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Review Article

# Thermoresponsive hydrogels in biomedical applications
## A seven-year update

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ABSTRACT

Thermally responsive hydrogels modulate their gelation behavior upon temperature change. Aqueous solutions solidify into hydrogels when a critical temperature is reached. In biomedical applications, the change from ambient temperature to physiological temperature can be employed. Their potential as *in situ* forming biomaterials has rendered these hydrogels very attractive. Advances in drug delivery, tissue engineering and cell sheet engineering have been made in recent years with the use of thermoresponsive hydrogels. The scope of this article is to review the literature on thermosensitive hydrogels published over the past seven years. The article concentrates on natural polymers as well as synthetic polymers, including systems based on *N*-isopropylacrylamide (NIPAAm), poly(ethylene oxide)–b-poly(propylene oxide)–b-poly(ethylene oxide) (PEO–PPO–PEO), poly(ethylene glycol) (PEG)-biodegradable polyester copolymers, poly(organophosphazenes) and 2-(dimethylamino) ethyl methacrylate (DMAEMA).



## 1. Introduction

Hydrogels are a class of materials characterized by their ability to imbibe water and swell in aqueous environments. Their highly hydrated nature resemblances the extracellular matrix and is one of the reasons for their popularity within the biomedical community [1]. One method of hydrogel preparation is the covalent crosslinking of a precursor solution [2]. Alternatively, physical hydrogels can form when a specific stimulus like temperature is applied, or in the presence of ions [3,4]. Thermally responsive hydrogels are particularly interesting because their gelation and changes in swelling can be triggered by temperature change. In biomedical applications, this can be accomplished through temperature increase from ambient to physiological. These systems allow for *in situ* hydrogel formation, where a biomaterial can be delivered in solution in a minimally invasive manner and solidify inside the body. Hydrogel formation for many systems happens almost instantaneously once the gelation temperature is reached. Additionally, when temperature is the only stimulus, the need for a chemical initiator system is eliminated, which results in a milder process.

Many thermoresponsive polymers utilized in biomedical applications exhibit a lower critical solution temperature (LCST) [5] and form a gel upon temperature increase. The process is reversible, and the polymers return into solution when the temperature is lowered below the LCST. In the case of a thermoresponsive polymer that has been additionally chemically crosslinked, the hydrogel will exhibit increased swelling below the LCST [6]. There are also some thermosensitive polymers that gel below a certain temperature and are soluble above it [7], and these polymers are denoted as having upper critical solution temperature (UCST) behavior. Some previous review articles summarize very well the theories and mechanisms behind thermogelation [6,8–10]. Several experimental techniques are employed for the determination of phase, or sol–gel transition, among those cloud point measurement [11–13], differential scanning calorimetry [11,14], and rheology [13]. It needs to be noted here that the value of the transition temperature may vary slightly depending on the experimental method used, as different stages of the gelation process may be measured by each technique [15].

This work serves as an update to a review article on thermoresponsive hydrogels published in 2008 [9]. Some of the challenges with this type of hydrogels involved precise control over the LCST and gelation kinetics, stability and mechanical properties as well as degradation profiles. Studies included herewith demonstrate efforts towards this goal by exploring the effects of composition, architecture, and functionalization, among other parameters. This article highlights the progress in the synthesis and fabrication of hydrogels which allow for controlled delivery and have the potential to be biointeractive, and covers advances

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in the literature published between 2007 and 2014. Thermally responsive hydrogel systems based on natural polymers, *N*-isopropylacrylamide (NIPAAm), poly(ethylene oxide)–b-poly (propylene oxide)–b-poly(ethylene oxide) (PEO–PPO–PEO), poly (ethylene glycol) (PEG)-biodegradable polyester copolymers, poly(organophosphazenes) and 2-(dimethylamino) ethyl methacrylate (DMAEMA) are showcased.

## 2. Natural polymers

Several natural polymers demonstrate thermally sensitive properties. Hydrogel systems based on polysaccharides (cellulose, chitosan and xyloglucan) and proteins (gelatin) are presented in the next sections.

### 2.1. Cellulose derivatives

Cellulose is the main structural component of plant cell walls and is widely abundant in nature. Cellulose is insoluble in water due to strong hydrogen bonding between hydroxyl groups. It can be made water soluble with substitution of the hydrogen on the hydroxyl groups with more hydrophobic units such as methyl or hydroxypropyl groups [16]. Metolose®, which is commercially available as methylcellulose and hydroxylpropylmethylcellulose, exhibits thermally responsive behavior. Generally, Metolose® aqueous solutions initially develop a drop in viscosity upon temperature increase, and when heated further, some formulations solidify into a hydrogel [17]. The material has been recently investigated as a drug carrier. Csoka et al. [17] examined the effect of salts on the transition temperature of Metolose®. They showed that in a physiological temperature range the viscosity of the formulation hydroxypropylmethylcellulose decreased, enabling drug delivery. Drug release from the gel was shown to be temperature-dependent. Further studies [18] determined the salt concentration necessary for gel formation close to 37 °C. The hydrogel showed adhesion to mucosal surfaces, with promise as a bioadhesive drug carrier for the oesophagus. In another approach to modulate the gelation temperature of methylcellulose closer to physiologic, Kim et al. [19] used enzymatic degradation to lower its molecular weight. When used loaded with protein drugs in a diabetic animal model, a reduction in blood glucose over several days was observed. By controlling the physical properties of the gel, gelation temperature and time as well as viscosity could be optimized and controlled release was obtained.

