POLYMER COMPOSITIONS FOR USE IN EMBOLOTHERAPY OR FOR THE PREPARATION OF INJECTABLE THERAPEUTIC DEPOTS

FIELD OF INVENTION

The present invention relates to the use of compositions comprising thermosensitive block co-polymers in embolotherapy, including chemoembolisation.

In some aspects, the invention relates to the use of the disclosed compositions in the preparation of therapeutic depots for use by injection.

BACKGROUND ART

Embolic materials are used extensively in vascular interventional radiology. Such materials are typically introduced into blood vessels by syringe or catheter, to block or reduce blood flow, to cause tissue necrosis by reducing the supply of oxygen, nutrients and other components to tissue, or to prevent loss of blood. These materials are typically delivered in combination with a contrast agent which renders the delivered bolus visible to imaging devices such as CT scanners. Embolic materials have been employed to treat a variety of conditions such as neuro or peripheral vascular diseases, such as aneurysm, arteriovenous malformation and fistula; uterine fibroids, hyper-vascular tumours, to treat benign prostate hyperplasia, to induce weight loss in overweight patients and to prevent trauma haemorrhage.

Some embolic agents are liquid-based materials which polymerise, solidify, precipitate or change phase in the target vessel to form an occlusion. In one approach, a DMSO solution of ethylene vinylalcohol is used. The DMSO dissipates in the bloodstream causing the polymer to precipitate, occluding the vessel. The intravascular delivery of solvents, such as DMSO, is problematic and can cause *i.a.* endothelial damage, vessel spasm and pain (Saeed Kilani M, Izaaryene J, Cohen F, et al. Ethylene vinyl alcohol copolymer (Onyx®) in peripheral interventional radiology: Indications, advantages and limitations. Diagn Interv Imaging. 2015; 96 (4): 319–326. doi: 10.1016/j.diii.2014.11.030). In another approach, a shear-thinning biomaterial is used (WO2023/055959). The viscosity of the embolic agent decreases while injected and goes back to its initial value once in the vessel. As the viscosity remains quite high under shear, the product is not compatible with catheters under 2.4 Fr and requires a 25 N injection force in 2.8 Fr catheter (Albadawi H, Altun I, Hu J, Zhang Z, Panda A, Kim HJ, Khademhosseini A, Oklu R. Nanocomposite Hydrogel with Tantalum Microparticles for Rapid Endovascular Hemostasis. Adv Sci (Weinh). 2020; 8 (1): 2003327). In yet another approach, still in development, a polymer solution containing oppositely charged polyelectrolytes undergoes an *in situ* solidification triggered by a ionic strength decrease (WO2022/140513). Once again, the product requires up to 26 N (14 lb) to be injected in a 2.8 Fr catheter with a 1 mL/min flow rate, depending on the product formulation, leading to much higher forces in smaller catheters (Stewart RJ, Sima M, Karz

J, Jones JP. Material characterization of GPX®: A versatile in situ solidifying embolic platform technology. Front Bioeng. Biotechnol. 2023,; 11: 1095148. doi: 10.3389/fbioe.2023.1095148).

In a further approach, aqueous liquid embolic agents have been developed taking advantage of the properties of temperature-sensitive polymers, which undergo a phase transition at temperatures above their lower critical solution temperature (LCST) from a soluble hydrated state to an insoluble, partially, or totally dehydrated state. Useful polymers have an LCST of below 37°C, and remain in their soluble state outside the body, whilst they rapidly become insoluble upon exposure to body tissues at 37°C.

The rapid phase transition may present a challenge for the use of these polymers in embolotherapy because the narrow-gauge catheters required for these procedures allow the polymer to transition within the catheter, possibly hindering delivery. A further challenge related to the use of narrow-gauge catheters is the viscosity of polymer solutions. High viscosity leads to a high back pressure on the delivery device (typically a syringe) requiring an increased delivery force, which in turn can lead to a loss of fine control over delivery and even bursting of joints in the delivery system. Furthermore, high viscosity also leads to slow delivery in which case the phase transition may occur within the catheter and, without optimized mechanical properties, may cause clogging.

Depending on the embolized territory and the patient specificities, the physician may want to favour either a proximal or a more distal embolisation and select the liquid embolic accordingly. In one approach, a mixture of glue and ethiodized oil is used and prepared in theatre, where the ratio between the 2 products can be adjusted depending on the desired distality as adding more ethiodized oil will delay the polymerization in situ (Comby PO, Guillen K, Chevallier O, Lenfant M, Pellegrinelli J, Falvo N, Midulla M, Loffroy R. Endovascular Use of Cyanoacrylate-Lipiodol Mixture for Peripheral Embolization: Properties, Techniques, Pitfalls, and Applications. J Clin Med. 2021; 10 (19): 4320. doi: 10.3390/jcm10194320). In most cases, the physician will need to select beforehand the embolic liquid formulation and/or concentration without the opportunity to adjust it extemporaneously. In one approach, a DMSO solution of ethylene vinylalcohol can be proposed with different polymer concentrations to prolong the solvent diffusion. However, these approaches do not allow to modulate the mechanical properties of the solidified embolic agent.

Embolization may be carried out using embolisation microspheres that are hydrogels exhibiting viscoelastic properties. In some extent, mechanical properties of the microspheres are correlated to their flow behaviours and thereby their embolizing efficiency. Embosphere® microspheres are more rigid than Hepasphere® microspheres, as supported by their elastic modulus (19.3 ± 4.97 vs. 9.64 ± 2.46 kPa respectively; Hidaka, K.; Nakamura, M.; Osuga, K.; Miyazaki, H.; Wada, S, Elastic characteristics of microspherical embolic agents used for vascular interventional radiology, J Mech Biomed Mater. 2010; 3 ; 497-503. doi: 10.1016/j.jmbbm.2010.05.004). This is correlated to different postembolisation vascular distributions; Hepasphere® allows for a more distal embolisation as compared to

Embosphere® (Bilbao, J.I.; Luis, E. de; Jalón, J.A.G. de; Martino, A. de; Lozano, M.D.; Cuesta, A.M. de la; et al. Comparative study of four different spherical embolic particles in an animal model: a morphologic and histologic evaluation, J Vasc Interv Radiol. 2008; 19(11), 1625-1638. doi: 10.1016/j.jvir.2008.07.014).

NIPAAM-HEMA copolymers have been developed as embolic agents; these polymers are hydrogels above their LCST but have low gel strength. To increase the gel strength, a high concentration of polymer is used, which in turn often causes catheter blockage due to low LCST and low elasticity. In order to overcome deliverability issues, the polymers are used at a low concentration, however this leads to weak and soft gels, which break up when used in large blood vessels under high shear, may "creep" or move distally and tend to embolise distal to their point of delivery. Moreover, gel fragmentation can lead to off-target embolisations, which are dangerous to the patient. Furthermore, some of these responsive polymers tend to exhibit significant contraction of the polymer after phase change with the concomitant loss of water from the gel and shrinkage which can contribute to mobility of the embolus.

It would be desirable to develop liquid embolics that can be formulated easily at low temperatures, around 20°C for example. It would be desirable that such liquid embolics exhibit good gel strength *in situ*, and can be delivered through a broad range of microcatheters, including small inner diameter microcatheters, for example 1.7 Fr. It would be desirable to have a delivery pressure that still enables a fine control of the embolic agent positioning and of the delivered volume. It would be further desirable that such liquid embolics rapidly undergo a transition when in contact with blood or other tissues to form a robust gel without fragmentation or shrinkage. Furthermore, it would be desirable for the physician to be able to adjust easily and on the spot the mechanical properties of the embolic agent, for instance by simple dilution or mixing, to fit with the patient pathology. It would also be desirable to be able to visualise such polymers readily in the body by a variety of means and to use the gel form for drug delivery if required. While visualisation of such polymer in the body per procedure is desirable, the long-term visualisation could be unbeneficial as it may hinder the visualisation of other body structure. In such case, a decrease in visualisation ability over time could be favourable. Liquid embolics can be associated with therapeutic products, allowing drug deposition *in vivo* as the composition undergo a rapid transition upon delivery. The liquid-to-solid transition triggers the retention of the therapeutic product in the gel; this latter allowing a sustain drug release over time. It would be desirable to have liquid embolics showing reliable and/or reproducible manufacturing properties.

The polymers, compositions and other aspects of the invention described herein address one of more of the issues identified above.

DESCRIPTION

In a first aspect, the disclosure provides a composition for use in embolotherapy, and/or in the preparation of therapeutic depots for use by injection, comprising: a) a tri-arm co-polymer of

hydroxyethyl methacrylate (HEMA), hydroxypropyl methacrylate (HPMA) and N-isopropyl acrylamide (NIPAAM); and b) a contrast agent.

In another aspect, the disclosure provides a composition for use in embolotherapy, including chemoembolisation; and in the preparation of therapeutic depots for use by injection, comprising: a) a tri-arm co-polymer of hydroxyethyl methacrylate (HEMA), hydroxypropyl methacrylate (HPMA) and N-isopropyl acrylamide (NIPAAM); and b) a contrast agent.

In some embodiments, each arm of the tri-arm copolymer comprises HEMA, HPMA and NIPAAM.

In some embodiments, each arm of the tri-arm copolymer comprises a first block and a second block; wherein the first block consists of HEMA and HPMA and the second block consists of NIPAAM; and wherein the second block is either an extension of the first block or is grafted to the first block.

In some embodiments, the tri-arm copolymer is of the form:

$$I\text{-}[(HEMA\text{-}HPMA)NIPAAM]_3$$

wherein I is the residue of a trivalent initiator and the NIPAAM is in the form of a block or is grafted onto the (HEMA-HPMA) block.

In some embodiments, the tri-arm copolymer comprises HEMA, HPMA and NIPAAM, where the number of HEMA, HPMA and NIPAAM monomer units per arm are 75-105, 0.5-20 and 50-1500 respectively.

In some embodiments, the tri-arm copolymer comprises HEMA, HPMA and NIPAAM monomer units, wherein the second block is an extension of the first block and wherein the number of HEMA, HPMA and NIPAAM monomer units are 75-105, 0.5-20 and 100-250 respectively.

In some embodiments, the second block is an extension of the first block and the molar ratio of first block monomers to second block monomers per arm is 75.5-125 to 100-250.

In some embodiments, the second block is grafted to the first block and the molar ratio of first block monomers to second block monomers per arm is 75.5-125 to 50-1500.

In some embodiments, the second block is one or more grafts grafted onto the first block and wherein the number of HEMA, HPMA and NIPAAM monomer units are 75-105, 0.5-20 and 50-1500 respectively.

In some embodiments, a composition may have a contrast enhancement ability in at least one medical imaging modality configured to allow detection of the composition; said medical imaging modality selected from X-ray, Computed Tomography (CT), Photon-counting CT, Magnetic Resonance Imaging, Optical imaging and Nuclear medicine.

In some embodiments, the contrast agent is selected from the group consisting of radiopaque, paramagnetic, superparamagnetic, fluorescent and radiopharmaceutical contrast agents, or a combination of any two or more thereof.

Thanks to these characteristics, contrast agents of this disclosure provide an increase in contrast in at least one medical imaging modality wherein the increase in contrast is sufficient to allow detection of the composition; medical imaging modalities include, for example, X-ray, Computed Tomography (CT), Photon-counting CT, Magnetic Resonance Imaging, Optical Imaging and Nuclear Medicine. Such contrast agents include radiopaque, paramagnetic, superparamagnetic, fluorescent and radiopharmaceutical contrast agents or a combination of any two or more thereof.

