Abstract: Novel amphiphilic macromonomers that are capable of both physical crosslinking and chemical crosslinking are described herein. The combination of chemical crosslinking and physical crosslinking provides the ability to generate rapidly gelling hydrogels for many different applications. Moreover, the macromonomers may incorporate functional groups that allow for two different gelation mechanisms i.e. thermal gelation and ionic gelation, further improving mechanical stability of hydrogels formed from the disclosed macromonomers.
NOVEL INJECTABLE MACROMONOMERS
FOR THE FABRICATION OF HYDROGELS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Application Serial No. 60/745,595, filed April 25, 2006, herein incorporated by reference in its entirety for all purposes.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This work was supported by the National Institutes of Health (ROI DE1 5164) and Deutsche Forschungsgemeinschaft DFG (HA 4444/1-1).

BACKGROUND

Field of the Invention

[0003] This invention relates generally to the field of polymers. More specifically, the invention relates to injectable in situ crosslinkable macromonomers.

Background of the Invention

[0004] Smart polymers, polymeric materials that respond to environmental stimuli, have become attractive materials in biotechnology and medicine. In response to small changes in the environment, such polymers undergo strong conformational changes that result in rapid desolvation of the polymer molecules and phase separation of the solution. Functional groups have been identified and polymers synthesized that respond to a variety of stimuli, including changes in temperature, pH, osmotic pressure, ionic strength, pressure, and electric or magnetic field. Temperature-sensitive hydrogel-forming polymers are one of the most common among these materials and have been previously studied as temperature-regulated drug delivery systems and have also been investigated as matrices for injectable tissue engineering applications. In situ gel formation is a concept of great interest for tissue engineers as it enables the delivery of a hydrogel matrix encapsulating cells and/or growth factors to defects of any shape using minimally invasive surgical techniques. Various natural and synthetic polymers have been modified chemically with moieties for chemical crosslinking, including acrylic esters, methacrylic esters, cinnamoyl esters, fumaric esters, and vinylsulfone, to yield Injectable biodegradable matrices. In situ gel formation by radical polymerization of the electron-poor olefins can be induced photochemically or thermally without harming encapsulated cells. However, only low concentrations of radical initiators and crosslinking agents are tolerated by encapsulated cells in thermally induced crosslinking reactions.
Photocrosslinking, on the other hand, requires accessibility of the defect for a light source and hydrogel dimensions are limited to ensure homogenous polymerization. Irradiation times and doses also have to be carefully controlled to avoid detrimental effects of the curing light on cells or tissues.

[0005] During the fabrication of hydrogels or polymer networks by chemical crosslinking, certain important parameters such as gelation kinetics, crosslinking densities and resulting mechanical properties of the hydrogels can only be varied to a limited extent without compromising the cytocompatibility of the process. Cytocompatible chemical gelation methods, for instance, typically yield firm hydrogels after several minutes, while thermally induced gelation of thermosensitive polymer solutions occurs almost instantaneously once a certain temperature is reached. In order to employ thermosensitive polymers for cell encapsulation applications, the materials should have a low gel temperature and the thermally aggregated polymer chains should retain a significant amount of water. Polymers classes from which certain representatives have been shown to meet these characteristics include copolyethers of poly(ethylene glycol) (PEG) and poly(propylene glycol), copolyesters of PEG and poly(lactic acid) or poly(propylene fumarate), homo- and copolymers of poly(organophosphazenes), and copolymers of poly(N-isopropylacrylamide) (pNiPAAm). Combining functional groups for chemical and physical gelation within a macromolecule in a way that polymer solutions physically gel in response to physiological temperature upon injection and can be radically crosslinked at a slower kinetic in situ may yield superior materials with regard to gelation kinetics and ultimate mechanical properties. In addition, control over hydrogel properties might be improved through the combination of two mechanistically and kinetically independent gelation techniques additionally. Few materials possess such tandem gelation properties for biomedical applications.

[0006] Consequently, there is a need for injectable, biocompatible compositions that are capable of physical crosslinking and chemical crosslinking.

**BRIEF SUMMARY**

[0007] Novel amphiphilic macromonomers that are capable of both physical crosslinking and chemical crosslinking are described herein. The combination of chemical crosslinking and physical crosslinking provides the ability to generate rapidly gelling hydrogels for many different applications. Moreover, the macromonomers may incorporate functional groups that allow for two different gelation mechanisms *i.e.*, thermal gelation and ionic gelation, further improving
mechanical stability of hydrogels formed from the disclosed macromonomers. Further aspects and embodiments of the invention are described in more detail below.

[0008] In an embodiment, a macromonomer has the following formula:

![Chemical structure diagram](image)

wherein \(X\) is a fatty acid group, \(A_1, A_2,\) and \(A_3\) are each independently a thermo-responsive repeating unit, an ionic repeating unit, a hydrophilic repeating unit, or a hydroxy containing repeating unit. In addition, \(A_1, A_2,\) and \(A_3\) may be the same or different from one another. \(R^1\) and \(R^2\) are vinyl groups, which may be the same or different from one another.

[0009] In an embodiment, a method of making a macromonomer comprises providing a precursor comprising a pentaerythritol group coupled to at least one fatty acid group and at least one vinyl group. The method further comprises polymerizing the precursor with one or more monomers to form a macromonomer intermediate with one or more polymer branches. The one or more monomers comprises a thermo-responsive monomer or an ionic monomer. In addition, the method comprises coupling one or more vinyl groups to the macromonomer intermediate to make the macromonomer.

[0010] In another embodiment, a method for making a hydrogel comprises providing one or more of the disclosed macromonomers and crosslinking the one or more macromonomers to form the hydrogel.

