

## A THERMORESPONSIVE INJECTABLE HYDROGEL FOR INTRAVASCULAR ADMINISTRATION

### Related Application

[01] This application claims priority to Australian Provisional Patent Application No. 2024901241, filed on 1 May 2024. The content of AU'241 is incorporated herein by reference in its entirety.

### Field of the Invention

[02] The present invention relates to biologically-compatible polymers and the present inventors' surprising discovery that such polymers are flowable through interventional vascular catheters and micro catheters (1F-6F). The solutions transition into an adhesive hydrogel network upon their extrusion from the catheter and thereby elicit potential as agents for intravascular (arterial or venous) administration.

[03] The present invention further relates to the discovery that at concentrations between about 10 and 120 mg/mL and about 5 to 200 mg/mL, respectively, Applicant's proprietary polymers PNPFO and PPHO or their combination thereof show potential as advanced thermoresponsive carrier biomaterials for multiple pharmaceutical classes including: therapeutics, drugs, radioisotopes, *etc.*

[04] The present invention relates to the discovery that radiographic intravenous contrast agents can be incorporated within the formulation of PNPFO and PPHO solution for effective intravascular embolisation or as a delivery vehicle. This includes contrast media for digital subtraction angiography (DSA), computer tomography (CT) and fluoroscopy – examples include iodinated contrast media (*e.g.*, iohexol), tantalum, and bismuth-based materials (*e.g.*, bismuth chelate). Further, heavy metal chelates contrast media for MRI can also be incorporated into PNPFO and PPHO including gadolinium agents (*e.g.*, gadobutrol) or MRI nanoparticles.

[05] The invention further relates to applications of PNPFO and PPHO, advanced thermoresponsive biomaterials, for intravascular embolisation and carriers for radioisotopes. This includes all classes of clinically relevant medical radioisotopes comprising: Therapeutic Alpha (*e.g.*, Lead-212, Radium-223) and Beta emitters (*e.g.*, Iodine-131, Yttrium-90, Rhenium-186). Further, the present invention is applicable to diagnostic radioisotopes including: Gamma emitters (*e.g.*, Gallium-67, Technetium-99m) and Positron emitters (*e.g.*, Gallium-68, Fluorine-18) and theranostic pairs (*e.g.*, Lutetium-

177 and Gallium-68).

[06] The invention further relates to applications of PNPFO and PPHO, advanced thermoresponsive biomaterials, for intravascular delivery of different pharmaceutical classes (as above) or other agents for *in situ* cargo deposition/depot formation.

[07] Accordingly, the present invention is envisaged to find utility in medicine, more particularly, in interventional radiology, interventional oncology, vascular surgery, diagnostic radiology, nuclear medicine, radiation oncology and related specialty fields.

[08] Although the present invention will be described hereinafter with reference to its preferred embodiment, it will be appreciated by those of skill in the art that the spirit and scope of the invention may be embodied in many other forms.

### Background of the Invention

[09] Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

[010] Transcatheter arterial embolisation (TAE) as a minimally invasive surgical technique has been widely used since the 1970s [1,2]. Typical diseases that have been targeted for arterial embolisation include, but are not limited to gastrointestinal tract haemorrhage, bleeding from vascular malformations, neoplasms, redistribution of preoperative blood flow, varicoceles, or endoleaks, and tumours particularly hepatocellular carcinoma [2]. The choice and design of embolisation materials play a crucial role in the success of TAE and are a function of clinical, anatomical, and local flow factors at the target site. Such materials should be flowable through an anatomically/arterially compatible catheter size, demonstrate fast and effective gelation to fill or plug the target site upon application, adhere for a tuneable period of time, and if drugs are included, control the release of such drugs (*e.g.*, chemotherapy).

[011] Injectable polymeric embolic agents can be introduced in a controlled amount for partial or total filling of the target site [3]. Common liquid embolisation materials include Onyx™, TRUfill™, and EmboGel™. Onyx™ is made of a solution of ethylene-vinyl alcohol copolymer and tantalum powder (as contrast) in dimethyl sulfoxide and it has low thrombogenicity [4,5]. Onyx™ embolisation for aneurysms is generally impractical due to the risk of leakage of the embolisation into the parent artery, potentially leading to stroke [4]. TRUfill™ is an adhesive with a fast injection rate and is comprised of n-butyl cyanoacrylate which acts as an embolic agent when polymerised via an exothermic

reaction [6].

**[012]** Injectable hydrogels have been developed and used for TAE due to their low toxicity. For instance, EmboGel™ is the product of calcium chloride-induced polymerisation of a mixture of iohexol (contrast agent) and alginate. However, this mixture may partially reflux into the parent artery [7-9]. Other examples of injectable hydrogels that have been reported for embolisation application include shear-thinning materials [10,11], pH-responsive hydrogels [12,13], and thermoresponsive hydrogels [14-20].

**[013]** Matsumaru, *et al.*, reported on the application of thermosensitive polymers (copolymers of *N*-isopropylacrylamide and *N*-n-propylacrylamide) as embolic material for intravascular neurosurgery in 1996 [21]. Vernon and co-workers have developed an *N*-isopropylacrylamide (NIPAAm)-based copolymer system composed of poly(NIPAAm-*co*-HEMA-acrylate) functionalised with olefins and 3-mercapto propionate) for endovascular embolisation [14]. Later, they enhanced their system by the addition of thiol groups, in which poly(NIPAAm-*co*-cysteamine) (NC) reacted with poly (NIPAAm-*co*-HEMA-acrylate) or poly(NIPAAm-*co*-cysteamine-vinylsulfone) to construct a gel [15]. Zhao, *et al.*, also reported a temperature-sensitive blood-vessel-embolic material for interventional therapy which was based on poly(*N*-Isopropylacrylamide-*co*-butyl methacrylate) which was mixed with iohexol, a non-ionic X-ray contrast agent, which increased the volume-phase transition temperature of their system and decreases the critical gelation concentration [22]. Interestingly, they demonstrated that the addition of iohexol to pure PNIPAAm shifted the LCST from 32 °C to 46 °C, which resulted in no gelation at body temperature [22]. Later, they claimed that their poly(*N*-Isopropylacrylamide-*co*-butyl methacrylate) with iohexol could be modified and potentially be used as a bioactive embolic agent in endovascular therapy [16]. Shi, *et al.*, overcame the LCST issue with PNIPAAm by introducing a supramolecular copolymer hydrogel poly(*N*-acryloyl glycinamide-*co*-acrylamide) (PNAGA-PAAm) combined with iohexol. Their system was injected into the renal arteries of rabbits through a microcatheter, and they reported successful embolisation with no recanalisation after 8 weeks [17].