Radiopaque contrast agents are contrast agents that are opaque, or partially opaque to X-rays and therefore appear darker on X-ray based imaging systems than the surrounding tissue. Paramagnetic and superparamagnetic contrast agents alter the longitudinal (T1) or transversal (T2*) relaxation times of protons inside tissues as well as magnetic susceptibility and thereby increase the contrast of MRI images. Fluorescent contrast agents are optical contrast agents. Typically, they are caused to fluoresce in the presence of an enzyme or other feature that is diagnostic of a particular type of tissue and are often used to guide surgery. Radiopharmaceutical contrast agent contains radioactive isotopes that emit gamma rays or positrons which can be detected by gamma cameras in technique like Single Photon Emission Computed Tomography or by Positron Emission Tomography scanners.

In some embodiment, the radiopaque contrast agent is selected from iodinated contrast agents, optionally wherein the iodinated contrast agent is selected from an iodinated oil and a water-soluble iodinated contrast agent; tantalum, barium, bismuth and tungsten or compounds thereof; wherein the paramagnetic and superparamagnetic contrast agents are each selected from gadolinium and manganese compounds and iron oxide particles; wherein fluorescent contrast agents are selected from indocyanine green, patented blue, methylene blue, fluoresceine, isosulfan blue and lymphazurin; and wherein radiopharmaceutical contrast agents are selected from technetium-99, fluorine-18, iodine-131, indium-111, gallium-68, cupper-62, cupper-64, zirconium-89, rubidium-82, scandium-43, scandium-44, samarium-117; or a combination of any two or more of the foregoing.

In some embodiment, said contrast in at least one medical imaging modality is configured to allow detection of the composition during procedure, and wherein the composition is configured so that the contrast disappear in the body over time.

In some embodiment, embolic compositions including a contrast agent generate an increase in contrast allowing the detection of the compositions.

In some embodiment, the increase in contrast is sufficient to guide the procedure and could decrease over time to not hinder or compromise ulterior medical imaging testing.

In some embodiment, the composition is a monophasic composition or a biphasic composition.

In some embodiment, the composition may comprise between 0.1-50 %w/w of the tri-arm copolymer.

In some embodiment, the composition may be selected from an aqueous solution, and an emulsion.

In some embodiment, the composition may have a total iodine content greater than 10 mg/ml.

In some embodiment, the composition may have a total iodine content greater than 0.4% w/w.

In some embodiment, the composition may have a radiopacity greater than 100 HU.

In some embodiment, the radiopaque contrast agent may be an iodinated oil contrast agent.

In some embodiment, the iodinated oil contrast agent may be an ethiodized oil.

In some embodiment, the composition may have a viscosity of less than 500 Cp at 20°C.

In some embodiment, the composition may be an emulsion having a comparative stability of at least 1.1.

In some embodiments, the composition may be deliverable through a microcatheter of 1.7 Fr at 20 $^{\circ}$C with an injection force of less than or equal to 45 N, or less than or equal to 30 N at 20 $^{\circ}$C.

The composition may also be deliverable through catheters of greater than 1.7 Fr with an injection force of less than or equal to 45 N, or less than or equal to 30 N.

The compositions of the present disclosure are used in embolotherapy i.e. they are delivered to the lumen of a blood vessel in order to occlude the vessel, for the purposes of providing a therapeutic effect. In some embodiments, the composition comprises one or more therapeutic products and may be used in chemoembolisation and, in some embodiments, the composition comprises one or more therapeutic products and may be used as a depot, for delivery by injection. Such compositions may be described herein as (chemo)embolic agents, (chemo)embolic compositions or in the case of the latter, as therapeutic depots.

Therapeutic depots comprise the polymer compositions described herein and a therapeutic product. They are delivered to tissue by local injection and act as a reservoir of the product, which is released into the tissues over a period of time.

In a further aspect, the disclosure provides a composition as described herein for use in a method of embolotherapy, including chemoembolotherapy and/or for use as in the preparation of therapeutic depot for use by injection.

In a further aspect, the disclosure provides a method of embolising a blood vessel of a subject in need thereof, comprising delivering to a lumen of the blood vessel, an embolic composition as disclosed

herein. Such methods provide therapeutic effects that provide treatments for a variety of conditions as described further herein.

In a further aspect, the disclosure provides a process for preparing an embolic composition or therapeutic depot for use by injection, comprising combining a tri-arm co-polymer as described herein; optionally in the form of an aqueous composition, with a contrast agent. In the case of a therapeutic depot, or chemoembolic compositions, the compositions comprise one or more therapeutic products as described further herein.

In a further aspect, the disclosure provides a process for preparing a therapeutic depot for use by injection comprising combining a tri-arm co-polymer as described herein, optionally in the form of an aqueous composition, with a contrast agent and a therapeutic product.

In a further aspect, the disclosure provides a kit for the preparation of a composition as disclosed herein comprising a tri-arm copolymer as described herein, optionally in the form of an aqueous composition, and a contrast agent. In some embodiments, a kit for the preparation of an embolic composition comprises a first sealed container comprising a sterile tri-arm copolymer as described herein, optionally in the form of an aqueous composition; and a second sealed container comprising a sterile contrast agent; wherein, when combined the sterile tri-arm copolymer and the contrast agent provides a unit dose of an embolic composition. In a further aspect, the disclosure provides a kit for the preparation of a therapeutic product depot for use by injection, comprising a first sealed container comprising a sterile tri-arm copolymer as described herein and a second sealed container comprising a sterile contrast agent; wherein, when combined the sterile tri-arm copolymer and the contrast agent provide a unit dose of therapeutic product depot.

In a further aspect, the disclosure provides a kit according to the invention, wherein the contrast agent is an iodinated contrast agent.

In a further aspect, the disclosure provides a kit according to the invention, wherein the tri-arm copolymer is in the form of an aqueous composition.

The above kits may further include one or more therapeutic products.

DETAILED DESCRIPTION

Polymers for use in embolotherapy, as described in this disclosure, are tri-arm copolymers of HEMA, HPMA and NIPAAM. The polymers are thermosensitive, by which is meant that they undergo a phase transition at temperatures above their lower critical solution temperature (LCST) from a soluble hydrated state to an insoluble, less hydrated state. The LCST of these polymers is at or below 37°C, but above normal room temperatures (e.g. 20°C), so that they remain in solution outside the body, but rapidly become insoluble upon exposure to body tissues at 37°C.

In some embodiments, each arm of the tri-arm copolymer comprises HEMA, HPMA and NIPAAM, and in some embodiments, each arm of the tri-arm copolymer comprises a first block and a second block; wherein the first block consists of HEMA and HPMA and the second block consists of NIPAAM; wherein the second block is either an extension of the first block or is grafted to the first block. In some embodiments, the arms of the polymer are linked through the residue of a trivalent initiator, which is described further below.

Hydroxypropyl methacrylate (HPMA) as referred to herein, may be 3-hydroxypropyl methacrylate, 2-hydroxyisopropyl methacrylate, 1-hydroxy-2-propyl methacrylate, or 2-hydroxypropyl methacrylate, or may be a mixture of any two or more of these isomers.

The HEMA-HPMA block may be present as a block copolymer having a HEMA block and an HPMA block or it may be present as a statistical copolymer of HEMA and HPMA represented as *-st-* or *-co-*. Where the HEMA-HPMA block is present as a block copolymer, the blocks may be reversed as an (HPMA-HEMA) block.

NIPAAM grafts may be pendant from the HEMA monomers, the HPMA monomers or, preferably, both. Thus the (HEMA-HPMA) block may be a (HEMA-*b*-HPMA) block, (HPMA-*b*-HEMA) block or (HEMA-*co*-HPMA) block and the tri-arm copolymer may be (HEMA-HPMA)-*b*-NIPPAM or (HEMA-HPMA)-*g*-NIPAAM.

Tri-arm polymers described herein may be represented by the formula I:

$$I\text{-}[(HEMA\text{-}HPMA)NIPAAM]_3$$

FORMULA I

wherein I is the residue of a trivalent initiator and NIPAAM is in the form of a block extending from the (HEMA-HPMA) (or first) block, or is grafted onto the (HEMA-HPMA) block.

In one embodiment the tri-arm polymer may be of the formula II:

$$I\text{-}[(HEMA_X\text{-}HPMA_Y)NIPAAM_Z]_3$$

FORMULA II

Optionally wherein:
X is 75-105
Y is 0.5 to 20
Z is 50-1500 ;
I is the residue of a trivalent initiator; and NIPAAM is in the form of a block extending from the (HEMA-HPMA) block, or as one or more blocks present as one or more grafts on the (HEMA-HPMA) block.

Thus a tri-arm polymer may comprise between 75-105 monomers of HEMA; 0.5 to 20 monomers of HPMA and 50-1500 monomers of NIPAAM, per arm.

In one embodiment, a tri-arm polymer may comprise NIPAAM present as an extension of the (HEMA-HPMA) block. The polymer may be represented by the formula III:

$$I\text{-}[(HEMA_X\text{-}HPMA_Y)\text{-}b\text{-}NIPAAM_a]_3$$

FORMULA III

Wherein:
X is 75-105
Y is 0.5 to 20; and
a is 100 to 250.

Thus, where NIPAAM is present as an extension block, a tri-arm polymer may comprise between 75-105 monomers of HEMA; 0.5 to 20 monomers of HPMA and 100-250 monomers of NIPAAM, per arm.

In one embodiment, a tri-arm polymer may comprise NIPAAM present as one or more grafts on the (HEMA-HPMA) block, the polymer may be represented by the formula III:

$$I\text{-}[(HEMA_X\text{-}HPMA_Y)\text{-}g\text{-}(NIPAAM_b)_c]_3$$

FORMULA III

Wherein:
X is 75-105
Y is 0.5 to 20;
b is 10 to 30;
and
c is 5 to 50.

Thus, where NIPAAM is present as one or more grafts on the (HEMA-HPMA) block, a tri-arm polymer may comprise between 75-105 monomers of HEMA; 0.5 to 20 monomers of HPMA and 50-150 monomers of NIPAAM, per arm, wherein the NIPAAM may be present as 5 to 50 blocks of 10 to 30 monomers.

Polymers in which the NIPAAM is present as one or more grafts on the (HEMA-HPMA) block are preferred.

A further aspect of the invention provides a composition for use in embolotherapy, including chemoembolisation; and in the preparation of therapeutic depots for use by injection, comprising: a) a tri-arm co-polymer of hydroxyethyl methacrylate (HEMA), hydroxypropyl methacrylate (HPMA) and N-isopropyl acrylamide (NIPAAM); and b) a contrast agent; wherein the tri-arm co-polymer is prepared by a process comprising polymerizing HEMA monomer and HPMA monomer to provide First Blocks which are HEMA-HPMA blocks wherein polymerization is initiated with a polymerization initiator, I, having 3 sites of polymerization initiation; and reacting NIPAAM monomers with First Blocks to provide a block co-polymer comprising First HEMA-HPMA blocks and Second NIPAAM blocks. In some embodiments NIPAAM blocks are provided as grafts to the First Block. In some embodiments NIPAAM blocks are provided as extensions of the First block.