[0011] The foregoing has outlined rather broadly the features and technical advantages of the invention in order that the detailed description of the invention that follows may be better understood. Additional features and advantages of the invention will be described hereinafter that form the subject of the claims of the invention. It should be appreciated by those skilled in the art that the conception and the specific embodiments disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present invention. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope of the invention as set forth in the appended claims.
BRIEF DESCRIPTION OF THE DRAWINGS

For a detailed description of the preferred embodiments of the invention, reference will now be made to the accompanying drawings in which:

FIGURE 1 illustrates a synthetic scheme for a thermogelling macromonomer (TGM) from the bifunctional precursor pentaerythritol diacrylate monostearate (PEDAS) and the acrylic monomers N-isopropyl acrylamide (NiPAAm), acrylamide (AAm) and hydroxyethyl acrylate (HEA) by addition copolymerization;

FIGURE 2 illustrates a synthetic scheme for a calcium-binding macromonomer (CBM) from the bifunctional monomer PEDAS, the vinyl phosphonic acid monomer (VPA) and the acrylic monomers HEA by addition copolymerization;

FIGURE 3 illustrates a 1H-NMR spectrum of poly(PEDAS-s tat-NiPAAm-\textunderscore y\textsubscript{16}\textunderscore stat-HEA) and corresponding chemical structures of structural (a) poly(PEDAS-stat-NiPAAm\textsubscript{16}-stat-AAm\textsubscript{1}) and (b) poly\textsuperscript{EDAS-s}\textsuperscript{154}stat-NiPAAm\textsubscript{16}\textsuperscript{stat-HEA\textsubscript{14}}. Stat denotes statistical polymers and subscript numbers indicate theoretical comonomer ratios;

FIGURE 4 illustrates GPC traces of (I) NiPAAm, (II) PEDAS, (III) poly(octadecyl acrylate (ODA), stat-NiPAAm\textsubscript{154}-stat-AAm\textsubscript{5}-stat-HEA\textsubscript{16}), (IV) Poly(PEDAS, stat-NiPAAm\textsubscript{154}-stat-AAm\textsubscript{5}-stat-HEA\textsubscript{14}), (V) poly\textsuperscript{EDAS-s}\textsuperscript{154}stat-NiPAAm\textsubscript{14}-stat-AAA) (Subscript numbers indicate theoretical comonomer ratios);

FIGURE 5(A) shows representative rheograms of a thermogelling macromonomer and polyNiPAAm as recorded during a test for reversibility of the thermogelation;

FIGURE 5(B) is a plot of values for storage modulus (G') and complex viscosity $|\eta^*|$ as determined at the end of the gelation step (I), after 2 min at 37°C (II), after 5' into step IV (15°), and 60' into step IV (15°). Columns and error bar represent means ± standard deviation for n = 3;

FIGURE 6 shows onset temperature of the phase transition of TGMs in culture medium with (A) different initial AAm % contents, (B) different initial HEA% to AAm% ratios with constant NiPAAm, and (C) a molar ratio of PEDAS:NiPAAm:AAm:HEA 1:14:3:3. Error bars stand for means ± standard deviation for n=3; and

FIGURE 7 is a plot of the fraction of live fibroblasts as a function of 2-hydroxyethyl acrylate/acrylamide content in polymer composition (A) after exposure to TGM conditioned media (no dilution) for 24h, and (B) after direct exposure to a TGM gel layer for 24h. Error bars stand for means ± standard deviation for n=5.
NOTATION AND NOMENCLATURE

Certain terms are used throughout the following description and claims to refer to particular system components. This document does not intend to distinguish between components that differ in name but not function.

In the following discussion and in the claims, the terms "including" and "comprising" are used in an open-ended fashion, and thus should be interpreted to mean "including, but not limited to...". In addition, the term "coupled" should be interpreted to mean, but not limited to a covalent bond, hydrogen bond, ionic bond, or electrostatic bond. As used herein, the term "macromonomer" should be interpreted, but not limited to mean a polymer or oligomer that has at least one reactive group, which enables the macromonomer to act as a monomer. Each macromonomer molecule may be attached to the main chain of the final polymer by the reaction of the reactive group in the macromonomer molecule.

In addition, as defined herein, the term "chemical crosslinking" refers to covalent linkage of one polymer chain to another whereas the phrase "physical crosslinking" refers to the physical entanglement of polymer chains to cause gelation due to ionic and/or thermodynamic interactions.

DETAILED DESCRIPTION

In embodiments, an injectable macromonomer generally comprises a compound having the following formula:

![Formula 1](image)

wherein \( R^3 \) is a fatty acid group, \( A_1, A_2, \) and \( A_3 \) are each independently a thermo-responsive repeating unit, an ionic repeating unit, a hydrophilic repeating unit, or a hydroxy containing repeating unit. \( A_1, A_2, \) and \( A_3 \) may be the same or different from one another. \( R^1 \) and \( R^2 \) may comprise vinyl groups. Likewise, \( R^1 \) and \( R^2 \) may be the same or different from one another. The subscripts "m" and "n" are integers representing a multiplicity of repeating units of \( A_1, A_2, \) and \( A_3 \). Preferably, \( m \) and \( n \) are greater than 1. In an embodiment, \( m \) and \( n \) may each independently be in a range of from 1 to 10, alternatively from 1 to 5, alternatively from 1 to 3. The subscript "p" in an
integer representing the number of repeating units of the macromonomer. The "/" in the formula indicates that the sequence of \( A_1, A_2, \) and \( A_3 \) is random. Embodiments of the macromonomer are capable of thermogelation at a variety of temperatures depending on the amount and the composition of \( A_1, A_2, \) and \( A_3 \) incorporated in the macromonomer. As used herein, thermogelation is a property where a liquid compound becomes a solid at specific temperature known as the lower critical transition temperature (LCST). Furthermore, in other embodiments, the macromonomer is capable of ionic gelation in the presence of ions such as calcium ions.

[0025] As can be seen in Formula 1 above, embodiments of the macromonomer incorporate at least one pentaerythritol ester as a branching or junction point for polymer branches comprising repeating units \( A_1, A_2, \) and \( A_3 \). In one embodiment the junction point is a pentaerythritol diester. The pentaerythritol ester junction point typically is coupled to at least one polymer of \( A_1, A_2, \) and \( A_3, \) more preferably at least two polymer branches of \( A_1, A_2, \) and \( A_3 \). That is, embodiments of the macromonomer may have a pentaerythritol ester junction point with one or more polymer branches of \( A_1, A_2, \) and \( A_3 \).

[0026] Typically, the polymers of \( A_1, A_2, \) and \( A_3 \) are random copolymers, when \( A_1, A_2, \) and \( A_3 \) are different from each other. In particular, the random copolymers may be statistical random copolymers, meaning that the repeating units are distributed according to a statistical distribution. However, in some embodiments, the copolymers of \( A_1, A_2, \) and \( A_3 \) may be block copolymers of \( A_1, A_2, \) and \( A_3 \). In further embodiments, \( A_1, A_2, \) and \( A_3 \) may all comprise the same repeating unit such that the one or more polymer branches are homopolymers (i.e., polymers comprising a single type of repeating unit). For example, \( A_1, A_2, \) and \( A_3 \) may all comprise isopropyl aminocarbonyl ethylene repeating units. Thus, in such an example, the one or more polymer branches are homopolymers of poly(isopropyl acrylamide).

[0027] In an embodiment, the macromonomer may comprise more than one pentaerythritol ester branching point as shown below.

