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Review Article

Injectable Hydrogels: A Review of Injectability Mechanisms and Biomedical Applications

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Abstract

Hydrogels have been used for biomedical applications in recent decades. They are a perfect candidate for regenerative medicine as they resemble the extracellular matrix of native tissues. In addition, their highly hydrated structure makes them a suitable choice for drug and other therapeutics delivery. Injectable hydrogels have increasingly gained attention due to their capability for homogeneous mixing with cells and therapeutic agents, minimally invasive administration, and perfect defect filling. In this review, we discuss various mechanisms which facilitate injectability of hydrogels, including *in situ* gelling liquids, injectable gels, and injectable particles. Then, we explore the biomedical applications of injectable hydrogels, including tissue engineering, therapeutic agent delivery, and medical devices.

Keywords: injectable, hydrogel, *in situ* gelling, tissue engineering, drug delivery, nanoparticles

1. Introduction

Biomaterials, including polymeric, ceramic, and metallic materials, are used for biomedical applications for the preparation of pharmaceutical, biological, and medical products and devices. These materials are fabricated and consumed in solid, liquid, or gel-like (hydrogel) states.

Hydrogels are a hydrophilic, highly hydrated network of polymers that form a soft swollen structure [1]. Hydrogels may absorb volumes of water up to thousands of times their dry weight. Due to their similarity to the natural extracellular matrix (ECM) of living tissues, as well as their excellent capability of loading and releasing of therapeutic agents, they are good candidates for biomedical applications [2]. The sources of materials for the synthesis and preparation of hydrogels can be either synthetic or natural.

Hydrogels are called “physical” hydrogels if the polymeric network is formed by molecular entanglements and/or secondary forces, such as ionic, H-bonding, or hydrophobic forces. Physical hydrogels are structurally weak, and their gelation is reversible. Alternatively, in the so-called “chemical” hydrogels, polymeric networks are covalently linked by chemical bonds which are strong and irreversible [1].

Hydrogels may form *ex vivo* as a bulky, pre-shaped intact structures that can be implanted via open surgery. Alternately, they can be injected, administered easily using a needle or catheter, without the need for invasive surgery. Injectable systems are attractive for both patients and clinicians because they are more comfortable and involve less pain, and have a faster recovery period, lower costs, and fewer complications and side effects.

In this review, we discuss various mechanisms of injectability in hydrogels, and describe the major biomedical applications of injectable hydrogels. We conclude with by presenting the future directions of this field.

2. Injectability mechanisms

There are several mechanisms for the injectability of a material. These mechanisms are categorized in three main groups: *in situ* gelling liquids, injectable gels, and injectable particles. The mechanisms and their examples, along with applications, are summarized in Table 1. We will describe these mechanisms in the following sections.

2.1. *In situ* gelling liquids

These materials are normally flowable solution or liquid that can convert into gels upon injection into the body [3-5], as shown in Fig. 1(A). Gelation systems can be divided into covalent and non-covalent systems [3]. Non-covalent hydrogels are formed through non-covalent bonds between molecules *in situ*. These physical bonds are formed by external stimuli that act as a trigger, such as temperature, pH, or ionic strength, as well as ionic bonding and self-assembling peptides. On the other hand, covalent hydrogels are formed by chemical reactions between injected molecules, which are comprised of photo-crosslinking or covalent precursors.

Liquid materials that respond to temperature as a stimulus and convert from sol at room temperature to gel by temperature rise in the body are known as thermo-responsive or thermo-sensitive materials [6-8]. Some natural or synthetic polymers show

Table 1: Examples of hydrogels for injectability mechanisms and their biomedical applications.