In some embodiments the process comprises polymerising a combination of HEMA and HPMA monomers to form a first block comprising HEMA and HPMA monomers, for example of the formula 4, the proportion of HEMA monomers and HPMA monomers in the tri-arm polymer being dependent on *i.a.* the reaction conditions and quantities of reactants chosen.

$$I\text{-}(HEMA\text{-}HPMA)_3$$

FORMULA 4

and either

(a) extending an NIPAAM block from the First Block to form a polymer of the general formula 5

$$I\text{-}[(HEMA\text{-}HPMA)\text{-}NIPAAM]_3$$

FORMULA 5

or;

(b) extending at least one NIPAAM graft from the First Block to form a co-polymer comprising at least one graft of NIPAAM according to Formula 6;

$$I\text{-}[(HEMA\text{-}HPMA)]_3$$
$$\quad\quad\quad

NIPAAM grafts are distributed between both HEMA and HPMA monomers, the proportion of grafted HEMA monomers and grafted HPMA monomers and the number of NIPAAM monomers being dependent on *i.a.* the reaction conditions and quantities of reactants chosen.

Polymerisation may be carried out by a variety of approaches including RAFT, ATRP or conventional free radical polymerisation. In one embodiment the polymerisation is carried out by ATRP. In some embodiments the First Block that reacts with NIPAAM monomer is in the form of a macro-initiator.

In some embodiments the ATRP the process comprises the steps of:

(i) Polymerising a combination of HEMA and HPMA monomers in the presence of an initiator configured to support ATRP polymerisation from 3 functional groups to provide a star polymer comprising the residue of the initiator covalently bound to 3 (HEMA-HPMA) groups to provide a halogenated macro initiator comprising HEMA-HPMA blocks which comprise the residue of the initiator covalently bound to copies of either a HEMA polymer or a HEMA-HPMA copolymer, each copy comprising a terminal monomer comprising a Hal group, wherein Hal is Halogen selected from Cl and Br, preferably Br ;

(ii) reacting the halogenated macro initiator with NIPAAM monomer in a further ATRP reaction to provide a co-polymer comprising a First Block comprising HEMA and HPMA and Second Block consisting of NIPAAM monomer, wherein Second Block is an extension of the First block;

In some other embodiments the ATRP the process comprises the steps of:

(i) Polymerising a combination of HEMA and HPMA monomer in the presence of an initiator configured to support ATRP polymerisation from 3 functional groups to provide a star polymer comprising the residue of the initiator covalently bound to 3 (HEMA-HPMA) groups;

(ii) reacting the star polymer with an activated halogen compound, such as α-haloisobutyryl bromide (optionally in the presence of a base) to provide a halogenated macroinitiator in which halogen is pendant from both HEMA monomers and HPMA monomers;

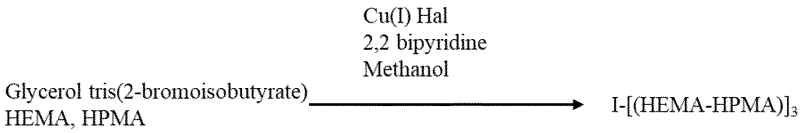
(iii) reacting the halogenated macro initiator with NIPAAM monomer in a further ATRP reaction to provide a co-polymer comprising a First Block comprising HEMA and HPMA and Second Block consisting of NIPAAM monomer, wherein second block is pendant from both HEMA monomers and HPMA monomers.

Typically the ATRP reaction takes place in the presence of a transition metal halide, typically copper(I) halide (e.g. copper (I) chloride or copper (I) bromide). The catalyst can be added to the reaction mixture

as a solid (eg as a powder) or may be solubilised before addition. The halogen will typically be the same as that which is present as a functional group on the initiator and the same as that present on the macro initiator. This may be chlorine or bromine but is typically bromine. The reaction typically takes place in the presence of a nitrogen-containing ligand, which binds the transition metal. Typically the reaction takes place in solution from which oxygen has been excluded.

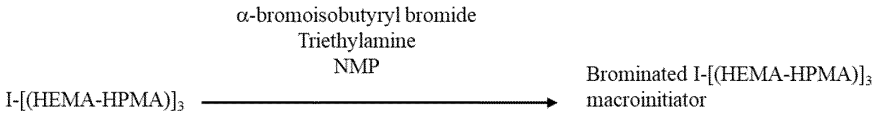
In some embodiments a process for the preparation of a comb-like tri-arm I-[(HEMA-HPMA)-graft-(NIPAAM)]$_3$ polymer comprises:

(i) Polymerising a combination of HEMA and HPMA monomers in the presence of a trifunctional initiator such as glycerol tris(2-bromoisobutyrate), Cu(I)halide and a nitrogen containing ligand such as 2,2'-bipyridine to provide a star I-[(HEMA-HPMA)]$_3$ polymer, (Scheme 1).



Scheme 1

(ii) reacting the star polymer with an activated alkyl bromine compound, such as α-bromoisobutyryl bromide in the presence of a base, such as triethylamine, to provide a brominated I-[(HEMA- HPMA)]$_3$ macroinitiator, (Scheme 2);



Scheme 2

(iii) reacting the brominated I-[(HEMA-HPMA)]$_3$ macroinitiator with NIPAAM monomer in a further ATRP reaction in the presence of a Cu(I)halide (eg Cu(I)Cl or Cu(I)Br) and a nitrogen containing ligand such as N,N,N',N'',N''-pentamethyldiehylenetriamine (PMDETA) in a solvent such as NMP, to provide an I-[(HEMA-HPMA)-graft-(NIPAAM)]$_3$ polymer (scheme 3).



Scheme 3

The process additionally includes the step of combining a polymer prepared according to the process with one or more contrast agents as described elsewhere herein. In one embodiment the polymer and contrast agent are combined in solution phase and the solution is dried to provide a dried composition of the polymer comprising a contrast agent.

In some embodiments HEMA monomer is reacted at an I:HEMA molar ratio of 1:(20 to 600)$_3$, HPMA monomer is reacted at an I:HPMA molar ratio of 1: (0 to 500)$_3$ and N monomer is reacted at an I:NIPAAM ratio of 1:(1 to 800)$_3$.

In some embodiments HEMA monomer may be reacted at an I:HEMA ratio of 1: (20-600)$_3$ preferably 1: (30 to 200)$_3$ more preferably 1:(60 to 120)$_3$ and yet more preferably at 1:(70 to 100)$_3$ mol/mol

In some embodiments HPMA monomer may be reacted at an I:HPMA ratio of 1: (0-500)$_3$, preferably 1:(2 to 100)$_3$, more preferably 1: (3-30)$_3$ mol/mol

In some embodiments NIPAAM monomer may be reacted at a rate of an I:NIPAAM ratio of 1:(1 to 800)$_3$, preferably 1:(3 to 600)$_3$, more preferably 1: (5 to 400)$_3$ mol/mol

In some embodiments the individual monomer species are reacted at a molar ratio of (HEMA+HPMA) to NIPAAM of 1:0.1 to 1:8; preferably 1:2 to 1:5 and particularly 1:2 to 1:4.

In some embodiments, of the First Block monomers reacted, 70% to 99.9% are HEMA monomers; preferably 80-99% or 80-99.9% are HEMA monomers.

HEMA-HPMA blocks may be (HEMA-*b*-HPMA) blocks (HPMA-*b*-HEMA) blocks or (HEMA-*co*-HPMA) blocks. The polymer may be HEMA-HPMA -*b*-NIPAAM or HEMA-HPMA-*g*-NIPAAM.

Polymerization may be carried out by RAFT, ATRP or conventional free radical polymerisation, but is preferably carried out by ATRP.

The person skilled in the art may adapt the process to synthesis block copolymers according to his common knowledge, notably regarding reactants and catalysts.

Bulk polymer compositions may comprise, in addition to a particular target copolymer, one or more additional polymer products such as other polymers described herein, polymers of differing molecular weight and polymers having alternative block patterns. Further, the target polymer considered as a bulk polymer, may have X, Y and Z values that are non-integers. Thus, synthesis may target a value for X, Y or Z within the bulk polymer which may be integers or any fractional value there between. Consequently, the skilled person will be aware that the values given for X, Y and Z in relation to bulk polymers herein may be considered to be ranges including all fractional values between the limits.

Such bulk compositions retain desirable properties, but may comprise, in addition to a particular target copolymer, one or more additional polymer products such as other polymers described herein, polymers

of differing molecular weight, polymers having alternative block patterns and so on. In some embodiments, such bulk compositions may comprise at least 30%, 40%, 50%, 60%, 70%, 80% or 90% target polymer by weight. Such bulk compositions are a further embodiment of the invention.

The formed gel has high storage modulus and can withstand more shear stress from blood flow. Contrary to some earlier polymers, it is not necessary to crosslink the polymers to modulate their properties.

The trivalent initiators of polymerisation (I) can be any suitable initiator known to those in the art particularly the initiator is one suitable for atom transfer radical polymerization (ATRP). Polymers typically retain the residue of the initiator, which links the three arms of the polymer. Herein the residue of the initiator is represented by "I" and the functional group (typically a halide) is lost from the initiator.

Polymer initiators typically comprise either alkyl bromides or alkyl chlorides comprising 3 halides. Example initiators include 1,1,1-tris(2-bromoisobutyryloxymethyl)ethane, glycerol tris(2-bromoisobutyrate), tris(2-bromopropanoic acid)1,2,3-propanetriyl ester, 2,2',2''-Nitrilotri(ethanol 2-methyl-2-bromopropanoate), and 3,4-bis[(2-bromo-2-methylpropanoyl)oxy]butyl 2-bromo-2-methylpropanoate.

Particularly preferred polymers include tri-arm polymers of the formula:

I-[(HEMA$_{75\text{-}105}$-HPMA$_{0.5\text{-}20}$)-*b*-NIPAAM$_{50\text{-}1500}$]$_3$

I-[(HEMA$_{75\text{-}105}$-HPMA$_{0.5\text{-}20}$)-*g*-(NIPAAM$_{10\text{-}30}$)$_{5\text{-}50}$]$_3$;

Particularly preferred polymers also include:

I-[(HEMA$_{75\text{-}105}$-HPMA$_{0.5\text{-}20}$)-*b*-NIPAAM$_{100\text{-}250}$]$_3$

I-[(HEMA$_{75\text{-}105}$-HPMA$_{0.5\text{-}20}$)-*g*-(NIPAAM$_{10\text{-}30}$)$_{5\text{-}50}$]$_3$

I-[(HEMA$_{75\text{-}105}$-HPMA$_{0.5\text{-}20}$)-*g*-(NIPAAM$_{10\text{-}60}$)$_{5\text{-}50}$]$_3$;

Additional particularly preferred polymers include:

I-[(HEMA$_{95\text{-}102}$-HPMA$_{1\text{-}5}$)-*g*-(NIPAAM$_{7\text{-}20}$)$_{7\text{-}30}$]$_3$;