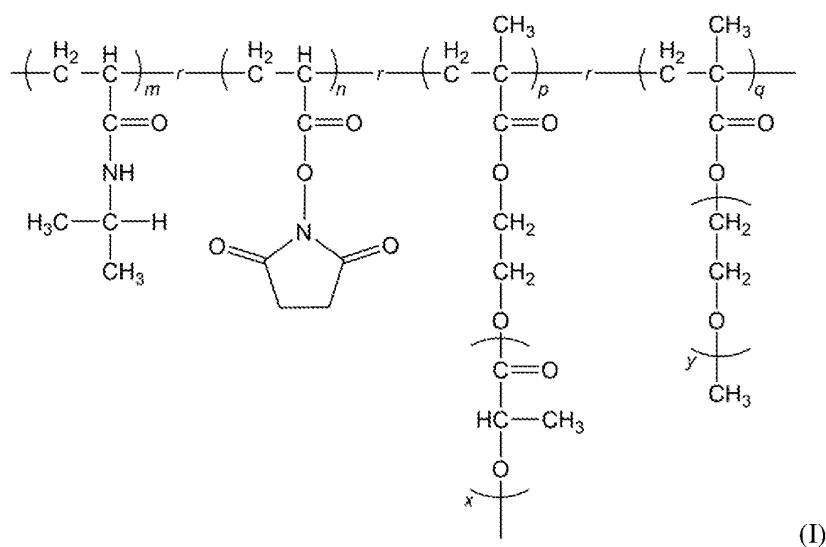
**[014]** In a different sector of the biomedical field, the present Applicant, Trimph IP Pty Ltd, of Sydney, Australia, has been active over the past decade in patenting a suite of biocompatible polymers for medical applications. Each of the patents and patent publications referred to herein are incorporated by reference in their respective entireties.

**[015]** WO 2013/091001 (PCT/AU2012/001566) relates to polymers, especially polymers

useful as hydrogels, and to the use of hydrogels for repair or restoration of tissue. In particular, the polymers and hydrogels of WO'001 can be used for the repair or restoration of cartilage, especially articular cartilage. The polymers comprise at least a monomer for binding water, a monomer for imparting mechanical properties and a monomer for binding to an extracellular protein. The hydrogels comprise a polymer comprising at least a monomer for binding water and a monomer for binding to an extracellular protein. Crosslinking polymers by binding of the extra-cellular matrix protein forms hydrogels.

**[016]** A preferred polymer disclosed in WO'001 is Poly(NIPAAm-*co*-NAS-*co*-(PLA/HEMA)-*co*-OEGMA), *i.e.*, "PNPHO". The polymer PNPHO preferably comprises OEGMA in an amount of from about 1 and about 15 mol%, PLA/HEMA in an amount of from about 5 and about 50 mol%, NAS in an amount of up to 15 mol%, and NIPAAm in an amount which makes up the remainder to 100% of the polymer composition, for example, from about 50 to about 85 mol%. For certainty, the percentages recited relate to the composition of the final polymer and not the feed amounts utilised when forming the polymer.

**[017]** A preferred form of the polymer PNPHO is a polymer of Formula (I), as drawn below. In addition, x is in the range of 1-1000 and y is in the range of 1-1000 and m, n, p, and q are in the range of 1-20. A person skilled in the art will be aware that the monomers A, B, C and D may be present in the polymer in any order, provided that the required water-binding, strengthening and/or cross-linking capabilities are achieved.



**[018]** WO 2017/035587 (PCT/AU2016/050817) discloses biocompatible materials useful for tissue regeneration and repair, wherein the bioactive polymer may be in the form of a hydrogel, for example a thermoresponsive hydrogel. The bioactive polymer and resulting hydrogel of WO'587 may be used for the regeneration of bone tissue. Accordingly, the reference teaches methods of treating a bone defect in a mammal, the methods comprising administering a therapeutically effective amount of a hydrogel formed by the bioactive polymer to the mammal to treat the bone defect.

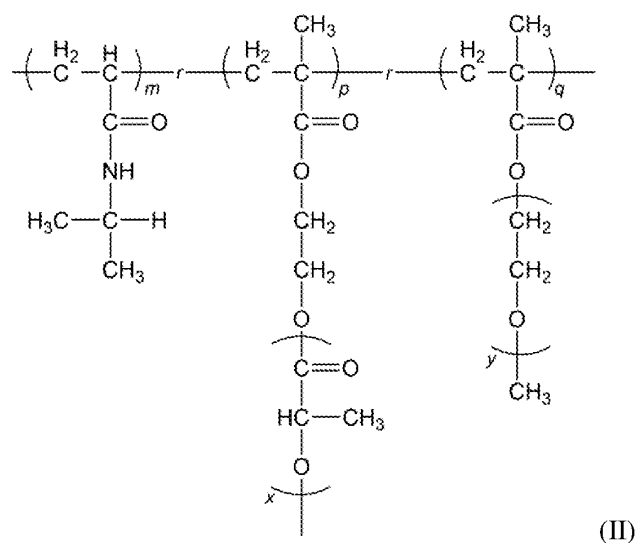
**[019]** WO 2017/015703 (PCT/AU2016/050653) discloses a polymer comprising at least one antiseptic/analgesic/anti-inflammatory monomeric unit in conjunction with at least three further monomeric units, the three further monomeric units eliciting properties selected from the group consisting of: temperature activation, water solubility, mechanical strength, protein/polysaccharide bonding capacity, and combinations thereof. In particular, WO'703 discloses a polymer, wherein the water-soluble monomeric unit is a hydrophilic ethylene glycol (OEGMA) moiety; the mechanical strength-conferring monomeric unit is polylactide-co-2-hydroxy-ethylmethyl acrylate (PLA/HEMA); the protein-reactive monomeric unit is an N-acryloxysuccinimide (NAS) moiety; and the thermosetting monomeric unit is an N- isopropyl acrylamide (NIPAAm) moiety. The antiseptic/analgesic/anti-inflammatory monomeric unit comprises a methacrylic ester derivative of salicylic acid (5-HMA or 4-HMA, or a combination thereof).