Injectability mechanism			Hydrogel	Application	Ref.
In situ gelling liquids					
	Non-covalent				
		Thermo-responsive	Chitosan/GP[a]	Growth factor delivery and chondrocyte encapsulation for cartilage tissue engineering	[10]
		pH-responsive	PCLA-PEG-PCLA[b]	Bone tissue engineering	[28]
		Ionic bonding	Alginate/$CaCl_2$	Cardiac tissue engineering	[33]
		Self-assembling peptide	MAX8 peptide	Drug (curcumin) delivery	[38]
	Covalent				
		Photo-crosslinking	Methacrylated gelatin	Cartilage tissue engineering	[42]
		Covalent precursors	Thiol-modified analog of heparin with thiol-modified HA[c] or CS[d] with PEGDA[e]	bFGF delivery[f]	[44]
Injectable gels			CMC/PEO blend	Dermal filler (as a medical device)	[98]
Injectable particles					
	Aggregated or self-assembled particles		Core: FITC-conjugated MSNs[g]; shell: HA[c]	Drug (anti-cancer) delivery	[56]
	Individual gel-like particles		Sodium alginate and cellulose nanofibers nanocomposite	Probiotics delivery	[61]

[a] glycerol phosphate disodium salt; [b] poly(ϵ-caprolactone-co-lactide)-poly(ethylene glycol)-poly(ϵ-caprolactone-co-lactide); [c] hyaluronic acid; [d] chondroitin sulfate; [e] poly(ethylene glycol) diacrylate; [f] basic fibroblast growth factor; [g] mesoporous silica nanoparticles

thermo-responsive properties. Natural biopolymers, such as cellulose derivatives [9], chitosan in combination with glycerol phosphate disodium salt [10], and gelatin [11], present a sol-gel transition behavior at specific temperatures. Several synthetic polymers undergo a similar phenomenon as well. Poly(N-isopropylacrylamide) (PNIPAAm) is a synthetic polymer with a sol-gel transition temperature of around 32˚C [12], which is fairly close to body temperature and, therefore, is a perfect choice for an injectable material [13]. The triblock copolymers of poly(ethylene oxide) (PEO or PEG) and poly(propylene oxide) (PPO), which are commercially available as Pluronic® or Poloxamer, are another example of well-known thermo-sensitive polymers for biomedical applications [14, 15].