Further preferred polymers include

I-[(HEMA$_{99}$-*st*-HPMA$_1$)-*b*-NIPAAM$_{100}$]$_3$

I-[(HEMA$_{80}$-*st*-HPMA$_{20}$)-*b*-NIPAAM$_{100}$]$_3$

I-[(HEMA$_{80}$-*st*-HPMA$_{20}$)-*g*-(NIPAAM$_{30}$)$_{30}$]$_3$

I-[(HEMA$_{80}$-*st*-HPMA$_{20}$)-*b*-NIPAAM$_{50}$]$_3$

I-[(HEMA$_{80}$-*st*-HPMA$_{20}$)-*g*-(NIPAAM$_{30}$)$_{10}$]$_3$

I-[(HEMA$_{99}$-*st*-HPMA$_1$)-*g*-(NIPAAM$_{30}$)$_{10}$]$_3$

I-[(HEMA$_{99}$-*st*-HPMA$_1$)-*g*-(NIPAAM$_{30}$)$_{30}$]$_3$
I-[(HEMA$_{99}$-*st*-HPMA$_1$)-*b*-NIPAAM$_{50}$]$_3$
I-[(HEMA$_{99}$-*st*-HPMA$_1$)-*b*-NIPAAM$_{200}$]$_3$
I-[(HEMA$_{80}$-*st*-HPMA$_{20}$)-*b*-NIPAAM$_{200}$]$_3$
I-[(HEMA$_{99}$-*st*-HPMA$_1$)-*b*-NIPAAM$_{250}$]$_3$
I-[(HEMA$_{80}$-*st*-HPMA$_{20}$)-*b*-NIPAAM$_{250}$]$_3$
I-[(HEMA$_{80}$-*st*-HPMA$_{20}$)-*g*-(NIPAAM$_{30}$)$_{50}$]$_3$
I-[(HEMA$_{99}$-*st*-HPMA$_1$)-*g*-(NIPAAM$_{30}$)$_{50}$]$_3$

Yet further preferred polymers include
I-[(HEMA$_{99}$-*st*-HPMA$_1$)-*g*-(NIPAAM$_8$)$_{25}$]$_3$) MT17
I-[(HEMA$_{99}$-*st*-HPMA$_3$)-*g*-(NIPAAM$_{16}$)$_9$]$_3$ MT20
I-[(HEMA$_{99}$-*st*-HPMA$_3$)-*g*-(NIPAAM$_{13}$)$_9$]$_3$ MT21/2
I-[(HEMA$_{99}$-st-HPMA$_1$)-g-(NIPAAM$_{20}$)$_9$]$_3$ MT21/3

The compositions disclosed herein are typically aqueous compositions, however dried compositions are also contemplated. An aqueous composition comprises water. In some embodiments, the tri-arm polymer is dissolved in or suspended in water or any water based media including saline or buffer (either as a solution or as molecular aggregates). Aqueous compositions may be at least 5% w/w polymer, more preferably at least 7%w/w, at least 10% w/w, at least 15%, at least 20% or higher. The composition may be up to 30%, or up to 50% w/w polymer. At temperatures below the LCST, for example at 25°C, the polymer may be dissolved (or substantially dissolved) in the aqueous composition. The polymer may be in the form of a molecular aggregates such as micelles or other self-assembled systems in the aqueous composition.

Contrast agents provide an increase in contrast in at least one medical imaging modality, for example in X-ray, Computed Tomography (CT), Photon-counting CT, Magnetic Resonance Imaging, Optical imaging and radioisotope imaging in nuclear medicine. Such contrast agents may be radiopaque, paramagnetic, superparamagnetic, fluorescent and radiopharmaceutical contrast agents, or a combination of any two or more thereof.

Radiopaque contrast agents include iodinated contrast agents such as iodinated oils and water-soluble iodinated contrast agents, but they also include metallic particles such as tantalum or tungsten particles, or radiopaque compounds of barium or bismuth, such as barium sulphate of bismuth subsalicylate.

Paramagnetic or superparamagnetic contrast agents include but not limited to iron oxide particles and gadolinium or manganese-based compounds. Contrast agents may also include combinations of any two or more of the above.

Fluorescent contrast agents include but are not limited to indocyanine green, patented blue, methylene blue, fluoresceine, isosulfan blue and lymphazurin, or combination thereof.

Radioisotopes may also be used as imaging agents including, but not limited to, technetium-99, fluorine-18, iodine-131, indium-111, copper-62 or copper 64, Zirconium 89 rubidium-82, scandium-43, scandium-44, samarium-117and galium-67 or combinations thereof.

In some embodiments, an iodinated contrast agent is used and the ratio of contrast agent to tri-arm copolymer or bulk polymer composition is between 0.05 to 10 on a dry weight for dry weight basis, preferably 0,5 to 10, more preferably 0.5 to 5.

In one aspect, the disclosure provides an aqueous composition for use in embolotherapy and/or in the preparation of therapeutic depots for use by injection comprising: a) a tri-arm copolymer as described herein; and b) an aqueous iodinated contrast agent.

In another aspect, the disclosure provides an aqueous composition for use in embolotherapy, including chemoembolotherapy and/or in the preparation of therapeutic depot for use by injection comprising: a) a tri-arm copolymer as described herein; and b) an aqueous iodinated contrast agent.

Aqueous (or water-soluble) contrast agents are typically polyiodinated compounds which may also be polyhydroxylated. Aqueous contrast agents include ionic contrast agents and non-ionic contrast agents. Non-ionic contrast agents are preferred. Aqueous contrast agents include iohexol, iodixanol, iopamidol, ioxilan, iopromide, iodixanol, iobitridol, ioversol, diatrizoate, metrizoic acid, iotalamic acid, ioxaglate, diatrizoate, metrizoic acid, iotalamic acid and ioxaglate.

The ratio of contrast agent to tri-arm copolymer or bulk polymer composition is between 0.05 to 10 on a dry weight for dry weight basis, preferably 0,5 to 10, more preferably 0.5 to 5.

A further aspect of the disclosure provides a biphasic composition for use in embolotherapy or chemoembolotherapy comprising: a) a tri-arm copolymer as described herein; and b) an iodinated oil, optionally wherein the composition is in the form of an emulsion. The emulsion optionally has a comparative stability of at least 1.1 when compared to an identical composition lacking the polymer.

In one embodiment, the contrast agent is an iodinated oil, such as an ethiodized oil, e.g. ethiodized poppy seed oil. In some embodiments, the composition is in the form of an emulsion, comprising an oil phase and an aqueous phase, in which the aqueous phase comprises a tri-arm copolymer or bulk polymer as described herein. In particular, the iodinated oil is an iodized poppy seed oil. Examples of such are available under the trade name Lipiodol® (or Lipiodol® Ultra-Fluid) (Guerbet, Paris, France), Vividol™

(Vivere imaging, Hyderabad, India), Hengrui ethiodized poppy seed oil injection, Fattiodol (Dongkook pharmaceutical) and Iodinated oil injection (Luyin pharmaceutical).

The ratio of oil to aqueous phase may be chosen to allow preparation of water-in-oil (W/O) or oil-in-water (O/W) emulsions. Oil-in-water-in-oil, or water-in-oil-in water emulsions may also be prepared. In some embodiments, the ratio of the aqueous polymer phase to oil is 100:1 to 1:100, preferably 10:1 to 1:10, more preferably 1:4 to 4:1.

In some embodiments of the emulsion, the polymer concentration in the aqueous solution may be 1%-20%, preferably 3%-15%, more preferably 5%-10%.

The emulsion may be prepared by conventional techniques, known to those in the art. Briefly, separate syringes containing the aqueous phase and the oil phase are connected to a 2, 3 or 4-way connector, (such as a stop cock) and the contents are briskly shuttled back and forth between two syringes to prepare the emulsion, to provide an embolic composition used in the treatment of (*inter alia*) hypervascular tumours such as hepatocellular carcinoma. The emulsion may be used without further formulation, but may comprise one or more pharmaceutical actives, particularly anti-cancer agents as described elsewhere herein.

The use of emulsions comprising iodinated oils is known and the technique has been used in the embolisation and chemoembolisation of tumours for some time. However, their correct extemporaneous preparation requires experience and skill. Poorly prepared emulsions may only be stable for a short period, before beginning to separate into an oil phase and an aqueous phase. This means that the emulsion cannot be prepared in advance and must be prepared in theatre immediately before use. Furthermore, the lack of longer-term stability of the emulsion contributes to its rapid phase separation *in situ* so that the embolisation effect may be short lived. In chemoembolisation, phase separation of the emulsion can also lead to burst release of the active ingredient into the blood stream, which increases off-target exposure to the active. The inventors have identified that oily compositions in which the aqueous phase comprises tri arm copolymers as described herein have much improved stability compared to the same composition lacking the polymer or composition.

The emulsion formulation may be tuned to have a range of viscosities and thermal response properties by altering the ratio of aqueous solution to ethiodized oil. Thus, in some embodiments, the formulation forms an oily fluid and in others a hydrogel, above the polymer LCST. In some embodiments, the formulation is formed as a string or as a globular deposit. The formulation may be used to direct the formulation towards a more distal or more proximal embolisation.

The iodinated oil or emulsion formulation may comprise one or more surfactants or emulsion stabilisers. Examples of such additives may include glucose, lactose, mannitol, ribitol, threitol, erythritol, sorbitan, volemitol, isomalt, maltitol, lactitol, cholesterol, polysorbate, sorbitan monolaurate, sorbitan

monostearate, sorbitan tristearate, decyl glucoside, lauryl glucoside, octyl glucoside, hydroxypropyl methylcellulose (HPMC), polyvinylpyrrolidone (PVP), polyvinyl alcohol, polyethylene glycol, polyethylene oxide, polyethylene oxide-co-polypropyleneoxide-co-polyethylene oxide (PEO-PPO-PEO), ethylene-vinyl alcohol (EVOH), polyacrylate, polymethacrylate, polyacrylamide, polymethacrylamide, acrylate polymer, polyamide, polysiloxane, polyester, polyurethane, polyvinyl ether, polyvinyl ester, polyglycerol methacrylate, poly(2-acrylamido-2-methylpropane sulfonic acid) or sodium salt, poly(2-acrylamido-2-methylpropane sulfonic acid) and it salts (eg sodium salt), poly(2-methacryloyloxyethyl phosphorylcholine), polyacrylic acid or its salts (such as sodium salt), polymethacrylic acid or its salts (such as sodium salt), microcrystalline cellulose, carboxymethyl starch sodium, croscarmellose sodium, magnesium stearate, , poloxamer, sodium lauryl sulfate, hypromellose acetate succinate, alginate, collagen, fibrin, chitosan, gelatin, hyaluronic acid, cyclodextrin, Laponite, sodium dodecyl sulfate, sodium lauryl sulfate, ammonium lauryl sulfate, docusate, and Triton X-100 amongst others.

The emulsion formulations may comprise compounding agents and/or additional components as described elsewhere herein including at least one chemotherapeutic as described elsewhere herein.

Stability of the emulsion may be measured by Static Multiple Light Scattering (SMLS) using a Turbiscan Tower system (Microtak USA). Back-scattering profiles are used to calculate the Turbiscan Stability Index (TSI) which is a measure of emulsion stability. For comparison purpose, a stability ratio may be calculated corresponding to control emulsion TSI divided by the test composition TSI. The presence of the polymers increases comparative stability by at least 0.1, or at least 0.2 or at least 0.3, so that comparative stability of the presently described emulsions is at least 1.1, or at least 1.2 or at least 1.3.

Without wishing to be bound by any theory, it is believed that at ambient temperature, the structured hydrophobic-hydrophilic copolymer chains rearrange at the interface of aqueous-oil emulsions and stabilise the formulation by reducing the surface energy between oil and water. The structure could allow the loading of different therapeutic agents, based on their hydrophobic or hydrophilic properties. At temperatures above the LCST (for example at body temperature – 37°C), the thermally responsive block is converted to a relatively more hydrophobic form and the original micellar or lamellar structure collapses. The increased hydrophobicity leads to the formation of a more rigid porous gel which functions as an embolisation barrier. Meanwhile, the oil contained inside the porous structure provides radiopacity which lasts considerably longer than that seen in traditional emulsions, or may be released as a delivery carrier for hydrophobic drugs, depending on the ratio of aqueous phase to oil and the polymer concentration in the aqueous phase.

