatory response, promoted wound healing remarkably.
Thermo-sensitive hydrogel that based on the synthesized glycidyl methacrylate modified hydroxypropyl chitin was able to be photocrosslinked by UV irradiation under physiological conditions to enhance the stability and mechanical strength of hydrogels. And it could stimulate mesenchymal stem cell proliferation and paracrine effects [80]. It was a promising method for delivering stem cells into the heart and skeletal muscle tissues to regenerate ischemic cardiovascular tissues.

There are so many studies about cartilage regeneration or bone regeneration. Poly (phosphazene) polymer, isoleucine ethyl ester, and PEG were used to formulate an amphiphilic thermo-sensitive hydrogel loaded with bone morphogenetic protein-2. It overcomes the demerits of the classical implantable materials as a promising protein delivery system with sustained-release properties [81]. A thermo-sensitive methylcellulose hydrogel based on calcium phosphate nanoparticles was proved to be used as an injectable hydrogel that suitable for bone regeneration [82]. The phenylalanine was synthesized with two triblock copolymers and alanine. The addition of phenylalanine could strengthen the mechanical properties and gelation behaviors of the polyaniline-based thermo-sensitive hydrogel. It is beneficial to cartilage regeneration on cartilage repair [83]. A thermo-sensitive hydrogel that was prepared based on methacrylate chondroitin sulfate and a glycol triblock copolymer was proved to be a promising biomaterial for cartilage 3D printing [84].

5. Conclusions
Thermo-sensitive hydrogel has been studied over four decades, and it has been widely used in pharmaceutical materials and cell culture due to its good temperature sensitivity, biocompatibility, and low toxicity. It is a promising drug delivery system for the treatment of many different kinds of diseases from ophthalmic disorders to cancer. And recently multi-responsive hydrogel has been attracting increasing concern in biomedical applications. Combinations of two or more stimulation-responsive materials more safely and effectively can be regarded as a potential study direction for researchers. Actually, some materials still have defects such as non-degradable, poor temperature sensitivity and so on. Therefore, novel combinations of the existing materials or the newly synthesized materials will contribute to the development of biocompatible targeting preparation for serving human beings, we still have a long way to go to get thermo-sensitive hydrogel be further researched.

Acknowledgements
This work was funded by the National Natural Science Foundation of China (No. 81503461) and the Postdoctoral Science Foundation of China (No.2016M591400).

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