**[020]** WO 2021/119727 (PCT/AU2020/051332) teaches a composition comprising a polymer and a natural or synthetic peptide or protein (NSPP) as Thymosin beta-4. The polymer comprises a first monomer for binding water, a second monomer for imparting mechanical properties, a third monomer for binding to an NSPP and a fourth monomer for imparting phase-transition behaviour. In particular, the composition forms an adhesive and flowable hydrogel upon administration into the body or onto the body surface, thereby assists in tissue repair and regeneration. Accordingly, WO'727 discloses methods of tissue repair and/or regeneration, the methods comprising administering the compositions by injection or by administering an aerosol, thereby to form a hydrogel at the body temperature of a mammal.

**[021]** Finally, WO 2023/201397 (PCT/AU2023/050329) discloses a new polymer, "PPHO", *i.e.*, Poly(N-isopropylacrylamide-*co*-(polylactide/2-hydroxy methacrylate)-*co*-(oligo (ethylene glycol) / Poly(NIPAAm-*co*-(PLA/HEMA)-*co*-OEGMA)).

**[022]** The polymer PPHO preferably comprises OEGMA in an amount of from about 1 and about 15 mol%, PLA/HEMA in an amount of from about 5 and about 50 mol%, and

NIPAAm in an amount which makes up the remainder to 100% of the polymer composition, for example, from about 50 to about 85 mol%. In preferred embodiments, PPHO comprises OEGMA in about 1 to 15 mol% and/or PLA/HEMA in about 15 to 50 mol% and/or NIPAAm in an amount of about 50 to 85 mol%. As above, the percentages recited herein relate to the composition of the final polymer and not the feed amounts utilised when forming the polymer. A representative polymer of PPHO (Formula (II)) is shown below.



**[023]** To date, all applications of the PNPPO and PPHO polymer have related to the use of the polymer solution for direct applications such as percutaneous injection, direct syringe extrusion and/or intranasal spray applications. Additionally, all hydrogels formed upon administration exhibit low intrinsic radio-opacity which limit their effective applications for a wide range of intra-arterial applications. Clinical translation of polymer hydrogel embolics into clinical practice fundamentally requires the clinician (*e.g.*, interventional/diagnostic radiologist, vascular surgeon, *etc.*) to visualise the material dynamically during application and delivery. Modern interventional radiology techniques rely on radiological “contrast” with image guided techniques. The predominant contrast media utilised in clinical practice are iodinated agents employed in angiography, computer tomography (CT) and fluoroscopy. MRI contrast media including gadolinium chelates offer alternative media in the context of Magnetic Resonance Imaging. Radioisotopes are widely used in nuclear medicine for diagnostic imaging and are also fundamental to many

emerging interventional imaging techniques and therapies.

**[024]** It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

**[025]** There is a general need in the art for new means of providing transcatheter arterial embolisation (TAE) therapy. As noted above, whereas the technique is widely practised and minimally invasive, many of the known TAE agents are somewhat sub-optimal.

**[026]** It is against this background that the present invention has been developed. Various embodiments of the present invention may find utility relating to one or more of the general needs identified above.

### **Summary of the Invention**

**[027]** The Applicant has surprisingly found that polymer solutions of PNPFO (10 mg/mL to 120 mg/mL) and PPHO (5 mg/mL to 200 mg/mL) are flowable through interventional vascular catheters and micro catheters (1F-6F). Contrast media may or may not be incorporated within the formulations. The resulting solutions transition into an adhesive hydrogel network upon their in-vivo intra-vascular extrusion from the catheter. For intra-vascular applications of these hydrogel systems, their visibility under imaging is required for their safe and accurate application.

**[028]** The chemical and/or ionic interaction between the PNPFO/PPHO polymer and the buffer/excipients used in the formulation of commercially available iodine-based contrast agents prevent the direct dissolution of the polymers within the contrast agents.

Surprisingly, the Applicant found that the initial dissolution of PNPFO and/or PPHO polymer at high concentration (*e.g.*, 200 to 400 mg/mL) in phosphate buffered saline (PBS), Hartmann's solution, or water, followed by their dilution with iohexol solution, resulted in the formation of PNPFO and PPHO solutions with high contrast agent content. The resulting solutions are flowable through microcatheters, *e.g.*, 3.3 French, and form a hydrogel upon their extrusion from the catheter.

**[029]** It is believed that PNPFO and PPHO, can be combined with any contrast agent, including but not limited to x-ray media for CT/angiography/fluoroscopy as well as heavy metal chelates for MRI.

**[030]** It is further believed that such combinations can facilitate the delivery of radioisotopes through catheters for radio-isotopic diagnostic imaging and targeted internal radioisotope therapy including directed PRRTs (peptide receptor radionuclide therapies). The thermoresponsive hydrogel-based combination is flowable (liquid) throughout the

catheter within the body and once applied to the target site forms a cohesive and adhesive hydrogel. The developed system allows for targeted image-guided delivery of diagnostic and therapeutic radioisotopes in interventional radiology and nuclear medicine.

**[031]** Thus, a PNPHO or PPHO based system can be applied for treating diseases or conditions including but not limited to gastrointestinal tract haemorrhage, bleeding from vascular malformations, neoplasms, redistribution of preoperative blood flow, varicoceles, or endoleaks, and tumours.

**[032]** In a broad form, the present invention relates to a flowable polymer solution for intra-arterial/intravascular administration. Preferably, the polymer is “PPHO”, *i.e.*, Poly(N-isopropylacrylamide-*co*-(polylactide/2-hydroxy methacrylate)-*co*-(oligo (ethylene glycol) / Poly(NIPAAm-*co*-(PLA/HEMA)-*co*-OEGMA) or “PNPHO”, *i.e.*, Poly(NIPAAm-*co*-NAS-*co*-(PLA/HEMA)-*co*-OEGMA).