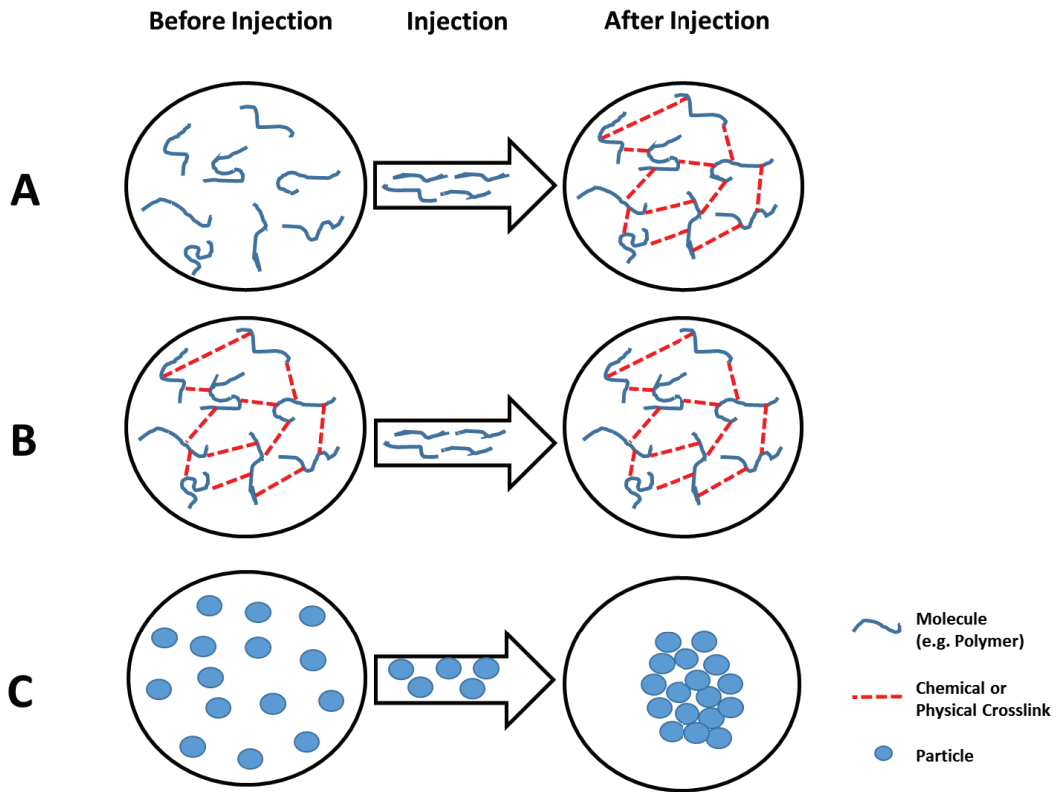

Figure 1: Schematic presentation of three injectability mechanisms. (A) "*In situ* gelling liquids" are found in a liquid state before and during injection and form a gel after injection. (B) "injectable gels" are found in gel form before injection. They are able to flow during injection due to shear forces and revert back to a gel after injection. (C) "Injectable particles" are immersed in a liquid phase before and during injection. They are self-assembled or aggregate to form a gel after injection.

For example, a Pluronic mixture was used as an easy handling and stable resident tissue adhesion barrier [16]. In another attempt, Choi *et al.* prepared an injectable dermal filler for soft tissue augmentation by combining levan with Pluronic and carboxymethyl cellulose [17]. Several block copolymers of PEG and biodegradable polyesters, such as PEG-poly(lactic-co-glycolic acid) (PEG-PLGA) [18] and PEG-poly(ϵ-caprolactone) (PEG-PCL) [19], also show thermogelling behavior. In a recent study, composites of PEG-PLGA-PEG/microspheres were developed. The microspheres were loaded with bupivacaine and dexmedetomidine for effective sustained analgesia in treatment of acute and chronic pain [20]. Encapsulated chondrocyte in an injectable blend of PCL-PEG-PCL with gelatin shows significantly increased SOX-6 and collagen-II gene expression, indicating it's potential for cartilage tissue engineering [21]. In addition, the combination of natural and synthetic polymers is being utilized by researchers to prepare thermo-responsive hydrogels. For example, a copolymer of chitosan and PNIPAAm was prepared by grafting PNIPAAm onto chitosan as a backbone [22, 23].

Another group of stimuli-responsive hydrogels are pH-responsive materials. These materials respond to pH changes upon injection into the body, or at a specific site within the body with a different pH value, such as the gastrointestinal tract, blood vessels, tumors, and the vagina. This response may lead to the conversion from a solution to a gel. Phase transition normally occurs due to the protonation/deprotonation of their ionizable pendant groups [24-26]. By copolymerization of PNIPAAm with poly(acrylic acid) (PAA), poly(propylacrylic acid) (PPAA), poly(N-isopropylmaleamic acid) (PNIPMAA), and poly(methacrylic acid) (PMAA), dual-responsive polymers were synthesized. These polymers are both pH- and thermo-responsive [27]. Dual-responsiveness could enhance the mechanical strength of the gel, which is a drawback of thermos-responsive hydrogels. Another example is poly(ϵ-caprolactone-co-lactide)-PEG-poly(ϵ-caprolactone-co-lactide) (PCLA-PEG-PCLA) [28]. Several natural polymers, such as chitosan, also show pH-sensitive properties owing to their free amino groups [29, 30].

Hydrogels with ionic crosslinking are another form of *in situ* gel-forming hydrogels. In this approach, a polymer with a surface charge is injected into a solution along with a counterion. The opposite charge encourages bridge formation between the polymer molecules and the counterions, resulting in the gelation of the solution [3]. The Alginate/$CaCl_2$ combination is the most common example of this group [31-33]. A complex formed by chitosan, a polycation, with negatively charged components, including anions, can also lead to ionic crosslinking [34].

Self-assembling peptides are another form of non-covalent hydrogels with potential for use in biomedical applications with injectability as a feature. Peptides are short chains of amino acids which can be assembled into various nanostructures, including vesicles and nanofibers, among others, through non-covalent physical bonds [35-38].

