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Microfluidics-assisted generation of stimuli-responsive hydrogels based on alginates incorporated with thermo-responsive and amphiphilic polymers as novel biomaterials

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ABSTRACT

We used a droplet-based microfluidics technique to produce monodisperse responsive alginate-block-polyetheramine copolymer microgels. The polyetheramine group (PEA), corresponding to a propylene oxide /ethylene oxide ratio (PO/EO) of 29/6 (Jeffamine® M2005), was condensed, via the amine link, to alginates with various mannuronic/guluronic acids ratios and using two alginate:jeffamine mass ratios. The size of the grafted-alginate microgels varied from 60 to 80 μm depending on the type of alginate used and the degree of substitution. The droplet-based microfluidics technique offered exquisite control of both the dimension and physical chemical properties of the grafted-alginate microgels. These microgels were therefore comparable to isolated grafted-alginate chains in retaining both their amphiphilic and thermo-sensitive properties. Amphiphilicity was demonstrated at the oil–water interface where grafted-alginate microgels were found to decrease interfacial tension by ~50%. The thermo-sensitivity of microgels was clearly demonstrated and a 10 to 20% reduction in size between was evidenced on increasing the temperature above the lower critical solution temperature (T_{LCST}) of Jeffamine. In addition, the reversibility of thermo-sensitivity was demonstrated by studying the oil–water affinity of microgels with temperature after Congo red labeling. Finally, droplet-based microfluidics was found to be a good and promising tool for generating responsive biobased hydrogels for drug delivery applications and potential new colloidal stabilizers for dispersed systems such as Pickering emulsions.

1. Introduction

One of the major challenges in the field of biomedical applications is to devise systems that encapsulate, transport and finally release, under controlled kinetics, an active at a specific site in the human body. Many systems are available to encapsulate and protect actives for oral administration (nanoparticles [1,2], polymeric micelles [3,4], hydrogels [5,6], liposomes [7,8],...), but few are currently trade-marked and commercialized due to the cost of large scale processing. [2] The most promising vehicles for therapeutic applications, however, are hydrogels: these systems have many advantages including ease of production from macro to nano scale, simple active formulations and administration procedures, no-organic solvent, sustained active release behavior and less-systemic toxicity. [9] Hydrogels are polymeric cross-linked three-dimensional networks able to absorb and retain large amounts of water thus providing good biocompatibility, while maintaining their shape. [5] Physical or chemical cross-linking of the polymeric chains prevents dissolution of the matrix and maintains mechanical integrity. The strong hydration state of hydrogels at equilibrium makes many of their physical properties similar to those of living tissues. There is thus great interest in using natural, synthetic and hybrid hydrogels in areas such as tissue engineering, drug delivery systems and bionanotechnology. [10,11] Moreover, when used as active delivery vehicles, hydrogels can incorporate stimuli-responsive elements that permit an "intelligent" delivery of the actives. [12,13] These systems swell or collapse in response to changes in the environmental conditions (temperature, pH, ionic strength, electric field, mechanical stress, enzymes,...) and are consequently used as "smart" materials for various biomedical applications. [14,15]

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The most popular stimuli-responsive polymers are those sensitive to pH and temperature, as both these factors are of physiological significance. Such polymers include poly-(*N*-isopropylacrylamide) (PNIPAAm), poly-(*N*-vinylcaprolactam) (PVCL) and block or random copolymers of ethylene oxide and propylene oxide (PEPO). Responsive hydrogels are therefore recognized to be of potential use in biomedical and pharmaceutical fields. [16]

Thermo-responsive polymers are a particular type of stimuli-responsive polymers characterized by temperature-dependent volume phase transition. In a positive thermo-reversible system, polymers with an upper critical solution temperature (UCST) shrink on cooling below the UCST while polymers with a lower critical solution temperature (LCST) contract on heating above the LCST. These negative thermo-reversible hydrogels can be tuned to be liquid at room temperature (20–25 °C) and to gel, as a result of the increase in temperature, when in contact with body fluids (36–37 °C). [16] Polymers with a LCST below the human body temperature therefore have a potential for injectable applications in tissue engineering.

The LCST transition is mainly characterized by a drastic change in the interactions between water molecules and the hydrophilic region of the polymer due to hydrogen bonding and hydrophobic interactions between the polymer chains. [17–19] Indeed, a high temperature weakens the hydrogen bonding, which may then modify hydration (water structure around the hydrophobic moieties of the polymer) and enhances the hydrophobic interactions. At low temperature, LCST polymer solutions are homogeneous whereas heating leads to phase separation up to a critical LCST value (for the lowest temperature at a fixed solute concentration), generally known as the cloud point temperature. [20] Additives such as salts [21], surfactants [22,23], solvents [24], and non-electrolytes [25] are known to affect this LCST property.