The polymer compositions disclosed herein make use of contrast agents to render them visible during imaging procedures. When using X-ray based techniques, the radiopacity of the composition increases

the contrast between the composition and the rest of the local image. In some embodiments therefore, the radiopacity of compositions is at least 100 Hounsfield units or at least 500 Hounsfield units (HU), in some embodiments at least 1000 HU and, in further embodiments, at least 2000 HU, 3000 HU or more.

Where an iodinated contrast agent is used, then the iodine content of the aqueous composition of biphasic composition is typically greater than 10 mg/ml or 50 mg/ml iodine and may be greater than 100 mg or 200 mg/ml iodine or more.

In a further aspect, the disclosure provides a dry embolic composition or dry therapeutic depot for use by injection, comprising (i) a tri-arm copolymer or bulk polymer as described herein; and (ii) a water-soluble contrast agent; wherein both the tri-arm copolymer and the water-soluble contrast agent are provided dry. Such dry compositions are intended to be re-constituted to provide the aqueous compositions described herein. Therapeutic products may be incorporated into the dried product where necessary or be incorporated later, after reconstitution. Consequently, the proportions of polymer and contrast agent suitable for aqueous compositions also apply to dry compositions.

Dry compositions comprise less than 0.1% water or other solvents w/w, preferably less than 0.01% water or other solvents w/w. This can be achieved by oven drying, spray-drying or freeze-drying for example, or by combining dried polymer and dried contrast agent.

In a further aspect, the disclosure also provides an embolic composition comprising (i) a tri-arm copolymer or bulk polymer as described herein; and (ii) an iodinated oil such as an iodized poppy seed oil (eg Lipiodol®); wherein the polymer is in dried form. Such compositions may comprise at least 5%, at least 10%, at least 15% or at least 20% w/w of polymer or bulk polymer. This can be achieved by combining the oil with dry polymer powder preferably having a size range between 1 μm and 500 μm preferably between 40 μm and 300 μm as judged by sieving to the appropriate size or range.

During the delivery of the composition into capillary vessels, for example, an embolisation initially occurs as a function of the polymer particle size, and the polymer would subsequently absorb water from blood and/or tissues as the oil liquid phase continues to percolate through the capillary bed thus forming an embolisation at 37°C.

The dry compositions may be compounded with anti-caking agents or dissolution enhancing agents such as glycerol, ethanol, mannitol, glucose, sorbitol, xylitol, trehalose, arabitol, galactitol, fucitol, iditol, inositol, lactose, fructose, sucrose, ribitol, threitol, erythritol, sorbitan, volemitol, isomalt, maltitol, lactitol, citric acid, succinic acid, urea, cholic acid, cholesterol, polysorbate, sorbitan monolaurate, sorbitan monostearate, sorbitan tristearate, decyl glucoside, lauryl glucoside, octyl glucoside, polyvinylpyrrolidone, low molecular weight polyethylene glycol, poloxamer, polyvinyl alcohol, poly(2-acrylamido-2-methylpropane sulfonic acid) or sodium salt, alginate, sodium dodecyl sulfate, sodium lauryl sulfate, ammonium lauryl sulfate, docusate, and triton X-100.

Dry compositions may also comprise a variety of additional components including pharmaceutically acceptable excipients such as small molecules or polymers, including ethanol, glycerol, DMSO, N-methylpyrrolidone, dimethylformamide, diethyl formamide, glucose, lactose, mannitol, hydroxypropylmethylcellulose (HPMC), polyvinylpyrrolidone (PVP), poly(2-Acrylamido-2-methylpropane sulfonic acid) and it salts (eg sodium salt), polyacrylic acid or its salts (such as sodium salt), polymethacrylic acid or its or its salts (such as sodium salt), microcrystalline cellulose, carboxymethyl starch sodium, croscarmellose sodium, magnesium stearate, polysorbate, poloxamer, sodium lauryl sulfate, hypromellose acetate succinate, alginate, collagen, fibrin, chitosan, gelatin, hyaluronic acid, and cyclodextrin amongst others.

In a further aspect, the disclosure provides a method for preparing an aqueous embolic composition comprising (i) providing a dried composition comprising a tri-arm copolymer or bulk polymer as described herein and a water-soluble contrast agent; and (ii) re-hydrating the polymer in a sterile aqueous medium at a temperature below the LCST of the polymer.

In a further aspect, the disclosure provides a method of preparing an aqueous embolic composition and/or therapeutic depots for use by injection, comprising a tri arm copolymer as described herein, and a contrast agent; the method comprising (i) providing a dried composition comprising the tri-arm copolymer but not the contrast agent; (ii) re-hydrating the polymer in a sterile aqueous medium at a temperature below the LCST of the polymer; and (iii) combining the rehydrated polymer with an iodinated contrast agent. The iodinated contrast agent may be an iodinated oil or a water-soluble contrast agent as described elsewhere herein. The water-soluble contrast agent may be in the form of a solution or a dry powder, particularly a solution. Therapeutic products may be incorporated into the composition product where necessary or be incorporated later, after reconstitution.

The polymer may be rehydrated below 25°C or below 10°C for example. Rehydration may include preparing a dispersion of the polymer (for example a dispersion of molecular aggregates), but more preferably comprises dissolving the polymer (or substantially all the polymer).

Where the iodinated contrast agent is an iodinated oil, combining the rehydrated polymer with an iodinated contrast agent may include forming an emulsion as described elsewhere herein.

In some embodiments, the embolic composition herein additionally comprises a therapeutic product for example a cytotoxic drug, an anti-glycolytic drug, an immunomodulatory agent, an anti-inflammatory drug, an anti-angiogenic medication, an immune checkpoint inhibitor, a radioisotope or any combination thereof.

Such agents are selected according to need, but in one approach the compositions may be used in interventional oncology applications, particularly as liquid chemo-embolic agents. In this case, the possible chemotherapeutic includes the anthracycline class such as doxorubicin, daunarubicin, epirubicin and idarubicin; the camptothecin class such as irinotecan, topotecan, and exatecan; the platins

such as cisplatin, oxaliplatin, carboplatin and miriplatin; mitomycin C, nucleoside analogues such as 5-fluorouracil, cytarabine, fludarabine and gemcitabine; multityrosine kinase inhibitors such as sorafenib, sunitinib, regorafenib, brivinb, dasetanib, bosutinib, erlotinib, gefitinib, imatinib and vandetinib; rapamycin; as well as biological actives such as nivolumab (Opdivo), pembrolizumab (Keytruda), ipilimumab, atezolizumab, avelumab, durvalumab, cemiplimab, dostarlimab, relatlimab, spartalizumab, aldesleukin, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon alfa-2a, interferon alfa-2b (Intron A®), Peginterferon alfa-2b (Sylatron®/PEG-Intron®), imiquimod, olaparib, Poly ICLC (Hiltonol®), pexidartinib; or any combination thereof.

Radiopharmaceuticals include but are not limited to Technetium-99, fluorine-18, iodine-131, indium-111, copper-62 or copper 64, copper-67, Zirconium 89 rubidium-82, scandium-43, scandium-44, Scandium-47, samarium-117 yttrium 90, lutecium-177, radium-223, Holnium-166, Actinium-225, Terbium-161, Rhenium-188 and Galium-67 or any combination thereof.

Typically, such copolymers or compositions are in a pharmaceutically acceptable form and may for example be sterile and/or pyrogen free. Depending on need, sterilisation may be achieved by heat, radiation sterilization or aseptic filtration for example, or may be achieved by reconstitution of a sterile dried composition using sterile aqueous solutions.

In a further aspect, the disclosure provides a composition for use in a method of embolotherapy comprising: a) a tri-arm copolymer of hydroxyethyl methacrylate (HEMA), 3-hydroxypropyl methacrylate (HPMA) and N-isopropyl methacrylamide (NIPAAM); and b) a contrast agent.

In a further aspect, the disclosure provides a method for the embolisation of a blood vessel in a patient in need thereof, comprising delivering to the lumen of the blood vessel an embolic composition comprising a tri-arm copolymer and a contrast agent as described herein.

In a further aspect, the disclosure provides a method for the treatment of a hypervascular tumour in a subject in need thereof, comprising delivering to a lumen of a blood vessel supplying the tumour, a composition comprising a) a tri-arm copolymer of HEMA, HPMA and NIPAAM; and b) a contrast agent.

In a further aspect, the disclosure provides a method of delivering a drug to a patient in need thereof, comprising delivering to the patient, by injection, a therapeutic depot as described herein.

In a further aspect, the disclosure provides a process for preparing an embolic composition comprising combining a tri-arm copolymer as described herein, optionally in the form of an aqueous composition, with a contrast agent.

In a further aspect, the disclosure provides a process for preparing a therapeutic depot for use by injection comprising combining a tri-arm copolymer, optionally in the form of an aqueous composition, with a contrast agent and a therapeutic product.

In some embodiments, the composition is an aqueous composition comprising a tri arm copolymer or bulk polymer composition described herein which may be in the form of a solution or of a dispersion of molecular aggregates and allowing the composition to increase in temperature to a point above its LCST such as to increase the viscosity of the composition and thereby to embolise the blood vessel.

In some embodiments, the composition is in the form of an emulsion as described further elsewhere herein or in the form of a suspension of the polymer or polymer composition in an ethiodized oil as described elsewhere herein.

In some embodiments, the polymer forms a hydrogel above its LCST.

In some embodiments, embolotherapy is for the treatment of a hypervascular tumour, such as hepatocellular carcinoma (HCC), colorectal cancer or its metastases, neuroblastomas and neuroendocrine tumours; aneurysms, arteriovenous malformations and fistulas, uterine fibroids, treatment of benign prostate hyperplasia (BPH) by embolisation of the prostatic artery and vessels arising therefrom, for the management of pain, for example pain associated with osteoarthritis or a musculoskeletal disorder by embolisation of associated blood vessels, for example the relief of pain in the knee by embolisation of the genicular artery and vessels arising therefrom; for the treatment of overweight or obesity by embolisation of the gastric artery, to induce hypertrophy of a *"future remnant"* of liver before liver resection (portal vein embolisation), to treat varicoceles or pelvic congestion syndrome through pelvic vein embolisation or to treat haemorrhage.

In a further aspect, the disclosure provides a kit for the preparation of an embolic composition as disclosed herein, comprising a first sealed container comprising a sterile tri-arm copolymer as described herein; optionally in the form of an aqueous composition; and a second sealed container comprising a sterile contrast agent as described herein; wherein, when combined the sterile tri-arm copolymer and the contrast agent provides a unit dose of an embolic composition. A unit dose may be from 1 to 20, particularly from 5 to 10 ml.

In a further aspect, the disclosure provides a kit for the preparation of a therapeutic product depot, comprising a first sealed container comprising a sterile tri-arm copolymer and a second sealed container comprising a sterile contrast agent; wherein, when combined the sterile tri-arm copolymer and the contrast agent provides a unit dose of drug depot. A unit dose may be from 0.1 to 50 ml, particularly from 1 to 20 ml or 5 to 10 ml.

By way of example only, one or both of the sealed containers may be an ampule, a vial, optionally a vial sealed with a pierceable septum cap, or a syringe.