**[033]** According to a first aspect of the present invention there is provided a flowable polymer solution for intravascular (arterial and venous) administration comprising the polymer and at least one solvent, the polymer comprising:

**[034]** a first monomer for binding water;

**[035]** a second monomer for imparting mechanical properties;

**[036]** optionally, a third monomer for binding to a natural or synthetic peptide or protein (NSPP); and

**[037]** a fourth monomer for imparting thermoresponsive phase-transition behaviour.

**[038]** In an embodiment, the polymer solution further comprises at least one radiographic intravenous contrast agent.

**[039]** In an embodiment, the at least one radiographic intravenous contrast agent is selected from contrast media for digital subtraction angiography (DSA), computer tomography (CT) and fluoroscopy such as iodinated contrast media, tantalum, and bismuth-based materials (*e.g.*, bismuth chelate), heavy metal chelates contrast media for MRI including gadolinium agents (*e.g.*, gadobutrol) or MRI nanoparticles.

**[040]** In an embodiment, the at least one contrast agent is an iodine-based contrast agent.

**[041]** In an embodiment, the iodine-based contrast agent is iohexol (1-N,3-N-bis(2,3-dihydroxypropyl)-5-[N-(2,3-dihydroxypropyl)acetamido]-2,4,6-triiodobenzene-1,3-dicarboxamide).

**[042]** In an embodiment, the at least one solvent comprises phosphate buffered saline (PBS) and/or water.

**[043]** In an embodiment, the polymer is Poly(NIPAAm-*co*-NAS-*co*-(PLA/HEMA)-*co*-

OEGMA), *i.e.*, “PNPHO”. The polymer PNPCHO preferably comprises OEGMA in an amount of from about 1 and about 15 mol%, PLA/HEMA in an amount of from about 5 and about 50 mol%, NAS in an amount of up to 15 mol%, and NIPAAm in an amount which makes up the remainder to 100% of the polymer composition, for example, from about 50 to about 85 mol%.

**[044]** In an embodiment, the polymer is Poly(N-isopropylacrylamide-*co*-(poly(lactide/2-hydroxy methacrylate)-*co*-(oligo (ethylene glycol) / Poly(NIPAAm-*co*-(PLA/HEMA)-*co*-OEGMA) *i.e.*, “PPHO”. The polymer PPHO preferably comprises OEGMA in an amount of from about 1 and about 15 mol%, PLA/HEMA in an amount of from about 5 and about 50 mol%, and NIPAAm in an amount which makes up the remainder to 100% of the polymer composition, for example, from about 50 to about 85 mol%.

**[045]** In an embodiment, the first monomer is selected from: polyethers, polyvinyl alcohol (PVA); poly(vinyl pyrrolidone) (PVP); poly(amino acids) and dextran.

**[046]** In an embodiment, the polyethers are selected from: polyethylene glycol (PEG), oligo(ethylene glycol) (OEG), polyethylene oxide (PEO), polyethylene oxide-*co*-propylene oxide (PPO), *co*-polyethylene oxide block or random copolymers thereof.

**[047]** In an embodiment, the first monomer is oligo (ethylene) glycol monomethyl ether methacrylate (OEGMA).

**[048]** In an embodiment, the second monomer is a methacrylate, or a random *co*-polymer comprising a methacrylate.

**[049]** In an embodiment, the second monomer is selected from: hydroxyethyl methacrylate (HEMA), a hydroxyethyl methacrylate poly(lactic acid) copolymer (PLA/HEMA), poly(lactic acid), poly(caprolactone), poly(glycolide), poly(glycolide-*co*-lactide) or poly(glycolide-*co*-caprolactone).

**[050]** In an embodiment, the second monomer is hydroxyethyl methacrylate poly(lactic acid) (PLA/HEMA).

**[051]** In an embodiment, the third monomer has electrophilic functional groups for binding to the NSPP.

**[052]** In an embodiment, the third monomer is selected from: N-hydroxysulfosuccinimide (SNHS), N-hydroxyethoxylated succinimide (ENHS), and N-acryloxysuccinimide (NAS).

**[053]** In an embodiment, the third monomer is N-acryloxysuccinimide (NAS).

**[054]** In an embodiment, the fourth monomer has a lower critical solution temperature (LCST) less than about 37 °C.

**[055]** In an embodiment, the fourth monomer is selected from: poly(ethylene

oxide)/poly(propylene oxide) and poly(N-isopropylacrylamide) (PNIPAAm) homopolymers and copolymers.

[056] In an embodiment, the fourth monomer is (N-isopropylacrylamide) (NIPAAm).

[057] In an embodiment, the polymer comprises the first monomer in an amount of from about 1 to about 15 mol%.

[058] In an embodiment, the polymer comprises the second monomer in an amount of from about 5 to about 50 mol%.

[059] In an embodiment, the polymer comprises the third monomer in an amount of from about 0 to about 15 mol%.

[060] In an embodiment, the polymer comprises the fourth monomer in an amount of from about 50 to about 85 mol%.

[061] In an embodiment, the polymer comprises: the first monomer in an amount of from about 1 to about 15 mol%; the second monomer in an amount of from about 5 to about 50 mol%; the third monomer in an amount of 0 to about 15 mol%; and the fourth monomer in an amount which makes up the remainder to 100% of the polymer.

[062] In an embodiment, the first monomer is OEGMA; the second monomer is PLA/HEMA; the third monomer is NAS; and the fourth monomer is NIPAAm,

[063] wherein the polymer comprises: OEGMA in an amount of from about 1 to about 15 mol%; PLA/HEMA in an amount of from 5 to about 50 mol%; NAS in an amount of from 0 to about 15 mol%; and NIPAAm in an amount of up to about 85 mol%.

[064] In an embodiment, the third monomer is present. In this embodiment, the polymer is Poly(NIPAAm-co-NAS-co-(PLA/HEMA)-co-OEGMA), *i.e.*, “PNPHO”. The polymer PNPHO preferably comprises OEGMA in an amount of from about 1 and about 15 mol%, PLA/HEMA in an amount of from about 5 and about 50 mol%, NAS in an amount of up to 15 mol%, and NIPAAm in an amount which makes up the remainder to 100% of the polymer composition, for example, from about 50 to about 85 mol%.

[065] In an embodiment, the polymer solution can be mixed *in situ* with one or more cargo compounds selected from therapeutics, drugs or isotopes, or a combination thereof prior to administration to dilute the polymer solution accordingly.

[066] In an embodiment, the one or more cargo compounds are mixed with the polymer solution via direct syringe-syringe mixing.