Covalently bound hydrogels have also increasingly gained attention for use in biomedical applications. Unlike the aforementioned hydrogels, covalent hydrogels are not reversible and are unable to revert back to their liquid state. Furthermore, they are mechanically stronger and more stable [3]. As such, they can be used in a wider range of tissues as scaffolds. Photo-crosslinking is one of the most widely used methods of covalent bonding between molecules in order to form hydrogels after injection. In this method, a photoinitiator is mixed with the desired monomers or macromolecules and injected as a solution. Free radicals are produced by exposure to light, and these free radicals attack the monomers to initiate polymerization. The reaction results in a polymeric network of hydrogel [39]. Light (often UV light) is provided to the injection site by inserting pencil-sized tubing instruments that contain lighting system as usual in medical scope devices. This light is transmitted via fiber optics. Various

molecules have been modified to obtain photo-crosslinkable molecules, including PEG diacrylate (PEG-DA) [40], gelatin methaacryloyl (GelMA) [41, 42], and methacrylated glycol chitosan (MeGC) [43].

In situ covalent bonds can also occur through some biocompatible reactions with slow kinetics. Precursors are mixed *ex vivo* and injected while the mixture is in a liquid state. The reaction continues at the targeted site in the body and gelation occurs as a result. Various chemical reactions, such as acrylate homopolymerization, Michael type addition, oxime linkage, and Schiff base polymerization, as well as their valuable subset, click reactions, are used in this approach [3, 4, 44].

Several *in situ* gelling liquids are available as a product in the market. For instance, BST-CarGel®, marketed by Smith & Nephew Co., is a thermo-responsive hydrogel composed of chitosan and glycerophosphate buffer solution. The solution is mixed and injected into knee lesion after microfracturing (a bone marrow stimulation procedure) to provide a superior structural framework for penetrating cell ingrowth in cartilage repair [45, 46].

2.2. Injectable gels

Certain gels are formed *ex vivo*, however, they can be also injected due to their shear-thinning properties and their ability to regain their hydrogel form upon relaxation post-injection [47]. This concept is illustrated in Fig. 1(B). Therefore, unlike *in situ* gelling hydrogels, gelation of the shear-thinning gels occurs prior to injection via one of the previously described physical or chemical crosslinking methods. They able to flow via the application of shear stress, commonly by using needles or catheters. The process of returning to the intact gel form is known as self-healing. The mechanisms of shear-thinning and self-healing vary in different systems.

For instance, an injectable gel is obtained by blending hyaluronic acid (HA) with methylcellulose (MC), which are able to flow under shear stress [48]. This blend was prepared for the delivery of therapeutic agents for the repair of spinal cord injuries. A blend of chitosan and silicate (Laponite) cross-linked poly(ethylene oxide) (PEO) also showed a similar behavior and was found to be a promising material for bone tissue engineering [49, 50]. A composite of nanofibers in hyaluronic acid was found to form a gel at room temperature. Nanofiber was composed of PCL fibers grafted with PAA and surface-modified with maleimide groups. The resulting gel could be injected for use as a soft tissue filling and in regeneration [51]. The self-assembly of complex inclusions composed of cyclodextrin and biodegradable block copolymers are another form of

shear-thinning hydrogels. Cyclodextrins form a cone-like structure, where the linear polymers penetrate the inner side of the conic structures to obtain complex inclusions. Complex inclusions can be further aggregated to create hydrogels [52].

The most popular example of injectable gels in the market is Juvéderm^{TM} (Allergan plc.). Juvéderm^{TM} is a hyaluronic acid gel which is injected as a dermal filler for the correction of facial wrinkles and folds [53, 54]. NovaBone Putty® (NovaBone Co.) is another commercialized injectable gel currently found on the market. It is a synthetic bone grafting material which is provided in pre-filled syringes for easy delivery to the bone defect sites. This product is composed of bioactive glass 45S5 microparticles, which are osteoconductive as well as osteoinductive bioceramic materials [55].

2.3. Injectable particles

In addition to the two aforementioned groups of injectable systems, particles can also be injected while immersed in a liquid phase. Particle sizes can be nano-, micro- or macro-scaled, depending on the desired outcomes. Two major subsets of injectable particles are found in literature.