The kit may additionally comprise one or more sterile syringes and/or one or more connectors or 2, 3 or 4-way valves suitable for fluidly connecting two syringes.

FIGURES

Figure 1 shows NMR spectra of (A) the polymer precursor from Example 2, (B) block copolymer MT2 from Example 4, (C) the macroinitiator of Example 3 and (D) grafted copolymer MT5, Example 5) each in deuterated methanol, 400MHz.

Figure 2 shows the appearance of the cast formed during injection into a flow model. (A) Polymer MT4, 7%w/w in saline, prepared as an emulsion with 50%vol ethiodized oil. (B) Polymer MT5, 7%w/w in ioversol 240. (C) Polymer MT6, 10%w/w in saline prepared as an emulsion with 50%vol ethiodized oil.

Figure 3 shows micro computed tomography images of example polymers (A) prepared as a solution in 7% in ioversol 240 and (B) prepared as a 10% solution in saline + 50%vol ethiodized oil.

Figure 4 is a temperature v modulus graph overlaying G'(storage modulus) and G" (loss modulus) plots for tri-arm polymers prepared according to examples 7 and 8.

Figure 5 compares the G' value for selected polymer compositions comprising either aqueous contrast agent (Optiray 240) or iodinated oil (Lipiodol) in an emulsion formulation.

EXAMPLES

In examples, formulae for polymers and intermediates are idealised assuming the reaction runs to completion. In the examples below, HPMA is a mixture of isomers hydroxypropyl methacrylates and hydroxyisopropyl methacrylates as outlined further above. Whilst the polymer comprises either grafted NIPAAM blocks or NIPAAM-blocks present as an extension of the HEMA-HPMA block, it may be the case that, during synthesis of graft polymers, a small number of NIPAAM monomers couple to and extend the HEMA-HPMA block.

SYNTHETIC EXAMPLES

Example 1: Preparation of tri-arm initiator glycerol tris(2-bromoisobutyrate)

Anhydrous glycerol 5 g (0.054 mol) was charged into a 250 mL round bottom flask, followed by 23 mL of triethylamine (0.168 mol) and 50 mL of anhydrous N-methyl pyrrolidinone. The flask was placed in an ice-water bath and stirred with a magnetic stirrer for 20 min. Then 38 g (0.165 mol) of α-bromoisobutyryl bromide was added dropwise through a dropping funnel over a period of about 30 min. The reaction was stirred at room temperature overnight. The reaction mixture was then filtered to remove triethylamine salt, then the solution was thoroughly mixed with saturated aqueous NaCl solution. Ethyl acetate was used to extract the water solution three times, and the combined organic phase was dried with $MgSO_4$ overnight. The ethyl acetate solution was removed with a rotary

evaporator, and the product vacuum dried for 24 hr to provide a pale-yellow wax-like solid. The ^{1}H NMR spectrum for this product is shown in Figure 1).

Example 2: Synthesis of tri-arm poly(HEMA$_{80}$-co-HPMA$_{20}$)

In a 500 mL three-neck round bottom flask, 1.5 g (2.78 mmol) of initiator prepared in Example 1 was mixed with 2-hydroxyethyl methacrylate 86.9 g (667 mmol) and 24.1 g (167 mmol) of hydroxypropyl methacrylate in 110 mL of methanol. The solution was degassed with nitrogen for 60 min, then 1.2 g (8.34 mmol) of CuBr and 2.61 g (16.7 mmol) of 2,2'-bipyridine were added under nitrogen flow to start polymerisation. After about 20 hr, the reaction was stopped by exposure to the air and diluted with methanol. The mixture was precipitated into deionised water to remove copper catalyst and solid precipitation was received. The solid was dissolved in MeOH and precipitated into water. The extraction was repeated twice. The received polymer was vacuum dried at 40°C overnight and 107 g of solid was received.

Example 3: Synthesis of poly (I-(HEMA$_{80}$-co-HPMA$_{20}$)$_3$) macroinitiator

In a 250 mL round bottom flask, 20 g (0.42 mmol) of tri-arm poly(I-(HEMA-HPMA)$_3$) obtained from Example 2 was dissolved into 80 mL of anhydrous N-methyl pyrrolidinone (NMP) under magnetic stirring, followed by adding 2.3 mL of triethylamine. The flask was placed in an ice-water bath, then 3.48 g of α-bromoisobutyryl bromide to target 10 unit of bromide per arm was added dropwise through a syringe over about 20 min. The reaction solution became turbid quickly and was stirred at room temperature overnight. The reaction mixture was precipitated dropwise into deionised water 500 mL to remove NMP and triethylamine salt. The collected solid was dissolved in 60 mL of NMP and the precipitation was repeated once. The solid was collected and vacuum dried at 40°C for two days.

Example 4: Synthesis of tri-arm poly(I-(HEMA$_{80}$-co-HPMA$_{20}$)-block-(NIPAAM$_{100}$))$_3$ by ATRP

In a 500 mL of three-neck round bottom flask, 24 g of polymer precursor prepared from Example 2 was dissolved in 60 mL of methanol, followed by dissolving 20.2 g of NIPAAM. The mixture was degassed with nitrogen for 60 min, then 0.26 g of CuBr and 0.46 g of Me4Cyclam were added to start the polymerisation. The reaction was stirred under nitrogen atmosphere for 16 hr. After exposed to the air, methanol 200 mL was added to dilute the mixture which was then mixed with water for ultrafiltration to remove catalysts and unreacted monomer. The final solution was concentrated and freeze-dried to obtain solid polymer.

Example 5: Synthesis of comb-like tri-arm poly(I-(HEMA$_{80}$-co-HPMA$_{20}$)-graft-(NIPAAM$_{30}$)$_{10}$)$_3$ by ATRP

In a 1 L of three-neck round bottom flask, 15 g of poly(HEMA-HPMA) macroinitiator prepared in Example 3 and 34 g of NIPAAM monomer were dissolved in 250 mL of NMP. The mixture was degassed with nitrogen for 60 min, then CuBr 1.44 g and PMDETA 1.74 g were added to start the

polymerisation. The reaction was left running overnight. The mixture was then ultra-filtrated against deionised water to remove unreacted monomer and catalysts. The solution was freeze dried and polymer solid was received.

Example 6: ^{1}H-NMR

Nuclear magnetic resonance (NMR) data was collected using a Bruker AVANCE III HD 400 MHz, deuterated methanol as solvent. Based on the spectra, the compositions of HEMA-HPMA-NIPAAM polymer were calculated and the HEMA:HPMA:NIPAAM ratio were listed for the comparison with targets in Table 1 below.

MT1 to MT8 relate to tri-arm polymers, whilst MT9 is a linear polymer, as a comparator.

Table 1

Entry	Target polymer	HEMA:HPMA:NIPAAM Target ratio	HEMA:HPMA:NIPAAM Ratio by NMR
MT1	I-[(HEMA$_{99}$-*st*-HPMA$_{1}$)-*b*-NIPAAM$_{100}$]$_3$	1:0.01:1.01	1:0.02:1.17
MT2	I-[(HEMA$_{80}$-*st*-HPMA$_{20}$)-*b*-NIPAAM$_{100}$]$_3$	1:0.25:1.25	1:0.28:1.40
MT3	I-[(HEMA$_{80}$-*st*-HPMA$_{20}$)-*g*-(NIPAAM30)$_{30}$]$_3$	1:0.25:11.25	1:0.28:9.54
MT4	I-[(HEMA$_{80}$-*st*-HPMA$_{20}$)-*b*-NIPAAM$_{50}$]$_3$	1:0.25:0.63	1:0.28:0.61
MT5	I-[(HEMA$_{80}$-*st*-HPMA$_{20}$)-*g*-(NIPAAM$_{30}$)$_{10}$]$_3$	1:0.25:3.75	1:0.28:3.11
MT6	I-[(HEMA$_{99}$-*st*-HPMA$_{1}$)-*g*-(NIPAAM$_{30}$)$_{10}$]$_3$	1:0.01:3.03	1:0.02:1.94
MT7	I-[(HEMA$_{99}$-*st*-HPMA$_{1}$)-*g*-(NIPAAM$_{30}$)$_{30}$]$_3$	1:0.01:9.09	1:0.02:6.46
MT8	I-[(HEMA$_{99}$-*st*-HPMA$_{1}$)-*b*-NIPAAM$_{50}$]$_3$	1:0.01:0.51	1:0.02:0.57
MT9	NIPAAM$_{200}$-HEMA$_{100}$-NIPAAM$_{200}$	1:0:4	1:0.02:1.04

The NMR spectra of the precursor from Example 2, the block copolymer from Example 4 (MT2), the macroinitiator from Example 3 and the grafted copolymer from Example 5, (MT5) are shown in Figure .

Example 7: Synthesis of grafted tri-arm polymers by ATRP with alternative catalysts

A typical grafted polymer synthesis is described here. In a 500 mL two-neck round bottom flask, 15.0 g of tri-arm poly(HEMA-HPMA) macroinitiator with an average of 10 bromides in each arm, prepared according to Example 3, and 34.7 g of NIPAAM monomer were dissolved in a mixture of 310 mL of NMP and water. Ligand PMDETA 1.77 g was also added to the solution, and the mixture was degassed with nitrogen for 1 hour. Then CuBr powder 1.46 g was added to start the polymerisation and the reaction was left running overnight. The mixture was ultra-filtrated against deionised water to remove unreacted monomer, catalysts and organic solvent. The final polymer solution was freeze-dried and

polymer solid was received. For grafted polymers showing up to 25 bromides in each arm, the person skilled in the art shall adapt the process according to his common knowledge in the field.

A typical synthesis using an alternative catalyst is as follows. In a two-neck round bottom flask, 9.0 g of tri-arm poly(HEMA-HPMA) macroinitiator with an average of 10 bromides in each arm, and 20.8 g of NIPAAM monomer were dissolved in a mixture of 120 mL of NMP and water. Cu(I)Cl powder 0.61g and ligand Me_6TREN 1.41g was dissolved in an NMP and water mixture in a separate flask. The two solutions were degassed with nitrogen separately for 1-2 hr, followed by adding the copper catalyst/ligand into the macroinitiator and NIPAAM monomer flask to start the polymerisation. The reaction was left running for 16 hr and the mixture was then ultra-filtrated against deionised water to remove unreacted monomer, organic solvent and catalysts. The final solution was freeze-dried and polymer solid was received.

The obtained polymers were characterised by NMR as described above and by gel permeation chromatography or size exclusion chromatography (GPC/SEC) to obtain structure, composition and MW distribution data. GPC/SEC measurements with triple-SEC analysis were carried out on an Agilent 390-MDS system using DMF solvent.

In the above manner MY17, MT20 and MT21/2 were prepared. MT17 and MT21/2 were prepared using a copper chloride catalyst and MT20 was prepared using a copper bromide catalyst and their structure confirmed by NMR.