Over the past few years, great attention has been focused on developing stimuli-responsive polymeric gels with unique properties such as biocompatibility, biodegradability and biological functionality, for biomedical applications. These responsive hydrogels can be prepared by combining thermos-responsive polymers with a natural-based polymeric component. [26–29] A number of polysaccharides have thus been combined with thermo-responsive materials, such as chitosan, pullulan [30–35], alginate [9,36–38], cellulose [36] and dextran [36].

The copolymers commonly grafted to hydrophilic polymers, and used to generate thermo-sensitive and amphiphilic properties, consist of polyetheramine chains containing both hydrophilic and hydrophobic groups.[9,18,35–37,39,40] These synthetic block copolymers are made up of hydrophilic poly(ethylene oxide) (EO) and hydrophobic poly(propylene oxide) (PO) chains and are of particular importance in the production of polyurea coating technologies, epoxy applications and pigment formulations. Grafting copolymers with different PO/EO ratios to hydrophilic polymers, via condensation reactions, will modify their swelling properties and LCST behavior. [16]

Apart from the temperature-sensitive properties found in LCST polymers, an addition of a copolymer may also confer an amphiphilic property useful for stabilization applications in dispersed systems. The hydrophilic polymer will display new surfactant interfacial properties after grafting with a copolymer. [30,35,40]

With the rapid rise of bionanotechnology, “responsive micro and nanogels”, as compared to conventional macroscopic gels, have attracted great interest due to their faster response to selective external stimuli. Since the characteristic gel swelling time is proportional to the square of the linear dimension of the hydrogel, [41] the simple strategy of using small-sized microgel particles will lead to a significant decrease in response time. However, for certain applications, like actuators in a tube of fixed diameter, the size of the microgel cannot be used as a control variable. By chemically grafting the polymer with linear side chains, the response time for the entire hydrogel can be reduced in comparison to a hydrogel with ungrafted polymer networks. [42]

We propose a novel approach for the design of thermo-sensitive and amphiphilic alginate-based polyelectrolyte microgels. The strategy is based on microfluidics soft lithography technology, which we have already used, to create new biobased functional microparticles. [43,44] The droplets-based microfluidics approach offers a number of advantages over conventional flow control technology since it can be used to produce monodisperse spherical and non-spherical polymeric microparticles with diameters ranging from several tens to several hundreds of microns and a diverse range of shapes. [45–48] Alginate, as a renewable resource, and jeffamine, as a source of stimuli-responsive polymer, were therefore used to form monodisperse thermo-sensitive and amphiphilic microgels.

2. Experimental

2.1. Reagents and solvents

1-[3-Dimethylaminopropyl]-3-ethylcarbodiimide hydrochloride (EDC, 98%, Sigma–Aldrich) and *N*-hydroxysuccinimide (NHS, 98%, Acros) were used as received for the covalent coupling of Jeffamine® M-2005 on alginates. Water was purified with a Millipore system combining an inverse osmosis membrane (Milli RO) and ion exchange resins (Milli Q). Sunflower seed oil was purchased from Fluka (France). Sodium acetate, calcium chloride, calcium carbonate, acetic acid and span 80 were purchased from Sigma-Aldrich (France).

2.2. Polymer Precursors (see Table 1)

Sodium alginate powders were kindly donated by the Brothier company (Laboratoire Brothier, Fontevraud L’abbaye, France) and were used without further purification. Jeffamine® M-2005, an amino terminated poly(ethylene oxide-co-propylene oxide) (PEO-co-PO) with a PO/EO ratio of 29/6, was kindly supplied by Huntsman (Belgium). The pKa of the Jeffamine® M-2005 amino-group is around 9.2. The LCST of a 10 wt% Jeffamine solution at pH < pKa is around 20 °C .

2.3. Alginate grafting reaction

The coupling reaction between the alginate carboxyl groups and the terminal amine of Jeffamine® 2005 was carried out in cold water using EDC/NHS as coupling reagent. The grafting conditions tested were for an alginate/Jeffamine mass ratio of (1:1) or (1:2). For the (1:1) ratio, 10 g of sodium alginate (50 mmol of urinate units) were dissolved under stirring in 700 mL of deionized water overnight. 10 g of Jeffamine® M-2005 (5 mmol) was separately dissolved under stirring in 100 mL of cold deionized water to get a homogeneous solution and the pH was then adjusted to around 7 with hydrogen chloride 1 M. This step limited the increase in pH when Jeffamine was added to the alginate solution. The Jeffamine solution was slowly added to the alginate solution at cold temperature and the pH was controlled again and, if necessary, was adjusted to 7–7.5. After mixing for 1 h under stirring, 0.46 g of NHS (4 mmol) and 2.48 g of EDC (16 mmol), dissolved separately in 5 mL of deionized water, were slowly added to the reaction solution. The reaction was allowed to proceed overnight at cold temperature. Two processes were then used to purify the grafted-alginates: (i) precipitation by mixing the grafted-alginates under vigorous stirring with ethanol at room temperature. The precipitates were