![Diagram of macromonomer structure](image-url)
Thus, in embodiments, \( p \) may be an integer ranging from 1 to 5, preferably from 1 to 3. The subscript "\( o \)" like subscripts "\( m \)" and "\( n \)" denote an integer multiplicity of repeating units, \( A_1, A_2, \) and \( A_3 \). In an embodiment, \( o \) is equal to or \( >1 \), alternatively in a range of from 1 to 10, alternatively from 1 to 5, alternatively from 1 to 3. In embodiments where \( p \) is greater than 2, the macromonomer may comprise a branched structure 141 as shown in Figure 1.

[0028] In embodiments, the macromonomer may have a number average molecular weight ranging from about 500 \( M_n \) to about 20,000 \( M_n \), preferably ranging from about 1,000 \( M_n \) to about 10,000 \( M_n \), more preferably from about 1,500 \( M_n \) to about 7,500 \( M_n \). The molecular weight of the macromonomer may be varied by adjusting the initiator concentration or the initial concentration of \( A_1, A_2, \) and \( A_3 \) during the copolymerization described below. As shown in Formula 1, the polymer branches of \( A_1, A_2, \) and \( A_3 \) may each have \( m \) and \( n \) number of repeating units. Each polymer branch of \( A_1, A_2, \) and \( A_3 \) may have different molecular weights (i.e. different numbers of repeating units). The number of \( A_1, A_2, \) and \( A_3 \) repeating units incorporated into each copolymer branch may be varied by adjusting the initial concentration of each monomer used in the polymerization reaction.

[0029] According to one embodiment, \( X \) may comprise a fatty acid group. \( X \) serves to impart hydrophobicity to the macromonomer. As used herein, a fatty acid group is a functional group having a carboxyl group and a long chain aliphatic tail. The long chain aliphatic tail may be saturated or unsaturated. In addition, the long chain aliphatic tail may be branched or unbranched. In embodiments, the aliphatic tail may comprise from 2 carbons to 22 carbons, preferably from 8 carbons to 24 carbons, more preferably from 14 carbons to 20 carbons. Examples of suitable fatty acid groups include without limitation, a stearic group, a palmitic group, a myristic group, a lauric group, a capric group, a caprylic group, a caproic group, a butyric group, or their derivatives.

[0030] Embodiments of the macromonomer also comprise at least one polymer of the repeating units, \( A_1, A_2, \) and \( A_3 \) as shown in the formula above. \( A_1, A_2, \) and \( A_3 \) may comprise a thermo-responsive repeating unit. As used herein, a thermo-responsive repeating unit is any repeating unit when incorporated into a polymer or macromonomer imparts LCST behavior to the polymer or macromonomer. Examples of a thermo-responsive repeating unit include without limitation, an alkyl aminocarbonyl ethylene repeating unit, an alkyl aminocarbonyl alkylethylene repeating unit, an alkyl oxycarbonyl ethylene repeating unit, an alkyl oxycarbonyl alkylethylene repeating unit or an alkylolxy ethylene repeating unit.
In an embodiment, the thermo-responsive repeating unit has the following formula:

\[
R^4 \quad \text{X} \quad \text{C-CH}_2 \quad \text{R}^5
\]

Formula 2

wherein \( R^4 \) comprises an alkyl group, \( X \) is an amide group (\( i.e. \) \(-\text{C}=\text{O}\text{NH}\)) , a carboxylate (\( i.e. \) \(-\text{C}=\text{O}\text{O}\)) group, or an ether group (\( i.e., \text{O-O} \)), and \( R^5 \) is hydrogen or a methyl group. The alkyl group may be a branched or unbranched alkyl group having from 1 to 8 carbon atoms. However, the alkyl group may comprise any number of carbon atoms.

In a particular embodiment, the thermo-responsive repeating unit is an alkyl aminocarbonyl ethylene repeating unit. Examples of alkyl aminocarbonyl ethylene repeating units include without limitation, isopropyl aminocarbonyl ethylene, butyl aminocarbonyl ethylene, isobutyl aminocarbonyl ethylene, propyl aminocarbonyl ethylene, and the like. The alkyl aminocarbonyl ethylene repeating units may be derived from a number of different monomers such as without limitation, isopropyl acrylamide, isobutyl acrylamide, dimethyl acrylamide, etc.

In other embodiments, \( A_1, A_2, \) and \( A_3 \) may independently comprise an ionic repeating unit. As used herein, an ionic repeating unit is a repeating unit that has either a negative or positive charge. The ionic repeating unit generally comprises an acidic functional group. For example, in one embodiment, \( A_1 \) comprises a phosphono ethylene repeating unit (\(-\text{C}=\text{P}(=\text{O})(\text{OH})_2\) ). An ionic repeating unit provides a charge to the macromonomer, thus allowing gels to be formed from embodiments of the macromonomer by binding ions such as without limitation, calcium, magnesium, barium, or copper. This is known as ionic gelation. Thus, the incorporation of an ionic repeating unit imparts another mechanism by which the macromonomer may form a gel or a polymer network. Other examples of ionic repeating units include without limitation, a carboxy ethylene repeating unit, a carboxy alkylethylene repeating unit, a phosphonoxyethyloxy carbonyl methylethylene repeating unit, a sulfinylethylene repeating unit, a sulfoxylethylene repeating unit, other sulfo-, sulfino-, and phosphono- derived repeating units, a dicarboxy ethylene repeating unit, and similar \( \alpha, \beta \) carboxy repeating units and \( \alpha, \beta \) dicarboxy repeating units.

Each of \( A_1, A_2, \) and \( A_3 \) may independently comprise a hydrophilic repeating unit. As used herein, a hydrophilic repeating unit is any repeating unit known to those of skill in the art to increase water solubility of a polymer. The hydrophilic repeating unit may comprise a
pyrrolidinone ethylene repeating unit, an oxyethylene repeating unit, a methoxy carbonyl ethylene repeating unit, or their derivatives. In an embodiment, the hydrophilic repeating unit is an unsubstituted aminocarbonyl ethylene repeating unit. In other words, using Formula 2 shown above, R⁴ and R⁵ are hydrogen atoms and X is an unsubstituted amide group. As used herein, the term unsubstituted refers to a functional group with no other substituents coupled to it. Without being limited by theory, the hydrophilic repeating unit may be used to increase or decrease the LCST of the macromonomer as well as imparting hydrophilicity to the macromonomer.