[067] In an embodiment, the syringes for mixing cool down the polymer solution and one or more cargo compounds during mixing.

[068] In an embodiment, the syringes for mixing protect the operator against radiation

exposure.

**[069]** In an embodiment, the polymer solution has a concentration of polymer between about 10 mg/mL and about 120 mg/mL.

**[070]** Preferably, the polymer solution has a concentration of polymer of about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 or about 120 mg/mL.

**[071]** In an embodiment, the third monomer is absent. In this embodiment, the polymer is Poly(N-isopropylacrylamide-*co*-(polylactide/2-hydroxy methacrylate)-*co*-(oligo (ethylene glycol) / Poly(NIPAAm-*co*-(PLA/HEMA)-*co*-OEGMA) *i.e.*, “PPHO”. The polymer PPHO preferably comprises OEGMA in an amount of from about 1 and about 15 mol%, PLA/HEMA in an amount of from about 5 and about 50 mol%, and NIPAAm in an amount which makes up the remainder to 100% of the polymer composition, for example, from about 50 to about 85 mol%.

**[072]** In an embodiment, the polymer solution has a concentration of polymer between about 5 mg/mL and about 200 mg/mL.

**[073]** Preferably, the polymer solution has a concentration of polymer of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, or about 200 mg/mL.

**[074]** In an embodiment, the polymer is PNPCHO or PPHO. In this embodiment, administering the polymer at a treatment site is performed by injection through a catheter.

**[075]** In an embodiment, the catheter is a fine gauge catheter, such as a French 3.3 mm catheter, or similar.

**[076]** In an embodiment, the intra-arterial/intravascular administration is part of a

transcatheter arterial embolisation (TEA) procedure.

[077] In an embodiment, the polymer solution comprises both the polymer with the third monomer present (*i.e.*, PNPFO) and the polymer with the third monomer absent (*i.e.*, PPHO).

[078] In an embodiment, the polymer solution comprises about 10 mg/mL of PNPFO to ionically bond with a compound and about 50 mg/mL PPHO to provide adhesion and flowability. It will be appreciated that altering the relative proportions of PNPFO and PPHO allows these characteristics to be tuned according to the specific circumstances.

[079] According to a second aspect of the invention there is provided a method of performing a transcatheter arterial embolisation (TEA) procedure, the method comprising administering to a subject in need of such treatment a polymer solution according to the first aspect of the present invention.

[080] In an embodiment, administering the polymer solution is performed at body temperature.

[081] In an embodiment, the thermoresponsive polymer solution forms an adhesive hydrogel network upon extrusion from a catheter.

[082] According to a third aspect of the invention there is provided use of a polymer solution according to the first aspect of the present invention in the manufacture of a medicament or delivery vehicle for administration via a transcatheter arterial embolisation (TEA) procedure.

[083] In an embodiment, administering the polymer solution is performed at body temperature.

[084] In an embodiment, the thermoresponsive polymer solution forms an adhesive hydrogel network upon extrusion from a catheter.

[085] According to a fourth aspect of the invention there is provided a kit for forming a flowable polymer solution for intra-arterial/intravascular administration, the kit comprising a polymer comprising:

[086] a first monomer for binding water;

[087] a second monomer for imparting mechanical properties;

[088] optionally, a third monomer for binding to a natural or synthetic peptide or protein (NSPP);

[089] a fourth monomer for imparting thermoresponsive phase-transition behaviour; and

[090] at least one solvent for forming a solution of the polymer; and

[091] means for administering the polymer solution; and

[092] optionally instructions for administering the polymer solution via a transcatheter arterial embolisation (TEA) procedure.

### **Definitions and Nomenclature**

[093] In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out below. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only and is not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one having ordinary skill in the art to which the invention pertains.

[094] Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise”, “comprising”, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”.

[095] The terms “preferred” and “preferably” refer to embodiments of the invention that may afford certain benefits, under certain circumstances. However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful and is not intended to exclude other embodiments from the scope of the invention.

[096] As used herein a wording defining the limits of a range or length such as, for example, “from 1 to 5” means any integer from 1 to 5, *i.e.*, 1, 2, 3, 4 and 5. In other words, any range defined by two integers explicitly mentioned is meant to comprise and disclose any integer defining the limits and any integer comprised in the range.

[097] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein are to be understood as modified in all instances by the term “about”. The examples are not intended to limit the scope of the invention. In what follows, or where otherwise indicated, “%” will mean “weight %”, “ratio” will mean “weight ratio” and “parts” will mean “weight parts”.

[098] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard variations found in their respective testing measurements.

[099] The present specification uses the following abbreviations:

|          |                                                                                                                                                                                                                                                                           |
|----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SEHNS    | N-hydroxyethoxylated succinimide                                                                                                                                                                                                                                          |
| HEMA     | Hydroxyethyl methacrylate                                                                                                                                                                                                                                                 |
| LA       | Lactic acid                                                                                                                                                                                                                                                               |
| NAS      | N-acryloxysuccinimide                                                                                                                                                                                                                                                     |
| NIPAAm   | N-isopropylacrylamide                                                                                                                                                                                                                                                     |
| NSPP     | Natural or synthetic peptide or protein                                                                                                                                                                                                                                   |
| OEG      | Oligo(ethylene glycol)                                                                                                                                                                                                                                                    |
| OEGMA    | Oligo(ethylene) glycol monomethyl ether methacrylate                                                                                                                                                                                                                      |
| PBS      | Phosphate-buffered saline                                                                                                                                                                                                                                                 |
| PEG      | Polyethylene glycol                                                                                                                                                                                                                                                       |
| PEO      | Polyethylene oxide                                                                                                                                                                                                                                                        |
| PLA      | Poly(lactic acid)                                                                                                                                                                                                                                                         |
| PLA/HEMA | Hydroxyethyl methacrylate poly(lactic acid)                                                                                                                                                                                                                               |
| PPO      | Polyethylene oxide- <i>co</i> -propylene oxide                                                                                                                                                                                                                            |
| PVA      | Polyvinyl alcohol                                                                                                                                                                                                                                                         |
| PVP      | Poly(vinyl pyrrolidone)                                                                                                                                                                                                                                                   |
| PNPHO    | Poly(N-isopropylacrylamide- <i>co</i> -(N-acryloxysuccinimide)- <i>co</i> -(polylactide/2-hydroxy methacrylate)- <i>co</i> -(oligo (ethylene glycol)) / Poly(NIPAAm- <i>co</i> -NAS- <i>co</i> -(PLA/HEMA)- <i>co</i> -OEGMA), <i>e.g.</i> , Formula (I) as defined above |
| PPHO     | Poly(N-isopropylacrylamide- <i>co</i> -(polylactide/2-hydroxy methacrylate)- <i>co</i> -(oligo (ethylene glycol)) / Poly(NIPAAm- <i>co</i> -(PLA/HEMA)- <i>co</i> -OEGMA), <i>e.g.</i> , Formula (II) as defined above                                                    |