Particles can aggregate or self-assembled *in situ* to form an intact gel after injection (Fig. 1(C)). Nanoparticles with a core-shell structure can be synthesized to produce a core composed of FITC-conjugated mesoporous silica nanoparticles (MSNs) and a shell formed by a layer of hyaluronic acid (HA). HA targets tumors and FITC indicates the tumor location upon imaging. Then, the nanoparticles aggregate at a lower pH around the tumors due to their pH-responsiveness and the loaded drug releases upon HA enzymatic degradation [56]. The thermo-responsive nanogel poly(N-isopropylacrylamide-co-2-hydroxyethyl methacrylate-co-2-dimethylaminoethyl methacrylate) (P(NIPAM-HEMA-DMAEMA)) is synthesized via the emulsion copolymerization technique. Above the volume phase transition temperature (VPTT), the nanogel aggregates and surrounds cells in a 3D manner, mimicking the native microenvironment [57]. The VPTT is a specific temperature at which thermo-sensitive polymers experience a drastic change in their degree of swelling (or de-swelling), resulting in change in the size of microgels and nanogels [58]. Oppositely-charged PLGA-based nanoparticles were prepared by coating the particles with either polyvinylamine or poly(ethylene-co-maleic acid) to induce positive or negative charges, respectively. The particles can form colloidal gels once the external injection force is removed due to inter-particle electrostatic interactions. This system has been used for the release of dexamethasone in cranial defects, as well as a three dimensional support for bone regeneration [59]. A similar concept was employed

to prepare oppositely-charged PLGA-Chitosan and PLGA-alginate nanoparticle mixtures for use as an injectable scaffold for tissue engineering [60].

Alternatively, particles may possess a gel-like structure individually, without any aggregation or self-assembling after injection. These injectable gel-like particles could be used for the delivery of medicinal agents. For example, gel macrospheres made of sodium alginate and cellulose nanofibers were used to deliver probiotics into the intestine. The pH-responsiveness of sodium alginate allows for the probiotic release in the neutral pH of the intestine, whereas it is unable to be released in the acidic conditions of the stomach [61].

3. Applications

Injectable hydrogels have widespread use in biomedical applications, including tissue engineering and regenerative medicine, drug and other therapeutic agents delivery, and medical devices, among others. The different applications are discussed in this section.

3.1. Tissue engineering

Tissue engineering is a multidisciplinary field of science and technology that employs both engineering and biology to regenerate failed or damaged tissues. Briefly, porous scaffolds are made using biocompatible and biodegradable materials that are seeded with autologous or allogenic cells. The cell-laden constructs are then cultured *in vitro* for maturation or implanted directly into the targeted site in patients [62]. Injectable hydrogels are one of the most interesting biomaterials for use as scaffolds in tissue engineering. Cells are mixed homogeneously with liquid, gel, or particles *ex vivo*. The resulting mixture can be delivered into the defect site via a minimally invasive procedure without the need for open surgery. The hydrogel is then formed in the body and molded into the required shape. It thus provides a suitable three dimensional microenvironment for the cells that is similar to their natural niche within the extracellular matrix (ECM) of the tissues [4].

Injectable hydrogels have been used for the regeneration of a variety of tissues, such as bone [63, 64], cartilage [65-67], skin [68], nervous system [69-71] and cardiac tissue [72-74]. Various natural and synthetic biomaterials have been synthesized to this end. For instance, chitosan [75], collagen/gelatin [76], alginate [77], fibrin [78], elastin [79], heparin [80], hyaluronic acid [81], PEG [82, 83], PCL [84], and PLGA [85] have been previously used for cartilage tissue engineering.

All of the biomaterials used for tissue engineering need to meet certain essential requirements. They must be biocompatible and non-cytotoxic, as well as have a suitable rate of biodegradability that correlated with the neo-tissue regeneration rate. Injectable hydrogels, when used for tissue engineering, must meet several additional criteria, including an acceptable gelation time (in the case of *in situ* gelling liquids and injectable particles), as well as adequate mechanical properties. The gelation time should not be too fast in order to provide the solution with sufficient time to pass through the needle and fill the targeted cavity, and should not be too slow in order to avoid cells (or other components of the solution) from settling. In each hydrogel system, various parameters may affect the gelation time, such that each system should be modified by adapting these parameters. Ganji *et al.* found that gelation time decreases by increasing Gp salt (glycerophosphate disodium salt) concentration, temperature, and the concentration and DDA (degree of deacetylation of chitosan) of chitosan solutions in thermo-responsive chitosan/Gp system [86]. For an injectable hyaluronic acid-based hydrogel, gelation time is significantly influenced by the ratio of aldehyde and amino groups introduced to the hyaluronic acid backbone [87]. Subsequent crosslinking with genipin results in improved mechanical properties [87]. The addition of a reinforcing agent to create a composite hydrogel is another strategy used to enhance the mechanical performance of gels. For example, the incorporation of cellulose nanocrystals to a hyaluronic acid-based hydrogel could increase the mechanical strength up to 135% [88]. A similar approach has been used by Boyer *et al*., who introduced Laponite nanoparticles into silated hydroxylpropylmethyl cellulose to improve its mechanical stability for use in cartilage tissue engineering [89]. The incorporation of micro- or nanoparticles may be performed to enhance the biological properties of hydrogels. Ingavle *et al.* embedded RGD-alginate and RGD-hyaluronate hydrogels with apatite-coated PLG microspheres as an osteoconductive component to improve their bone healing ability [90].