[Q035] In an embodiment, A₁, A₂, and A₃ may independently comprise a hydroxy-containing repeating unit. As used herein, a hydroxy-containing repeating unit is any repeating unit having a pendant hydroxy functional group. The hydroxy-containing repeating unit may have the following formula.

\[
\text{HO}\begin{array}{c}
\text{X}\\
\text{CH}_2
\end{array}
\text{R}
\]

wherein R⁶ comprises an alkyl group or a hydrogen, and X is an amide group, an alkyl carboxylate group, an alkyl group, or an ether group. Furthermore, X may comprise a branched or unbranched alkyl group having from 1 to 8 carbon atoms. However, the alkyl group may comprise any number of carbon atoms. Without being limited by theory, the hydroxy-containing repeating unit may provide further functional groups for polymerization or modification.

[0036] In an embodiment, the hydroxy-containing repeating unit is a hydroxyalkyl oxycarbonyl ethylene repeating unit. The hydroxyalkyl oxycarbonyl ethylene repeating unit may have the following formula:

\[
\text{HO}\begin{array}{c}
\text{O}\\
\text{R}^7
\end{array}
\text{CH}_2
\text{O}
\]

wherein R⁷ comprises a branched or unbranched alkyl group. The alkyl group may have from 1 to 8 carbon atoms. Examples of hydroxyalkyloxycarbonyl ethylene repeating units include without limitation, hydroxyethyloxycarbonyl ethylene, hydroxybutyloxycarbonyl ethylene,
hydroxypropyloxy carbonyl ethylene, hydroxyethyloxycarbonyl methylethylene, hydroxymethyloxycarbonyl methylethylene, hydroxy-poly(oxyethylene)oxycarbonyl ethylene, etc. The hydroxy group in the hydroxyalkyloxy carbonyl ethylene repeating unit provides further functional groups to the macromonomer for vinyl group modification. In other embodiments, the hydroxy-containing repeating unit may comprise a hydroxyalkyl aminocarbonyl ethylene repeating unit,

[0037] Referring to Formula 1, R¹ and R² each may comprise a vinyl group. As used herein, a vinyl group is any functional group containing a carbon-carbon double bond (-C=CH₂). R¹ and R² provide the ability for the macromonomer to be chemically crosslinked in addition to being physically crosslinked. In embodiments, R¹ and R² may comprise the same functional group or different functional groups. Examples of suitable vinyl groups include without limitation, an acrylate group, a methacrylate group, a fumarate group, a cinnamoyl group, etc.

[0038] As mentioned briefly above, embodiments of the macromonomer possess several advantageous features and properties. Specifically, the macromonomer is capable of both physical and chemical crosslinking. With respect to physical crosslinking, embodiments of the macromonomer exhibit thermogelation. That is, the macromonomer may physically crosslink with an increase in temperature. In other words, while soluble below a characteristic temperature, solutions of the macromonomers undergo thermally induced phase separation above their lower critical solution temperature (LCST). Without being limited to theory, it is believed that the phenomenon of polymer aggregation at LCST is an entropically driven process. Due to strong, specifically oriented hydrogen bonds, the entropy of the polymer solution is smaller than that of the two-phase polymer and water system. An increase in solution temperature renders the entropic contribution to overcome the positive enthalpy term and the free energy of association to become negative, thus favoring polymer desolvation and colloidal aggregation. This phase transition may lead to gel formation or polymer precipitation. Thus, in certain embodiments, the macromonomer may have a LCST ranging from 0°C to about 100°C, preferably from about 15°C to about 60°C, more preferably from about 25°C to about 40°C. In an embodiment, the macromonomer has an LCST less than or equal to the body temperature of a mammal (e.g., a human) such that the macromonomer forms a hydrogel in vivo after injection to the body.

[0039] Embodiments of the macromonomer may be crosslinked to form hydrogels useful for many different applications including biomedical, drug delivery, cell encapsulation, and tissue
engineering applications. Hydrogels formed by the crosslinking of the macromonomers may comprise a variety of water contents depending on the initial concentration of macromonomers used. In embodiments, a hydrogel may be formed by the crosslinking of at least one of the disclosed macromonomers having an ionic repeating unit and at least one macromonomer having a thermo-responsive repeating unit. In further embodiments, hydrogels may be formed by the addition of one or more crosslinking agents to the macromonomers. As used herein, crosslinking agents are any compounds known to those of skill in the art which are capable of covalently linking polymer chains together. Examples of suitable crosslinking agents include without limitation, acrylates, diacylates, dimethacrylates, bisacrylamides, or combinations thereof. In a specific embodiment, the crosslinking agent may be a poly(ethylene glycol) (PEG) diacrylate or ethylene glycol diacrylate. The PEG diacrylate may be of any suitable molecular weight.

[0040] Referring to Figure 1, in an embodiment, a method of making a macromonomer comprises providing a precursor 101 comprising a pentaerythritol group coupled to at least one fatty acid and at least one vinyl group. The fatty acid group may comprise any functional group as described above for X. In an embodiment, the pentaerythritol group is coupled to at least two vinyl groups. Preferably, the pentaerythritol group is coupled to two acrylate groups. The precursor 101 may be dissolved in a solvent such as without limitation, tetrahydrofuran (THF) or any other suitable solvent.

[0041] In at least one embodiment, the precursor 101 is polymerized with one or more monomers 121, 123, 125 to form a macromonomer intermediate 131. The one or more monomers may include a first monomer 121, a second monomer 123, and a third monomer 125. However, in some embodiments, more than three monomers may be used. The concentration ratio of precursor 101 to the one or more monomers may be about 1:5 to about 1:50, preferably about 1:10 to about 1:30, more preferably about 1:20. Nevertheless, the concentration ratio of precursor 101 to monomers may comprise any suitable ratio.

[0042] The first monomer 121 may be a thermo-responsive monomer. As used herein, thermo-responsive monomers are compounds that polymerize to form compounds with LCST behavior. In an embodiment, the thermo-responsive monomer is an alkyl acrylamide. As mentioned previously above, examples of alkyl acrylamide monomers that may be copolymerized with the precursor include without limitation, isopropyl acrylamide, isobutyl acrylamide, dimethyl acrylamide, etc.
Furtheπnore, the thermo-responsive monomer may comprise an alkyl methacrylamide, an alkyl acrylate, an alkyl methacrylate, an alkyl vinylimidazole, a vinyl alkyl ether, or their derivatives.

[0043] In addition, the first monomer 121 may be an ionic monomer. As used herein, an ionic monomer may be a monomer with charged or ionic groups. Ionic monomers may be polymerized to form polymers with charged or ionic groups. Examples of ionic monomers that may be copolymerized with the precursor include without limitation, acrylic acid, methacrylic acid, vinylsulfonic acid, vinylphosphonic acid, maleic acid, fumaric acid, itaconic acid, mesaconic acid, citraconic acid, ethylene glycol methacrylate phosphate, and similar α, β unsaturated carboxylic acids and α, β dicarboxylic acids.