### Brief Description of the Drawings

[0100] Preferred embodiments of the invention will now be described, by way of example only, with reference to the accompanying drawings in which:

[0101] *Figure 1* relates to *Example 7*, below. In this example, 22.5 mL of “TL200” (defined below) was diluted with 7.5 mL of 350 mg I/mL to achieve a PPHO concentration of 150 mg/mL and 90 mg I/mL. The resulting solution was then injected subcutaneously in a warm cadaveric porcine model. In total nearly 5 mL of the product was injected. After administration, a CT-scan of the site was used to verify the presence of the hydrogel and its thermoresponsive and radiopaque properties. Injection of 5 mL of TL150-IOP090 in a cadaveric subcutaneous model forms a hydrogel (white gel, shown in black arrows) at the

administration site (*Figure 1(a)*). A CT-scan of the injected site showed the presence of radiopaque white structures at the administration site, shown by white arrows (*Figure 1(b)*).

**[0102]** *Figure 2* relates to *Example 8*, below. The results confirmed that it is feasible to form injectable/flowable formulations with different Hounsfield units to match the required clinical needs. A linear regression with  $R^2 = 0.973$  is achieved, allowing further product formulations.

**[0103]** *Figure 3* relates to *Example 9*, below. In this example, the “TP050/TL50-IOP45” solution was used to test the flowability of the formulation through medical catheters (Excelsior XT-27). To simulate the physiological condition, the catheter was submerged in a pre-warmed PBS for 5 minutes. Thereafter, the catheter was primed with warmed water for injection. The solution was delivered through the catheter, using a standard syringe for injection. *Figure 3* confirms that the solution flows through the catheter, forming a cohesive string of hydrogel once reaching the physiological temperature.

**[0104]** *Figure 4* relates to *Example 11*, below. In this example, the “TP100-IOP45” solution was used to test the flowability of the formulation through medical catheters as described. To simulate the physiological condition, the catheter was submerged in a pre-warmed PBS for 5 minutes. Thereafter, the catheter was primed with warmed water for injection. The solution was delivered through the catheter, using a standard syringe for injection. *Figure 4* confirms that the solution flows through the catheter, forming a cohesive string of hydrogel once reaching the physiological temperature.

**[0105]** *Figure 5* relates to *Example 12*, below. In this example, the “TP050/TL50-IOP240” solution was used to test the flowability of the formulation through catheters and an *in vitro* vessel model under fluoroscopy imaging. Phosphate buffer solution (PBS) was pumped at 37 °C into the simulated *in vitro* vessel model through a pump. For this study, a 2F Trevo catheter was used. TP050/TL50-IOP240 solution was delivered through the catheter, using a standard syringe for injection. The result, shown in *Figure 5(a)*, confirmed that the solution flows through the catheter and forms a solid white coil-like gel at the tip of the catheter upon entering the flow within the vessel model. Also, the formation of the gel was observable through live fluoroscopy. Similar results were observed by passing TL050/TP050-IOP225 through a 2.9F Maestro catheter, as shown in *Figure 5(b)*.

**[0106]** *Figure 6* relates to *Example 13*, below. In this example, the “TL050/TP050-IOP240” solution was used to test the flowability of the formulation through a 2F Trevo

catheter (as an example) within microvessels in an *ex vivo* renal porcine model. The results, shown in *Figure 6(a)* and *Figure 6(b)*, confirmed that the formulation was flowable and can flow within the targeted microvessels, visible under live fluoroscopy imaging.

### Detailed Description of the Embodiments

[0107] The present invention will now be more fully described with reference to the accompanying examples and drawings. It should be understood, however, that the description following is illustrative only and should not be taken in any way as a restriction on the generality of the invention described above.

[0108] Reference will now be made in detail to certain embodiments of the invention. While the invention will be described in conjunction with the embodiments, it will be understood that the intention is not to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present invention as defined by the claims.

[0109] One skilled in the art will recognise many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. The present invention is in no way limited to the methods and materials described.

[0110] It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

[0111] Disclosed broadly herein is the use of a biocompatible polymer for administration to a treatment site at or proximal with a target tissue (*e.g.*, the human prostate), the polymer thereby forming a hydrogel at body temperature to provide physical spacing between the tissue and nearby cells/tissues/organs (*e.g.*, the human rectum). Such physical spacing/isolation of the prostate from the rectum allows radiation therapy to focus on the isolated target tissue without substantially affecting the cells/tissues/organs spaced from it by the polymer hydrogel.

### Polymers

[0112] The term “polymer”, as used herein, refers to a large molecule (macromolecule) composed of repeating structural units (monomers). These subunits are typically connected by covalent chemical bonds. Polymers can be linear or branched polymers. Preferably, the

polymers of the present invention are copolymers comprising three or more different monomers. For example, in one embodiment, the polymer of the present invention includes a first water-binding monomer, a second monomer that is capable of imparting mechanical properties to the polymer, and a third monomer that has a functional group for binding to an NSPP.

**[0113]** The term “monomer”, as used herein, refers to a structural unit that can be combined to form a polymer, but that itself may also be a polymer, or a derivative of a monomer or polymer. Monomers of this type are herein also referred to as “macromonomers”. Herein, a “macromonomer” is a polymer or oligomer the molecules of which each have one end-group that acts as a monomeric molecule, so that each polymer or oligomer molecule contributes only a single monomer unit to a chain of the product polymer.

**[0114]** The polymer of the present invention comprises: a first monomer for binding water; a second monomer for imparting mechanical properties to the polymer; an optional third monomer for binding to a natural or synthetic peptide or protein (NSPP); and a fourth monomer for imparting phase-transition behaviour.