I-[(HEMA$_{99}$-*st*-HPMA$_1$)-*g*-(NIPAAM$_8$)$_{25}$]$_3$ (MT17)

I-[(HEMA$_{99}$-*st*-HPMA$_3$)-*g*-(NIPAAM$_{16}$)$_9$]$_3$ (MT20)

I-[(HEMA$_{99}$-*st*-HPMA$_3$)-*g*-(NIPAAM$_{13}$)$_9$]$_3$ (MT21/2)

Example 8; Synthesis of I-[(HEMA$_{99}$-*st*-HPMA$_1$)-*g*-(NIPAAM$_{20}$)$_9$]$_3$

In a 500 mL three-neck round bottom flask, 1.5 g (2.78 mmol) of initiator prepared in Example 1 was mixed with 2-hydroxyethyl methacrylate 107.6 g (826 mmol) and 1.2 g (8 mmol) of hydroxypropyl methacrylate in 110 mL of methanol. The solution was degassed with nitrogen for 60 min, then 1.2 g (8.34 mmol) of CuBr and 2.61 g (16.7 mmol) of 2,2'-bipyridine were added under nitrogen flow to start polymerisation. After about 20 hr, the reaction was stopped by exposure to the air and diluted with methanol. The mixture was precipitated into deionised water to remove copper catalyst and solid precipitation was received. The solid was dissolved in MeOH and precipitated into water. The extraction was repeated twice. The received polymer was vacuum dried at 40°C overnight and 99 g of solid was received.

Synthesis of poly (I-(HEMA$_{99}$-co-HPMA$_1$)$_3$) macroinitiator

In a 250 mL round bottom flask, 40 g (1.1 mmol) of tri-arm poly(I-(HEMA-HPMA)$_3$) obtained from above was dissolved into 160 mL of anhydrous N-methyl pyrrolidinone (NMP) under magnetic stirring,

followed by adding 5.0 mL of triethylamine. The flask was placed in an ice-water bath, then 7.62 g of α-bromoisobutyryl bromide to target 10 unit of bromide per arm was added dropwise through a syringe over about 20 min. The reaction solution became turbid quickly and was stirred at room temperature overnight. The reaction mixture was precipitated dropwise into deionised water 500 mL to remove NMP and triethylamine salt. The collected solid was dissolved in 180 mL of NMP and the precipitation and water washing was repeated thrice. The solid was collected and vacuum dried at 40 °C for two days, which yield 42 g of solid.

Synthesis of triarm polymer poly(I-(HEMA$_{99}$-co-HPMA$_1$)- graft-(NIPAAM$_{20}$)$_9$)$_3$ (MT21/3)by ATRP

Similar as the NIPAAM polymer synthesis process aforementioned, MT21/3 was synthesised using more concentrated reactants. In a two-neck round bottom flask, 9.0 g of tri-arm poly(HEMA-HPMA) macroinitiator with average 10 bromide in each arm, and 20.8 g of NIPAAM monomer were dissolved in the mixture of around 78 mL of NMP and water. CuCl powder 0.61g and ligand Me$_6$TREN 1.41g was dissolved in around 47 mL of NMP and water mixture in a separated flask. The two solutions were degassed with nitrogen separately for 1-2 hr, followed by adding the copper catalyst/ligand into the macroinitiator and NIPAAM monomer flask which was kept in an ice water bath to start the polymerisation. The reaction was left running for 16 hr and the mixture was then ultra-filtrated against deionised water to remove unreacted monomer, organic solvent and catalysts. The final solution was freeze-dried and polymer solid was received. Structure was confirmed by NMR as above.

FURTHER EXAMPLES

Example 7: Polymer solubilization for in vitro testing.

2.1 g of the dried polymer is progressively added in a 50 mL Schott bottle containing 27.9 g of the aqueous contrast agent ioversol (Optiray 240, Guerbet, Paris) to prepare a 7%w/w solution. The bottle is placed in an ice bath and magnetically stirred until a uniform solution is obtained (several hours). Depending on the polymer, the resulting solution is either transparent, translucent, or slightly whitish, and of low viscosity.

Example 8: Preparation of ethiodized oil-based emulsion.

3 g of the dried polymer is progressively added in a 50 mL Schott bottle containing 27 g of saline (OTEC NaCl 0,9%) to prepare a 10%w/w solution. The bottle is placed in an ice bath and magnetically stirred until a uniform solution is obtained. Depending on the polymer, the resulting solution is either transparent, translucent, or slightly whitish, and of low viscosity. To prepare the 7% w/w polymer saline solution, the same protocol is applied but with 2.1 g of polymer and 27.9 g of saline.

For the emulsion preparation, 2 mL of ethiodized oil (Lipiodol®, Guerbet) is withdrawn with a 5 mL syringe (Terumo Luer-lock®) and 2 mL of the polymer saline solution is withdrawn similarly. Both

syringes are connected to a 3-way stopcock (BBraun Discofix™). 10 back-and forth transfers between syringes are carried out to shear the mixture and create the emulsion.

Example 9: Evaluation of emulsion stability

To determine the emulsions' stability, approximately 4 mL of the emulsion is transferred into a small vial to proceed with a stability analysis using a Turbiscan tower equipment (Formulaction Turbiscan Tri-lab) at 20°C. Light scattering readings are taken over the height of the vial over time which allows the detection of droplet migration and/or coalescence.

The back-scattering profiles are further treated to calculate the Turbiscan Stability Index (TSI) which corresponds to the integration of the backscattering curves. The larger the TSI the more unstable to emulsion is.

For comparison purpose, a stability ratio was calculated corresponding to the emulsion saline/ethiodized oil TSI divided by the composition TSI. The presence of the polymers increases the emulsion stability up to 5-fold after 1 h, compared to the emulsion without polymer.

Table 2

Polymer	Polymer concentration in saline (%w/w)	TSI 1h	Stability ratio (vs no polymer)
No polymer	0%	8.6	NA
MT3	7%	2.6	3.3
MT3	10%	4.9	1.8
MT4	7%	6.6	1.3
MT5	10%	3.0	2.9
MT6	10%	2.1	4.1
MT7	7%	1.6	5.4
MT8	7%	2.8	3.1

NA : not applicable

Example 10: Evaluation of interfacial tension

Drop shape analyses were carried out with a DSA30S (Kruss) to determine the interfacial tension between ethiodized oil and the polymer aqueous solutions (polymer concentration 0,1 wt%). More precisely, an ethiodized oil droplet (~20 µL) is pushed vertically in a cuvette containing the polymer solution at 20°C. The droplet profile, captured by a camera, is fitted with the Young-Laplace equation to obtain the interfacial tension (ADVANCE software). The data (table 3) show that the polymers act as surfactants as the interfacial tension is significantly reduced.

Table 3

Polymer	Interfacial tension (mN/m)
No polymer	24.4 ± 0.8
MT1	11.6 ± 0.2
MT2	11.4 ± 0.1
MT3	10.5 ± 0.1
MT5	10.9 ± 0.1
MT6	11.1 ± 0.1
MT7	11.0 ± 0.1

Example 10: Quantitative assessment of ease of deliverability in 1.7 Fr microcatheter.

The setup to assess the ease of deliverability comprises a flow model made of two silicon tubes, a water bath heated at 37°C, a pump (Masterflex) to create a 1 ml/min flow. A dynamometer (Mark-10 - ESM-303 and M5-20) is used to apply a fixed flow rate (1 ml/min) while measuring and recording the force applied to the syringe. A 1.7 Fr microcatheter (Merit Pursue) is partially submerged in a heated bath at 37°C (70 cm out of 130 cm) and introduced in the flow model. A 1mL-syringe is filled with the embolic composition, settled in the dynamometer holder and connected to the microcatheter. A camera (Dino lite, Dinocapture) is placed over the flow model to record the product exiting the catheter and the cast formation.

All formulations are easily injectable though 1.7 Fr microcatheter, with the maximal injection force below 26.1 N (see table 4). In each case this is below the "comfortable" range for human hands (~30 N).

Table 4

Polymer	Formulation	Required injection force (N)
MT1	7%w/w in ioversol 240	25.7
MT2	7%w/w in ioversol 240	26.1 ± 0.5
MT3	7%w/w in ioversol 240	8.3 ± 1.8
MT5	7%w/w in ioversol 240	7.8 ± 2.0
MT6	7%w/w in ioversol 240	8.0 ± 3.4
MT7	7%w/w in ioversol 240	14.4 ± 0.9
MT1	5%w/w in saline, 50%vol ethiodized oil	10.3 ± 0.5
MT2	5%w/w in saline, 50%vol ethiodized oil	9.8 ± 1.3
MT3	10%w/w in saline, 50%vol ethiodized oil	13.0 ± 1.3
MT3	7%w/w in saline, 50%vol ethiodized oil	8.6 ± 0.8
MT4	10%w/w in saline, 50%vol ethiodized oil	13.0 ± 0.8
MT5	10%w/w in saline, 50%vol ethiodized oil	7.9 ± 0.7
MT6	10%w/w in saline, 50%vol ethiodized oil	8.2
MT7	10%w/w in saline, 50%vol ethiodized oil	10.1 ± 1.9
MT7	7%w/w in saline, 50%vol ethiodized oil	6.3 ± 1.4
MT8	7%w/w in saline, 50%vol ethiodized oil	9.7 ± 0.6

Example 112: Qualitative assessment of ease of deliverability in 1.7 Fr microcatheter

Samples of the polymers were weighted into a 30 mL vial, aqueous contrast agent iohexol (Omnipaque™ 300, GE) to prepare a 10%w/w solution. The vial was placed in a cold-water bath and magnetically stirred until a uniform solution was obtained. The obtained polymer solution was transferred into a syringe for delivery test with a 1.7 Fr catheter. Ease of delivery was qualitatively assessed and the results are gathered in table 5 below.

Table 5

Polymer	Formulation	Ease of deliverability
MT6	10%w/w in iohexol 300	Easily deliverable using either fast (roughly 2 mL/min) or slow (0.5 mL/min) injection rate.
MT9	10%w/w in iohexol 300	Difficult delivery using either fast (roughly 2 mL/min) or slow (0.5 mL/min) injection rate.

Example 13: Catheter delivery of polymer – observations.

Samples of the polymers prepared according to Examples 7 and 8 were delivered through a 1.7 Fr catheter in a flow model according to Example 10. Observations are recorded in table 6 and cast appearance are illustrated by pictures in figure 2.

Table 6

Polymer	Formulation condition	Observations upon injection into flow model (N=3)
MT3	7%w/w in ioversol	Long cohesive segments
MT3	10%w/w in saline, 50%vol ethiodized oil	No visible segmentation, cohesive cast
MT3	7%w/w in saline, 50%vol ethiodized oil	No visible segmentation, cohesive cast
MT4	7%w/w in saline, 50%vol ethiodized oil	Cohesive cast
MT5	7%w/w in ioversol	Long cohesive segments
MT5	10%w/w in saline, 50%vol ethiodized oil	Long cohesive segments
MT6	7%w/w in ioversol	Long cohesive segments
MT6	10%w/w in saline, 50%vol ethiodized oil	No visible segmentation, cohesive cast
MT7	7%w/w in ioversol	No visible segmentation, cohesive cast
MT7	7%w/w in saline, 50%vol ethiodized oil	Long cohesive segments
MT8	7%w/w in saline, 50%vol ethiodized oil	Cohesive cast

Example 14: Radiopacity of polymer composition

Samples of the polymer compositions prepared according to Examples 7 and 8 were analysed by micro computed tomography (Perkin Elmer - QuantumGX2) at 37°C. Approximately 200 µL of the compositions were transferred in wells (NUNC - F8 maxisorp loose) to proceed with 3D-RX analysis at 37°C. The scan acquisition parameters were set as follows:

- Voltage 90 kV,
- Current CT 88 µA , live 40 µA
- Dose : 83 mGy
- FOV : 72 mm
- Scan mode : high resolution 4 min
- X-ray filter : Cu 0.06 mm + Al 0.5 mm.