[0044] The second monomer 123 is generally a hydrophilic monomer. The concentration of the second monomer 123 may be adjusted to control the LCST of the macromonomer and also to impart hydrophilicity (i.e. water solubility) to the macromonomer. Examples of hydrophilic monomers include without limitation, unsubstituted acrylamide, methyl methacrylate, methoxy ethylene glycol acrylate, N-vinyl pyrrolidinone, propylene oxide, ethylene oxide, and their derivatives.

[0045] The third monomer 125 may comprise a hydroxy-containing monomer such as without limitation, a hydroxyalkyl acrylate monomer. A hydroxy-containing monomer, as used herein, is any monomer when polymerized contains a hydroxy pendant group. Examples of hydroxyalkyl acrylate monomers which may be copolymerized with the precursor include without limitation, hydroxyethyl acrylate (HEA), hydroxybutyl acrylate, hydroxypropyl acrylate, hydroxyethyl methacrylate, hydroxymethyl acrylate, hydroxymethyl methacrylate, etc. In additional embodiments, the hydroxy-containing monomer may comprise a hydroxyalkyl methacrylate, a hydroxyalkyl acrylamide, a vinyl alcohol, or their derivatives.

[0046] In embodiments, the precursor 101 may be polymerized with any combination of the monomers described above. For example, as shown in Figure 2, the precursor 101 may be polymerized with only the first 121 and third 125 monomers. In particular, the precursor may be polymerized with an ionic monomer 121 (e.g., VPA) and a hydroxy-containing monomer 125 (e.g., HEA) to form a macromonomer with only ionic gelation properties. In addition, the precursor may be polymerized with just the first monomer i.e. only a thermo-responsive monomer or only an ionic monomer. In one embodiment as shown in Figure 1, the first monomer 121 is NiPAAm, the second monomer 123 is AAm, and the third monomer 125 is HEA. In an additional
embodiment, it is contemplated that the macromonomer may comprise an ionic monomer and a thermo-responsive monomer. In such an embodiment, the macromonomer may possess both ionic gelation and thermal gelation properties in a single composition. Thus, an advantage of the disclosed macromonomers is the ability to finely tailor their properties through the incorporation of different types of monomers.

[0047] In general, the precursor is polymerized with the monomers by radical polymerization. Specifically, an initiator is added to a solution of the precursor and the monomers. Examples of suitable initiators include without limitation, benzoyl peroxide, ammonium persulfate, hydrogen peroxide, potassium persulfate, photoinitiators, azobisisobutyronitrile (AIBN), azobis(cyclohexanecarbonitrile) (ABCN), or combinations thereof. However, any radical initiators known to those of skill in the art may be used. Polymerizing the monomers with the precursor forms the macromonomer intermediate.

[0048] The precursor and monomers may be copolymerized at raised temperatures. In embodiments, the precursor and the monomers may be copolymerized at a temperature ranging from about 20°C to about 120°C, preferably from about 40°C to about 80°C, more preferably from about 50°C to about 70°C. The number of repeating units of each type of monomer incorporated into the macromonomer may be adjusted by altering the concentration ratio of each monomer during copolymerization. In embodiments, the concentration ratio of the first monomer to second monomer to third monomer may be from about 20:0:0 to about 7:7:6, preferably about from about 18:2:2 to about 10:5:5, more preferably about 14:3:3. However, the concentration ratio of monomers may be adjusted to any value according to the desired application.

[0049] Referring back to Figure 1, once the macromonomer intermediate 131 has been synthesized, one or more vinyl groups may be coupled to the macromonomer intermediate 131 to form the macromonomer 141. In an embodiment, coupling at least one vinyl group to the macromonomer intermediate involves adding acryloyl chloride to a solution containing the macromonomer intermediate as shown in Figure 1. The solution may comprise a concentration of the macromonomer intermediate dissolved in an organic solvent and a base. In an embodiment, the base is triethylamine (TEA). Other compounds which may be used to modify the macromonomer intermediate include without limitation, glycidyl methacrylate, methacryoyl chloride, cinnamyl chloride, maleic anhydride, or methacrylic anhydride.
In an embodiment, a method of making a hydrogel comprises forming a solution of the disclosed macromonomers. To form the solution, an amount of macromonomer may be dissolved in a liquid such as water. In an embodiment, an amount of macromonomer may be dissolved in a commercial available culture medium such as without limitation, minimum essential media (MEM). An initiator may be added to the solution to initiate the chemical crosslinking reaction. Examples of suitable initiators include without limitation, peroxides, persulfates, azo compounds, halogen compounds, and the like. In particular, the initiator may be a water soluble redox initiation pair such as without limitation, ammonium persulfate (APS) and tetramethylethylenediamine (TMED). However, any water soluble redox initiators known to those of skill in the art may be used to initiate the crosslinking reaction. Alternatively, the hydrogel may be crosslinked via photo-crosslinking using ultra-violet (UV) light and a photoinitiator. In further embodiments, the hydrogel may be crosslinked via the addition of one or more crosslinking agents, as described above.

To form the crosslinked hydrogel, the macromonomer solution may be heated to the LCST of the macromonomer to cause physical gelation or crosslinking. The heating may also cause chemical crosslinking by initiating the free radical chain reaction by the formation of free radicals from the initiator.

To further illustrate various illustrative embodiments of the present invention, the following examples are provided.

**EXAMPLE 1**

*Materials.* Pentaerythritol diacrylate monostearate (PEDAS), octadecyl acrylate (ODA), N-isopropylacrylamide (NiPAAm), acrylamide (AAm), 2-hydroxyethylacrylate (HEA), 2,2'-azobis(2-methylpropionitrile) (azobisisobutyronitrile, AIBN), glycidyl methacrylate (GMA), 4-di(methylamino)pyridine (DMAP), ammonium persulfate (APS), and N,N,N',N'-tetramethylethane-1,2-diamine (TMED) were purchased from Sigma-Aldrich (Sigma, St. Louis, MO) and used as received. Solvents, tetrahydrofuran (THF), diethyl ether, and 2-propanol were obtained from Fisher Scientific (Pittsburgh, PA) in ACS grade.

*Macromonomer Synthesis:* Pentaerythritol monostearate diacrylate, N-isopropyl acrylamide, and acrylamide were dissolved in tetrahydrofuran at the desired ratio under nitrogen at 60°C. Radical polymerization was initiated by 2,2'-azobisisobutyronitrile (AIBN), and the system was refluxed over 16 h at 60°C. The thermogelling macromonomers (TGMs) were isolated and
purified by precipitation in diethyl ether. Calcium binding macromonomers were synthesized from PEDAS, vinylphosphonic acid, AAm, and 2-hydroxyethylmethacrylate (HEMA).