3.2. Delivery of therapeutic agents

Another extensive application of injectable hydrogels is their role as a carrier for the delivery of drugs, bioactive molecules, cells and other therapeutic agents. Hydrogels are a great candidate for delivery due to their water-swollen porous structure, which

provides an appropriate environment for bioactive molecules and cells, as well as allowing for their controlled release [25]. Hydrogels can also be designed for targeted delivery of therapeutic agents. These conventional hydrogels may contain toxic crosslinkers and catalysts, whereas some injectable hydrogels can be physically crosslinked to render them more biocompatible. Moreover, better homogenous encapsulation and minimal invasive administration are additional advantages of injectable hydrogels for this application [25, 26]. Among these, stimuli-responsive hydrogels can eject therapeutic drugs by undergoing changes in their physical or chemical conditions (e.g. shrinkage) in response to external changes to their environment [91].

For example, a previous study demonstrated that double-walled microparticles loaded with anticancer drugs and embedded in an injectable alginate hydrogel showed superior results compared with free drugs for the treatment of breast cancer [92]. An insulin-loaded injectable gel composed of carboxymethyl-hexanoyl chitosan and integrated lysozyme nanoparticles was used for the management of problems related to diabetes [93]. Jalili *et al.* prepared poly(*N*-isopropylacrylamide-*co*-acrylamide) (poly(NIPAM-*co*-AM)) and magnetic nanoparticles (MNPs) loaded with doxorubicin and entrapped them in shear-thinning GelMA network, and demonstrated that this magneto-thermal activation system had a good potential for the localized and on-demand delivery of therapeutics [94].

3.3. Medical devices

Some injectable hydrogels can be utilized as medical devices. For example, the nanocomposite of hydrophilized silica nanoparticles in hydrophobically modified poly(ethylene glycol) forms an *in situ* gelling hydrogel which was developed as an injectable accommodative intraocular lens [95]. Various injectable bone fillers are also categorized as medical devices by most regulatory authorities [96, 97]. Dermal fillers, which are injectables used for skin rejuvenation and the removal of facial wrinkles, are another application of injectable gels as a medical device [98]. Hyaluronic acid [99, 100] and collagen [101] are the most commonly used biomaterials in this area.

4. Conclusions and future perspectives

This review has focused on the different mechanisms of injectability in hydrogels. These mechanisms were categorized in three main groups: *in situ* gelling liquids, injectable gels, and injectable particles. Examples were provided for each of these groups. The

major applications of the injectable hydrogels were discussed, and their roles, requirements, advantages, and drawbacks were investigated.

Despite notable attempts to synthesize and fabricate injectable hydrogels, several challenges remain to be addressed. The mechanical strength of hydrogels, particularly physical hydrogels, needs to be improved. The cytotoxicity and biocompatibility of these gels must be assessed appropriately, according to recognized standards. When used as scaffolds, the biodegradation rate of the hydrogels should be adjusted in order to correlate with the tissue regeneration rate. The integration of the scaffolds with host tissue and the stability of the attachment within the interface of hydrogel and native tissue is another issue that requires further investigation. In the case of delivery applications, the sustained and acceptable release profile is another factor that needs further careful consideration.

Overall, this field is in need of novel hydrogel designs with tunable properties. As such, future studies should focus on the potential applications of composites and hybrids.

Conflicts of Interest

None

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