For data processing, signal measurements of each well were quantified using Myrian software by Intrasense version 2.9.13. Data is given in table 7.

Table 7

Polymer	Formulation	Calculated iodine conc. (mgI/ml)	Radiopacity (ADU) at 37°C
MT1	7%w/w in ioversol 240	240	4653 ± 791
MT2	7%w/w in ioversol 240	240	4011 ± 369
MT3	7%w/w in ioversol 240	240	3847 ± 350
MT5	7%w/w in ioversol 240	240	3796 ± 329
MT6	7%w/w in ioversol 240	240	4212 ± 293
MT7	7%w/w in ioversol 240	240	5743 ± 195
MT3	10%w/w in saline, 50%vol ethiodized oil	240	4352 ± 359
MT5	10%w/w in saline, 50%vol ethiodized oil	240	3701 ± 166
MT6	10%w/w in saline, 50%vol ethiodized oil	240	6042 ± 471
MT7	10%w/w in saline, 50%vol ethiodized oil	240	4466 ± 57
MT7	7%w/w in saline, 50%vol ethiodized oil	240	3745 ± 692

ADU = Arbitrary density units

Example 15: Rheology

Samples of the polymers prepared according to Examples 7 and 8 were characterized by rheology (Anton Paar rheometer MCR302e modular compact rheometer) using the oscillation method with a temperature ramp. All testing was performed with a 25 mm smooth stainless steel parallel plate. Plate gap/sample thickness was 0.5 mm. The results are shown in figure 4 and table 8.

Figure 4 shows that the sol-gel transition temperature is around 31 °C. At 20°C, all tri-arms based formulations are characterised by a viscosity lower than 388 Cp. At 37°C, storage (G') and loss moduli (G") are above 1,010 and 678 Pa respectively, which suggests that a solid gel could be formed when the sample is placed at body temperature. Storage moduli are greater than loss moduli; gel are viscoelastic solid-like material with a predominant elastic behaviour over viscous one.

Varying the nature of the iodinated contrast agent highly impacts the rheological properties of the gel; the storage moduli are increased by a factor of 4 to 8 by switching from the aqueous contrast agent (ioversol) to ethiodized oil. On the contrary, mechanical properties of the gel formed by the linear polymer formulation do not depend on the nature of the iodinated contrast agent as supported by MT9 data. See table 8 and figure 5.

Table 8

Code	Formulation	Complex viscosity at 20°C (Cp)	Mean G' at 37°C (Pa)	Mean G'' at 37°C (Pa)
MT1	7%w/w in ioversol 240	307 ± 14	1 010 ± 255	678 ± 234
MT3	7%w/w in ioversol 240	115 ± 2	2 080 ± 127	1 891 ± 29
MT3	7%w/w in saline 50%vol ethiodized oil	211 ± 16	16 858 ± 1 625	2 562 ± 190
MT4	7%w/w in saline 50%vol ethiodized oil	918 ±194*	1 296 ±52	474 ± 11
MT5	7%w/w in ioversol 240	74 ± 12	1 007 ± 15	925 ± 16
MT5	10%w/w in saline 50%vol ethiodized oil	255 ± 55	4 015 ± 1 399	1 654 ± 197
MT6	7%w/w in ioversol 240	71 ± 11	1 388 ± 255	1 058 ± 201
MT6	10%w/w in saline 50%vol ethiodized oil	388 ± 64	4 834 ± 656	1 551 ± 56
MT7	7%w/w in ioversol 240	212 ± 11	2 880 ± 139	2 261 ± 146
MT7	7%w/w in saline 50%vol ethiodized oil	236 ± 12	11 505 ± 929	1 857 ± 192
MT8	7%w/w in saline 50%vol ethiodized oil	170 ± 28	74 ± 6	42 ± 3

*: data at 15°C

Example 16: Behaviour of polymers in a vascular flow model

The model: A moulded vascular model is held at physiological temperature (37°C) in a water bath and is connected to a source of phosphate buffered saline (PBS) held in a second water bath at 37°C by tubing. The outlet of the vascular model returns the PBS to the source for recirculation. PBS is pumped via a peristaltic pump through tubing having an inline flow rate indicator, analogue pressure gauge, catheter introducer and a digital pressure gauge prior to the moulded vascular model. A catheter is directed into one of the vessels within the vascular model where polymer formulations are delivered and assessed for performance. PBS is recirculated back to the reservoir via a holding tank that is at a height of approximately 1 metre above the vascular model. This induces a pressure gradient for the pumped PBS flow to better reflect an *in vivo* blood pressure environment.

A number of attributes are qualitatively and quantitatively scored for these tests to determine relative performance including the following:

Creep: a quantitative measure of the extent a delivered formulation moves, migrates or flows in the vascular mould within a 15-minute time window after injection, captured as a timelapse video. The creep distance is determined using a visual reference of known dimensions that is present within the video frame and expressed in millimetres.

Proximal Ethiodised Oil (EO) Release: A qualitative score of the amount of EO generated during a delivery at the proximal end of a delivered formulation. Scoring is either 1,2 or 3 where:

1: No or minimal EO generated proximal to the delivered formulation

2: Some EO generated proximal to the delivered formulation more than (1) but unlikely to lead to false positive imaging under fluoroscopy.

3: Significant EO generated proximal to the delivered formulation that would lead to false positive imaging under fluoroscopy.

Distal EO Count: A count of EO droplets that separate and flow downstream of a delivered formulation over the length of the timelapse video.

Dye Perfusion Time: The time lag between a fluorescent dye contacting the proximal end of a delivered embolic formulation and perfusing out of the distal end of the delivered formulation, expressed in seconds and indicating occlusion efficiency.

Data obtained are shown in table 9:

Table 9

	Formulation	**Creep** (mm)	**Creep CoV** (%)	**Perfusion** (s)	**Perfusion CoV** (%)
MT17	7%w/w in ioversol 240	11,5	7%	1,8	n/a
MT20	7%w/w in ioversol 240	1,2	31%	11	32%
MT20	8%w/w in ioversol 240	0,6	5%	8,9	58%
MT21/3	7%w/w in ioversol 240	0,4	16%	12,6	8%

The present data sjow improved creep and perfusion scores.

CLAIMS

1. A composition for use in a method of embolotherapy and/or in the preparation of therapeutic depots for delivery by injection comprising:

a) a tri-arm copolymer of hydroxyethyl methacrylate (HEMA), 3-hydroxypropyl methacrylate (HPMA) and N-isopropyl methacrylamide (NIPAAM); wherein each arm of the tri-arm copolymer comprises a first block and a second block; wherein the first block consists of HEMA and HPMA and the second block consists of NIPAAM; and wherein the second block is either an extension of the first block or is grafted to the first block; and

b) a contrast agent.

2. A composition for use according to any preceding claim, wherein the tri-arm copolymer is of the formula:

$$I\text{-}[(HEMA_X\text{-}HPMA_Y)NIPAAM_Z]_3$$

wherein I is the residue of a trivalent initiator and the NIPAAM is in the form of an extension block or is one or more grafts grafted onto the (HEMA-HPMA) block.

3. A composition for use according to any preceding claim, wherein the number of HEMA, HPMA and NIPAAM monomer units are 75-105, 0.5-20 and 50-1500 respectively.

4. A composition for use according to any of claims 1 to 3, wherein the second block is an extension of the first block and wherein the number of HEMA, HPMA and NIPAAM monomer units are 75-105, 0.5-20 and 100-250 respectively.

5. A composition according to any of claims 1 to 3, wherein the second block is a is one or more grafts grafted onto the first block and wherein the number of HEMA, HPMA and NIPAAM monomer units are 75-105, 0.5-20 and 50-1500 respectively

6. A composition for use according to any preceding claim with contrast enhancement ability in at least one medical imaging modality configured to allow detection of the composition; said medical imaging modality selected from X-ray, Computed Tomography (CT), Photon-counting CT, Magnetic Resonance Imaging, Optical imaging and Nuclear medicine.

7. A composition for use according to claim 6, wherein said contrast in at least one medical imaging modality is configured to allow detection of the composition during procedure, and wherein the composition is configured so that the contrast disappear in the body over time.

8. A composition for use according to any preceding claim, wherein the contrast agent is selected from the group consisting of radiopaque, paramagnetic, superparamagnetic, fluorescent, radiopharmaceuticals contrast agents or a combination of any two or more thereof.

9. A composition for use according to claim 8, wherein the radiopaque contrast agent is selected from iodinated contrast agents, optionally wherein the iodinated contrast agent is selected from an iodinated oil and a water-soluble iodinated contrast agent; tantalum, barium, bismuth and tungsten or compounds thereof; wherein the paramagnetic and superparamagnetic contrast agents are each selected from gadolinium and manganese compounds and iron oxide particles; wherein fluorescent contrast agents are selected from indocyanine green, patented blue, methylene blue, fluoresceine, isosulfan blue and lymphazurin; and wherein radiopharmaceutical contrast agents are selected from technetium-99, fluorine-18, iodine-131, indium-111, gallium-68, cupper-62, cupper-64, zirconium-89, rubidium-82, scandium-43, scandium-44, samarium-117; or a combination of any two or more of the foregoing.

10. A composition for use according to any preceding claim, which is a monophasic composition or a biphasic composition.

11. A composition for use according to claim 11, comprising between 0.1-50%w/w of the tri-arm copolymer.

12. A composition for use according to any of claims 1 to 12, selected from an aqueous solution, and an emulsion.

13. A composition for use according to any preceding claim having a total iodine content greater than 0,4% w/w.

14. A composition for use according to any preceding claim, providing an increase of radiopacity greater than 100 HU.

15. A composition for use according to any preceding claim, wherein the radiopaque contrast agent is an iodinated oil contrast agent.

16. A composition for use according claim 15, wherein the iodinated oil contrast agent is an ethiodized oil.

17. A composition for use according to any preceding claim, having a viscosity of less than 500 Cp at 20°C.

18. A composition for use according to any of claims 10 to 17 which is an emulsion having a comparative stability of at least 1.1.

19. A composition for use according to any preceding claim which is deliverable through a microcatheter 1.7 Fr at 20 °C with an injection force of less than or equal to 45 N.

20. A method of embolising a blood vessel of a subject in need thereof, comprising delivering to a lumen of the blood vessel, an embolic composition according to any preceding claim.

21. A process for preparing an embolic composition comprising combining a tri-arm copolymer according to any of claims 1 to 6; optionally in the form of an aqueous composition, with a contrast agent.

22. A process for preparing a therapeutic depot for use by injection comprising combining a tri-arm copolymer according to claims 1 to 6, optionally in the form of an aqueous composition, with a contrast agent and a therapeutic product.

23. A kit for the preparation of an embolic composition comprising a first sealed container comprising a sterile tri-arm copolymer according to any of claims 1 to 6, optionally in the form of an aqueous composition; and a second sealed container comprising a sterile contrast agent; wherein, when combined the sterile tri-arm copolymer and the contrast agent provides a unit dose of an embolic composition.

24. A kit for the preparation of a therapeutic product depot for use by injection, comprising a first sealed container comprising a sterile tri-arm copolymer according to any of claims 1 to 6 and a second sealed container comprising a sterile contrast agent; wherein, when combined the sterile tri-arm copolymer and the contrast agent provides a unit dose of a therapeutic product depot.

25. A kit according to claim 23 or 24, wherein the contrast agent is an iodinated contrast agent.

26. A kit according to either of claims 23 to 25, wherein the tri-arm copolymer is in the form of an aqueous composition.