[0055] Macromonomer Characterization: Thermogelling macromonomers were characterized by gel permeation chromatography and NMR. Rheological characterization was performed on an oscillating rheometer at 1 Hz and a displacement of 0.1 mrad. The calcium binding efficacy of the CBMs was determined using a calcium assay.

[0056] Gelation properties. In a typical experiment, TGM and control samples were loaded, cooled to 5°C, presheared at a rate of 1 s⁻¹ for 1 min, and equilibrated for 15 min at 5°C. The viscoelastic properties of the samples were then recorded during a temperature sweep from 5°C to 65°C at a rate of 1°C/min at an observing frequency of 1 Hz and a displacement of 1x10⁻⁴ rad.

[0057] Reversibility of the thermogelation. Samples were loaded, cooled to 10°C, presheared at a rate of 1 s⁻¹ for 1 min, and equilibrated for 5 min at 10°C. The viscoelastic properties of the samples were then recorded during a set of different steps with a solvent trap installed. To gel the samples, a temperature sweep from 10°C to 37°C was performed at a rate of 4°C/min with a frequency of 1 Hz and a displacement of 1x10⁻⁴ rad (step I). The samples were kept at 37°C for 2 min while maintaining frequency and displacement at 1 Hz and 1x10⁻⁴ rad, respectively (step II). For the next 2 min the displacement was increased to 1.5x10⁻³ rad (step III). Thereafter, the temperature setting was automatically changes to 15°C and a time sweep was recorded over 90 min at a frequency of 1 Hz and a displacement of 1.5x10⁻³ rad (step IV). In a typical experiment, the temperature had equilibrated at 15°C after around 2.0 min into the time sweep. Storage modulus (G') and complex viscosity |η*| were analyzed at the end of step I and step II and at two time points during step IV (5' and 60').

[0058] Macromonomer crosslinking. Macromonomer solutions with a concentration of 12.5% (m/v) were prepared in α-MEM and loaded on the rheometer at 15. Before the geometry was lowered to gap size, 5 µl TMED and 30 µl of APS solution (100 mg/ml) or 30 µl of water were added. The loaded samples were presheared at a rate of 1 s⁻¹ for 1 min at 15°C before the viscoelastic properties were recorded in a two step protocol. A temperature sweep from 15°C to 37°C was performed at a rate of 5°C/min with a frequency of 1 Hz and a displacement of 1x10⁻⁴ rad (step I). Thereafter, the thermogel properties were monitored at 37°C over 15 min while maintaining oscillation frequency and displacement (step II).
Results. The resulting polymers were water-soluble at room temperature (PEDAS is typically insoluble in water). $^1$H-NMR spectroscopy of the TGMs showed typical signals of NiPAAm, HEA and PEDAS as shown in Figure 3. The most prominent signals were obtained from the stearic acid of PEDAS (0.85 and 1.25 ppm) and the isopropyl group of NiPAAm (1.15 and 4 ppm) (Figure 3). The signals between 1.6 and 2.2 ppm represent the polymerized C-C backbone. As verified from the quantification of NMR spectra, the theoretical monomer molar ratios are comparable to the actual molar analogies in the macromonomers, suggesting the successful incorporation of the monomers (Table 1).

| TGMs synthesized using PEDAS as a lipophilic element (branching possible) and octadecyl acrylate (ODA) as a monofunctional lipophilic component were compared. Referring to Figure 4, poly(PEDAS $\gamma$-stat-NiPAAm$_{15.4}$-stat-AAm$_3$-stat-HEA$_{16}$) (The numbers show theoretical molar monomer ratios in the macromonomer) (IV) compared with TGMs synthesized using ODA (no branching, lipophilic domain comparable to PEDAS), poly(ODA$_1$-stat-NiPAAm$_{15.4}$-stat-AAm$_3$-stat-HEA$_{16}$) (III), showed similar MW distribution which demonstrates that TGMs containing PEDAS are not significantly branched. This can be also seen under Table 1 which lists the molecular weights and polydispersity indices of the different TGM compositions. However, extensive branching occurred under certain conditions, e.g., high AAm feed, as shown in trace V (poly(PEDASi-stat-NiPAAm$_{15}$-stat-AAm$_6$)) and confirmed by molecular weight data (PI=5.2) as shown in Table 1. The asymmetrical peak shape and dispersity of the TGMs might in part be attributed to specific interactions of the lipophilic domains which might also be causing the broad trace that was found for the PEDAS monomer (II). Other acrylic monomers, here represented by

<table>
<thead>
<tr>
<th>Theoretical</th>
<th>$^1$H-NMR</th>
<th>GPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEDAS</td>
<td>NiPAAm</td>
<td>AAm</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>15.4</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>15.4</td>
<td>2.6</td>
</tr>
<tr>
<td>1</td>
<td>15.4</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>ODA: 1</td>
<td>15.4</td>
<td>3</td>
</tr>
</tbody>
</table>
NiPAAm (I), showed a narrow peak at high retention times (Fig. 4). These results indicate that the macromonomers may be branched, however their branching does not affect their solubility in water.

Different TGMs were synthesized by substituting different amounts of NiPAAm with acrylamide (AAm) and/or 2-hydroxyethyl acrylate (HEA) and varying their ratios. Solutions of the different TGMs in α-MEM were prepared and analyzed using an oscillating rheometer to determine their LCST and the changes in dynamic moduli (G', G''), complex viscosity, and loss angle (δ). A representative rheogram is depicted in Figure 5(A) comparing a TGM and a polyNiPAAAM control. Figure 5(B) shows values of storage modulus (G') and complex viscosity [η*] at the end of step I and step II and at two time points during step IV (5' and 60').

Figure 6 depicts the phase transition temperature of TGMs as determined by rheology and differential scanning calorimetry. For TGM synthesized from PEDAS, NiPAAm and AAm, a linear correlation was found between the acrylamide content of the macromonomer and the onset temperature of the phase transition (Figure 6(A)). Increased substitution with HEA yielded TGMs with decreasing transition temperatures (Figure 6(B)). A formulation with 15% molar AAm and 15% HEA (molar ratios PEDAS:NiPAAm:AAm:HEA 1:14:3:3) was found to have a transition close to physiologic temperature. Macroscopic observation of different macromonomer formulations at 37°C revealed gel formation within 30 min; the 14:3:3 formulation formed a gel that was stable for 2 hours at 37°C with minimal syneresis as compared to other synthetic compositions.