*First monomer: Water-binding monomer*

**[0115]** As discussed above, the advantages of the polymer of the present invention can be attributed, at least in part, to the particular components that make up the polymers of the present invention. A particularly advantageous property of the polymers of the present invention is their water-binding capacity. The presence of water in the polymer of the present invention provides an environment that resembles both that of the natural environment of the damaged tissue (which assists in tissue regeneration) and the required compression resistance to the polymer.

**[0116]** Accordingly, the preferred polymers used herein should include monomers or units that are able to bind water to such a capacity that a malleable structure is able to form when the polymer is hydrated. In addition, the structure thus formed should have the required compression resistance and resilience.

**[0117]** A person skilled in the art will understand that water-binding monomers need to be present in the polymers of the present invention in proportions that are sufficient to produce a polymer that fulfils these requirements. Generally, the proportion of water-binding monomers in the polymer is about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, about 1:20, about 1:30, about 1:40, about 1:50 molar ratio

of water binding:mechanical strength monomers. In fact, the water-binding monomers need to make the polymer not only hydrophilic, but impart much more significant water-binding capacities to the polymer. Accordingly, polymers in accordance with the present invention will have water-binding capacities of between about 70% and about 500%, between about 80% and about 400%, between about 90% and 300% or between about 100% and 200%. For example, the water-binding capacity of the polymers of the present invention is about 70%, about 80%, about 90%, about 100%, about 110%, about 120%, about 130%, about 140%, about 150%, about 160%, about 170%, about 180%, about 190%, about 200%, about 210%, about 220%, about 230%, about 240%, about 250%, about 260%, about 270%, about 280%, about 290%, about 300%, about 310%, about 320%, about 330%, about 340%, about 350%, about 360%, about 370%, about 380%, about 390%, about 400%, about 410%, about 420%, about 430%, about 440%, about 450%, about 460%, about 470%, about 480%, about 490%, or about 500%.

**[0118]** Suitable examples of water-binding monomers include those that can be synthesised into polymers such as polyethers (*e.g.*, alkylene polyoxides such as polyethylene glycol (PEG), oligo(ethylene glycol) (OEG), polyethylene oxide (PEO), polyethylene oxide-co-propylene oxide (PPO), co-polyethylene oxide block or random copolymers, polyvinyl alcohol (PVA)), poly(vinyl pyrrolidinone) (PVP), poly(amino acids) and dextran. The polyethers, and more particularly oligo(oxyalkylenes) (*e.g.*, OEG), are especially preferred, because they have the requisite water-binding capacity, are simple to synthesise and/or purchase, and are inert, in the sense that they illicit minimal or no immune response from the tissues into which they are placed.

**[0119]** In addition, any of a variety of hydrophilic functionalities can be used to make a monomer (and therefore a polymer formed from such a monomer) water soluble. For example, functional groups like phosphate, sulfate, quaternary amine, hydroxyl, amine, sulfonate and carboxylate, which are water soluble, may be incorporated into a monomer to make it water soluble.

**[0120]** Monomers may also be reacted with other compounds to form “macromonomers”. Thus, the first monomer may optionally be a macromonomer.

**[0121]** A preferred first monomer which is a macromonomer is oligo(ethyleneglycol) monomethyl ether methacrylate (OEGMA), which is a hydrophilic monomer composed of two hydrophilic monomers: ethylene glycol and methacrylate.

**[0122]** Preferably, the polymer comprises the first monomer in an amount of from about 1 to about 15 mol%. In various embodiments, the first monomer may be present in about 1,

about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, or about 15 mol%. In various embodiments, the first monomer may be present from about 1 to about 15, about 2 to about 14, about 3 to about 13, about 4 to about 12, about 5 to about 11, about 6 to about 10, about 7 to about 9, or about 8 mol%.

*Second monomer: Monomer imparting mechanical properties*

**[0123]** As discussed above, the advantageous properties of the polymer of the present invention can be attributed, in part, to the particular components that make up the polymers of the present invention. In some embodiments, the polymers of the present invention are able to contribute additional mechanical properties and adhesivity to the polymer of the present invention.

**[0124]** A person skilled in the art will understand that monomers capable of imparting mechanical properties to a polymer need to be present in the polymers of the present invention in proportions that are sufficient to produce a polymer having the desired mechanical properties. Generally, the proportion of “mechanical” monomers in the polymer is about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, about 1:20, about 1:30, about 1:40, about 1:50 molar ratio of water binding:mechanical strength monomers. Suitable examples of monomers that are capable of imparting mechanical properties (e.g. compression resistance) to a polymer include acrylates such as hydroxyethyl methacrylate (HEMA), polyesters such as poly(lactic acid), poly(caprolactone), poly(glycolide), and their random co-polymers (e.g. poly(glycolide-*co*-lactide) and poly(glycolide-*co*-caprolactone)).

**[0125]** Monomers may also be reacted with other compounds to form “macromonomers”. A preferred second monomer which is a macromonomer is hydroxyethyl methacrylate poly(lactic acid) (PLA/HEMA).

**[0126]** Preferably, the polymer comprises the second monomer in an amount of from about 1 to about 50 mol%. In various embodiments, the second monomer may be present in about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, or about 50 mol%.

[0127] In various embodiments, the second monomer may be present from about 1 to about 15, about 2 to about 49, about 3 to about 48, about 4 to about 47, about 5 to about 46, about 6 to about 45, about 7 to about 44, about 8 to about 43, about 9 to about 42, about 10 to about 41, about 11 to about 40, about 12 to about 39, about 13 to about 38, about 14 to about 37, about 15 to about 36, about 16 to about 35, about 17 to about 34, about 18 to about 33, about 19 to about 34, about 20 to about 33, about 21 to about 30, about 22 to about 29, about 23 to about 28, about 24 to about 27, or about 25 to about 26 mol%.

[0128] A person skilled in the art would understand that the amount of the second monomer occupies a broader range than the other monomers as mechanical strength and adhesivity are the critical factors in the present invention.

*Third monomer: NSPP-binding monomer*

[0129] As discussed above, the polymer used in the present invention can optionally be formed by combining the polymer with an NSPP. In order to effectively combine the polymer with the NSPP, preferably monomers or units that have a crosslinking ability are included in the polymer.