Methylcellulose has also been used in the fabrication of cell sheet fragments [20–22]. Cells cultured on methylcellulose formed a monolayer cell sheet at 37 °C. The monolayers could be detached from the hydrogel when cooled to ambient temperature due to the material's reversible phase transition and increased hydrophilicity. The cell sheets were subsequently fragmented in order to allow for their delivery via injection. Fragmented cell sheets provide the advantage of higher localization and retention when transplanted compared to dissociated cells [20]. The process of cell sheet fragment fabrication is illustrated in Fig. 1. In an effort to create a cell delivery platform for the treatment of myocardial infarction, this technology was employed with amniotic-fluid derived stem cells [22] and mesenchymal stem cells [21]. In both cases, cell differentiation towards cardiomyocyte-like cells and endothelial cells was observed *in vivo*. Cardiac function in a porcine model could be maintained after myocardial infarction.

### 2.2. Chitosan

Chitosan is a linear polysaccharide derived from chitin after a deacetylation process. Chitin is abundantly found in nature as a component in the skeletons of invertebrates [23]. Its use for biomedical applications has been widely reported over the past years. A thermally responsive hydrogel based on chitosan was introduced by Chenite et al. [24] with the addition of β-glycerophosphate. A recent review article [25] highlights the overall characteristics and applications of chitosan-glycerophosphate based hydrogel systems. Other types of phosphates [26,27] as well as phosphate-free polyols and polyoses [28] have been proposed as thermogelation agents alternative to β-glycerophosphate.

Chitosan has generated much research interest in the field of controlled delivery systems. A review article by Bhattarai et al. [29] summarizes the properties and use of chitosan hydrogels in drug delivery. A system based on chitosan was reported for the controlled delivery of doxorubicin [30]. Doxorubicin was chemically conjugated to acrylated chitosan, and formed a physical gel including free doxorubicin and acrylated Pluronic® when heated to physiological temperature. The hydrogel was subsequently chemically crosslinked, and doxorubicin release profiles *in vitro*

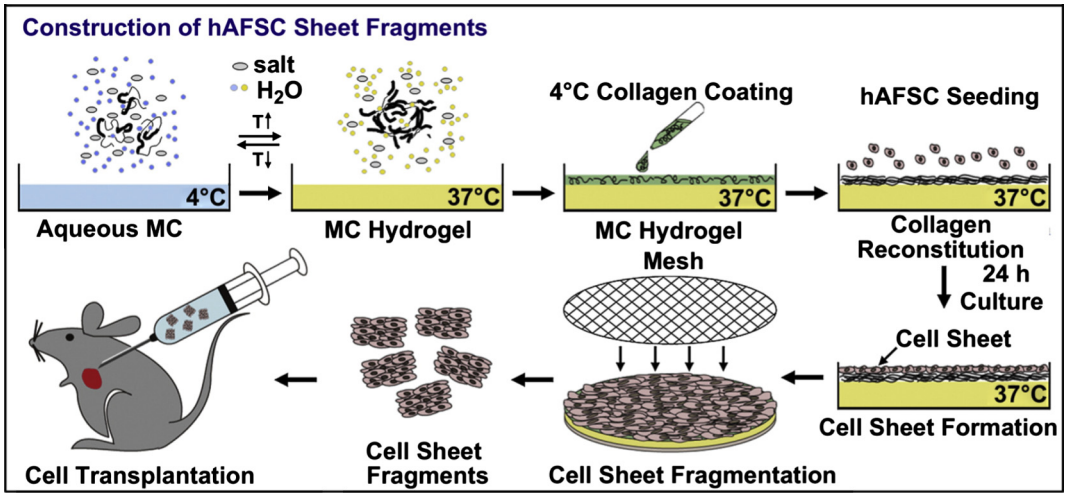


**Fig. 1.** Fabrication of cell sheet fragments derived from human amniotic fluid stem cells (hAFSC). Aqueous solution of methylcellulose (MC) was placed in a tissue culture dish at 4 °C and solidified after temperature increase to 37 °C. A layer of collagen was added to the hydrogel to enhance cellular attachment and cells were seeded onto the surface. After 24-h incubation, a continuous cell monolayer had formed. In order to lift, compress and fragment the cell sheet, a pre-sterilized mesh was used at room temperature. The cell sheet fragments could then be delivered via injection. Adapted from [22] with permission from Elsevier.