EXAMPLE 2

Two different types of studies were conducted to test the cytocompatibility of the thermogelling macromonomers. For the first study (a modified elution test), conditioned media were prepared by dissolving thermogelling macromonomers composed of PEDAS, NiPAAm, AAm and HEA in primary media. Stock solutions of 10% (w/v in DMEM) TGM conditioned media were prepared. After 24h, the solutions were heated to 37°C to gel the macromonomers. The supernatant was used either directly or after dilution with primary media (10- and 100-fold dilution) to test the effect of the macromonomers on the viability of rat fibroblasts (CRL.1764) following a protocol previously described in Timmer et al, Biomacromolecules 4, 1026-1033, (2003), herein incorporated by reference. For the second study (direct contact test), TGM solutions were prepared (10% w/v in DMEM) and added on CRL rat fibroblasts in a thin layer. Following
brief incubation at 37°C, the macromonomers gelled and media were added on top of the gel layer. The cells were incubated for 24h with the hydrogel and the cell viability was examined.

[0064] Figures 7(A)-(B) depict the cytotoxicity curves for different TGM compositions with respect to the ratio of the theoretical molar percentage in 2-hydroxyethyl acrylate to acrylamide with fibroblasts. For the first test, 10- and 100-fold dilutions gave viability fractions of above 0.9 (data not shown). Overall, the results indicate a minimal cytotoxicity induced by TGMs in various compositions. These preliminary studies as well as the proposed cytocompatibility assays utilize a cell line (CRL 1764 rat fibroblasts) to reduce the number of animals euthanized for cell harvest. Based on the cytocompatibility results, the formation of viable marrow stromal cell-hydrogel constructs with the disclosed macromonomers may be feasible.

[0065] The direct contact test were also performed using CBM formulations to evaluate the effect of varying the VPA:HEA ratio on cytocompatibility. CBMs with VPA:HEA ratios of 3:1, 1:1 and 1:3 demonstrated cytocompatibility comparable to that of TGMs and live controls. Based on preliminary data, the formation of viable marrow stromal cell-hydrogel constructs with calcium-binding macromonomers may be feasible.

[0066] While embodiments of this invention have been shown and described, modifications thereof can be made by one skilled in the art without departing from the spirit or teaching of this invention. The embodiments described herein are exemplary only and are not limiting. Many variations and modifications of the system and apparatus are possible and are within the scope of the invention. Accordingly, the scope of protection is not limited to the embodiments described herein, but is only limited by the claims which follow, the scope of which shall include all equivalents of the subject matter of the claims.
What is claimed is:

1. A macromonomer having the formula:

   ![Chemical Structure Diagram]

   wherein X is a fatty acid group, $A_1$, $A_2$, and $A_3$ are each independently a thermo-responsive repeating unit, a hydroxy repeating unit, a hydrophilic repeating unit, or an ionic repeating unit, wherein $A_1$, $A_2$, and $A_3$ may be the same or different from one another, and $R^1$ and $R^2$ are vinyl groups, wherein $R^1$ and $R^2$ may be the same or different from one another.

2. The macromonomer of claim 1 wherein said fatty acid group contains from 2 to 22 carbon atoms.

3. The macromonomer of claim 1 wherein said fatty acid group comprises a stearic group, a palmitic group, a myristic group, a lauric group, a capric group, a caprylic group, a caproic group, or a butyric group.

4. The macromonomer of claim 1 wherein said fatty acid group is an unsaturated fatty acid group.

5. The macromonomer of claim 1 wherein said thermo-responsive repeating unit is an alkyl aminocarbonyl ethylene repeating unit, an alkyl aminocarbonyl alkylethylene repeating unit, an alkyl oxycarbonyl ethylene repeating unit, an alkyl oxycarbonyl alkylethylene repeating unit, or an alkyl oxyoxy ethylene repeating unit.

6. The macromonomer of claim 5 wherein said alkyl aminocarbonyl ethylene repeating unit comprises a butyl aminocarbonyl ethylene repeating unit, an isobutyl aminocarbonyl ethylene
repeating unit, a propyl aminocarbonyl ethylene repeating unit, an isopropyl aminocarbonyl repeating unit, an ethyl aminocarbonyl ethylene repeating unit, or a methyl aminocarbonyl ethylene repeating unit.

7. The macromonomer of claim 1 wherein said thermo-responsive repeating unit comprises a repeating unit with the formula:

\[ \begin{array}{c}
\text{R}^4 \\
\text{X} \\
\text{C} - \text{CH}_2 \\
\text{R}^6 \\
\end{array} \]

wherein \( \text{R}^4 \) comprises an alkyl group, \( \text{X} \) is an amide group, a carboxylate group, or an ether group, and \( \text{R}^5 \) is hydrogen or a methyl group.

8. The macromonomer of claim 1 wherein said hydroxy-containing repeating unit is a hydroxy-containing repeating unit having the following formula:

\[ \begin{array}{c}
\text{HO} \\
\text{X} \\
\text{C} - \text{CH}_2 \\
\text{R}^6 \\
\end{array} \]

wherein \( \text{R}^6 \) comprises an alkyl group or a hydrogen, and \( \text{X} \) is an amide group, an alkyl carboxylate group, an alkyl group, or an alkyl ether group.

9. The macromonomer of claim 1 wherein said hydroxy-containing repeating unit is a hydroxyalkyloxy carbonyl ethylene repeating unit.

10. The macromonomer of claim 9 wherein said hydroxyalkyloxy carbonyl ethylene repeating unit comprises a hydroxyethyloxy carbonyl ethylene repeating unit, a hydroxybutyloxy carbonyl ethylene repeating unit, a hydroxypropyloxy carbonyl ethylene repeating unit, hydroxyethyloxy carbonyl methylethylene repeating unit, a hydroxymethoxy carbonyl methylethylene repeating unit, a hydroxy(poly(oxyethylene))oxycarbonyl ethylene repeating unit, or a hydroxymethoxy methylethylene repeating unit.
11. The macromonomer of claim 9 wherein said hydroxy alkyloxy carbonyl ethylene repeating unit comprises a repeating unit with the formula:

\[
\text{HO-R}^7 \quad \text{O-} \quad \text{O}
\]

wherein R\(^7\) comprises an alkyl group having from 1 to 8 carbon atoms.

12. The macromonomer of claim 1 wherein said ionic repeating unit comprises a carboxy ethylene repeating unit, a carboxy methylethylene repeating unit, a phosphonooxyethyloxycarbonyl methylethylene repeating unit, a carboxy alkyl ethylene repeating unit, a sulfoethylene repeating unit, a sulfinoethylene repeating unit, or a phosphonoethylene repeating unit.

13. The macromonomer of claim 1 wherein A\(_1\), A\(_2\), and A\(_3\) are all different.

14. The macromonomer of claim 1 wherein A\(_1\), A\(_2\), and A\(_3\) are all the same.

15. The macromonomer of claim 1 wherein A\(_1\) is thermo-responsive repeating unit, and A\(_2\) and A\(_3\) are hydrophilic repeating units.

16. The macromonomer of claim 1 wherein A\(_1\) is an ionic repeating unit, and A\(_2\) and A\(_3\) are hydroxy-containing repeating units.

17. The macromonomer of claim 1 wherein R\(^1\) and R\(^2\) comprise methacrylate or acrylate groups.

18. The macromonomer of claim 1 wherein R\(^1\) and R\(^2\) are the same.

19. The macromonomer of claim 1 wherein p is an integer ranging from 1 to 5.
20. The macromonomer of claim 1 having a lower critical solution temperature ranging from about 0°C to about 100°C.

21. The macromonomer of claim 1 having a number average molecular weight ranging from about 500 to about 15,000.

22. A hydrogel comprising one or more macromonomers of claim 1.

23. The hydrogel of claim 22 wherein the one or more macromonomers comprises at least one macromonomer having an ionic repeating unit and at least one macromonomer having a thermo-responsive unit.

24. The hydrogel of claim 22, further comprising one or more crosslinking agents.

25. A method of making a macromonomer comprising:
   a) providing a precursor comprising a pentaerythritol group coupled to at least one fatty acid group and at least one vinyl group;
   b) polymerizing the precursor with one or more monomers to form a macromonomer intermediate with one or more polymer branches, wherein the one or more monomers comprises a thermo-responsive monomer or an ionic monomer;
   c) coupling one or more vinyl groups to the macromonomer intermediate to make the macromonomer.

26. The method of claim 25 wherein the at least one fatty acid comprises a stearic group, a palmitic group, a myristic group, a lauric group, a capric group, a caprylic group, a caproic group, or a butyric group.

27. The method of claim 25 wherein the precursor comprises a pentaerythritol group coupled to two acrylate groups.
28. The method of claim 25 wherein the thermo-responsive monomer comprises an alkyl acrylamide, an alkyl methacrylamide, an alkyl acrylate, an alkyl methacrylate, an alkyl vinylimidazole, or a vinyl alkyl ether.

29. The method of claim 28 wherein the alkyl acrylamide comprises isopropyl acrylamide, isobutyl acrylamide, or dimethyl acrylamide.

30. The method of claim 25 wherein the ionic monomer comprises acrylic acid, methacrylic acid, vinylsulfonic acid, maleic acid, fumaric acid, itaconic acid, mesaconic acid, citraconic acid, ethylene glycol methacrylic phosphate, vinylsulfonic acid, or vinylphosphonic acid.

31. The method of claim 25 wherein the one or more monomers further comprises a hydrophilic monomer.

32. The method of claim 31 wherein the hydrophilic monomer comprises unsubstituted acrylamide, methyl methacrylate, methoxy ethylene glycol acrylate, N-vinyl pyrrolidinone, propylene oxide, or ethylene oxide.

33. The method of claim 25 wherein the one or more monomers further comprises a hydroxy-containing monomer.

34. The method of claim 33 wherein the hydroxy-containing monomer comprises a hydroxyalkyl acrylate, a hydroxyalkyl methacrylate, a hydroxyalkyl acrylamide, a poly(ethylene glycol) acrylate, or a vinyl alcohol.

35. The method of claim 34 wherein the hydroxyalkyl acrylate comprises hydroxyethyl acrylate, hydroxybutyl acrylate, hydroxypropyl acrylate, hydroxyethyl methacrylate, hydroxymethyl acrylate, or hydroxymethyl methacrylate.

36. The method of claim 25 wherein the one or more monomers further comprises a hydroxy-containing monomer and a hydrophilic monomer.
37. The method of claim 25 wherein b) comprises adding an initiator to initiate the polymerization reaction.

38. The method of claim 37 wherein the initiator comprises a peroxide, a persulfate, an azo compound, a redox initiator pair, or combinations thereof.

39. The method of claim 37 wherein the initiator is a water soluble initiator.

40. The method of claim 25 wherein c) comprises coupling acrylate or methacrylate groups to the macromonomer intermediate.

41. A method of making a hydrogel comprising:
   a) providing one or more macromonomers having the formula:

   \[
   \begin{align*}
   &R_2^2 \left[ \begin{array}{c}
   A_3 \\
   A_2 \\
   A_1
   \end{array} \right]_n \text{O} \text{O} \text{O} \text{OH} \\
   &\text{O} \text{O} \text{O} \text{X} \text{R}_1^1 \left[ \begin{array}{c}
   A_1 \\
   A_2 \\
   A_3
   \end{array} \right]_m \text{R}_2^2
   \end{align*}
   \]

   wherein X is a fatty acid group, A_1, A_2, and A_3 are each independently a thermo-responsive repeating unit, a hydroxy repeating unit, a hydrophilic repeating unit, or an ionic repeating unit, wherein A_1, A_2, and A_3 may be the same or different from one another, and R^1 and R^2 are vinyl groups, wherein R^1 and R^2 may be the same or different from one another; and
   b) crosslinking the one or more macromonomers to form the hydrogel.

42. The method of claim 41 wherein a) comprises dissolving the one or more macromonomers in a solvent to form a solution.

43. The method of claim 42 wherein the solvent comprises culture medium.
44. The method of claim 41 wherein the one or more macromonomers comprises at least one macromonomer having an ionic repeating unit and at least one macromonomer having a thermo-responsive repeating unit.

45. The method of claim 41 wherein b) comprises heating the one or more macromonomers to physically crosslink the one or more macromonomers by thermal gelation, adding ions to the one or more macromonomers to physically crosslink the one or more macromonomers by ionic gelation, adding an initiator to the one or more macromonomers to chemically crosslink the one or more macromonomers, or combinations thereof.

46. The method of claim 45 wherein the initiator comprises ammonium persulfate and N,N,N',N'-tetramethylethane-1,2-diamine.

47. The method of claim 41 wherein b) comprises heating the one or more macromonomers to a temperature of about 37°C.

48. The method of claim 41 wherein b) comprises adding calcium to the macromonomers.

49. The method of claim 41 wherein b) comprises adding one or more crosslinking agents to the macromonomers.
Figure 1
Figure 2
Figure 3
Figure 5
Figure 6
Figure 7