[0130] This crosslinking ability means that the polymers are able to bind to NSPPs and, by doing so, crosslink the NSPP to form polymer containing the NSPP. Alternatively, via a similar mechanism, the NSPPs act as the crosslinker, thereby crosslinking the polymer to form a polymer.

[0131] In order to produce a polymer that is capable of binding to NSPPs, a person skilled in the art will understand that monomers capable of binding to an NSPP need to be present in the polymers of the present invention in proportions that are sufficient to crosslink with an NSPP, such that a polymer can be formed in the presence of water. Generally, the proportion of “crosslinking” monomers in the polymer is at about 15:1, about 10:1, about 5:1, about 4:1, about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, about 1:15 of crosslinking monomer:water binding monomer.

[0132] Monomers that are capable of binding to NSPPs generally have either electrophilic or nucleophilic functional groups, such that a nucleophilic functional group on, for example, an NSPP may react with an electrophilic functional group on the monomer, to form a covalent bond.

[0133] Therefore, for example, if an NSPP has nucleophilic functional groups such as amines, the polymer may have electrophilic functional groups such as N-hydroxysuccinimides (NHS). Other electrophilic functional groups that are suitable for use

in the present invention are N-hydroxysulfosuccinimide (SNHS) and N-hydroxyethoxylated succinimide (ENHS). An example of a monomer of this type is N-acryloxysuccinimide (NAS). On the other hand, if an NSPP has electrophilic functional groups, then the polymer may have nucleophilic functional groups such as amines or thiols.

**[0134]** Preferably, the polymer comprises the third monomer in an amount of up to 15 mol%. In various embodiments, the third monomer may be present in about 0, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, or about 15 mol%. In various embodiments, the third monomer may be present from about 0 to about 1, about 1 to about 15, about 2 to about 14, about 3 to about 13, about 4 to about 12, about 5 to about 11, about 6 to about 10, about 7 to about 9, or about 8 mol%.

**[0135]** A skilled person would understand that the polymer may be formed from hydrophobic compositions, therefore the third monomer is optional in the polymer.

*Fourth monomer: Phase-transition monomer*

**[0136]** In another embodiment of the present invention, the polymer may further include a fourth monomer that is capable of imparting phase transition characteristics to the polymer, thereby ensuring post-administration stability of the polymer. Further, these phase-transition characteristics allow the polymers of the present invention to form polymer, of which various properties (such as viscosity) can be varied by altering factors such as pH and temperature. The polymers are designed such that the lower critical solution temperature (LCST) is below body temperature. Various thermo-responsive and injectable polymers including poly(ethylene oxide)/poly(propylene oxide) and poly(N-isopropylacrylamide) (PNIPAAm) copolymers are suitable for use in the present invention.

**[0137]** Generally, the proportion of phase-transition monomers in the polymer is at least about 3:1 molar ratio of phase-transition monomer:water binding monomer. This ratio can increase to, for example, about 10:1, about 11:1, about 12:1, about 13:1, about 14:1, about 15:1, about 16:1, about 17:1, about 18:1, about 19:1, about 20:1, about 25:1, about 30:1, about 35:1, about 40:1, about 45:1, about 50:1, about 55:1, about 60:1, about 65:1, about 70:1 molar ratio, about 75:1, about 80:1 and about 85:1 of phase-transition monomer:water binding monomer.

**[0138]** Preferably, the polymer comprises the fourth monomer in an amount which makes

up the remainder to 100% of the polymer composition. In an embodiment, the mol% of the fourth monomer can be up to about 85%, preferably, about 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84 or 85 mol%.

#### *Other polymer properties*

**[0139]** It will be understood by a person skilled in the art that, by combining different types of monomers, polymers can be produced that have a range of different properties. In addition, by incorporating particular monomers or functional groups into a pre-existing polymer, the properties of the polymer can be modified. For example, co-polymerisation of HEMA monomers with other monomers (such as methyl methacrylate) can be used to modify properties such as swelling and mechanical properties. Monomers may also be reacted with other compounds to form macromonomers (defined above) that are then included in the polymers of the present invention. For example, HEMA can be reacted with lactide to form a HEMA-poly-lactic acid polymer (PLA/HEMA), which itself can be used as a monomer in the polymers of the present invention. In addition, the monomers themselves may be combinations of monomer units, which are then incorporated into the polymer. An example of this type of monomer is oligo(ethylene glycol) monomethyl ether methacrylate (OEGMA), which is a hydrophilic monomer composed of two hydrophilic monomers: ethylene glycol and methacrylate.

**[0140]** The preferred polymers of the present invention may be further modified with one or more moieties and/or functional groups. Any moiety or functional group can be used in accordance with the present invention. In some embodiments, polymers may be modified with polyethylene glycol (PEG), with a carbohydrate, and/or with acyclic polyacetals derived from polysaccharides. In addition, as discussed above, hydrophilic groups can be incorporated into monomers (and therefore polymers) to increase the water-binding capacity of the polymer.

**[0141]** In terms of sequence, copolymers may be block copolymers, graft copolymers, random copolymers, blends, mixtures, and/or adducts of any of the foregoing and other polymers. Typically, polymers in accordance with the present invention are organic polymers. Preferably, the polymers of the present invention are biocompatible. In some embodiments, the polymers are biodegradable. In other embodiments, the polymers are both biocompatible and biodegradable.

**[0142]** The preferred polymers of the present invention may also include other monomers

in their structure. For example, the monomers may be polymers such as poly(vinyl alcohol) (PVA), polyesters, acrylic polymers and ionic polymers, or monomers of these.

**[0143]** If it is desired that the polymer be biodegradable or absorbable, one or more monomers having biodegradable linkages may be used. In the alternative, or in addition, the monomers may be chosen such that the product of the reaction between them results in a biodegradable linkage. For each approach, monomers and/or linkages may be chosen such that the resulting biodegradable polymer will degrade or be absorbed in a desired period of time, *e.g.*, from about 6 h to about 6 months. Preferably, the monomers and/or linkages are selected such that, when the polymer degrades under physiological conditions, the resulting products are non-toxic.

**[0144]** The biodegradable linkage may be chemically or enzymatically hydrolysable or absorbable. Illustrative chemically-hydrolysable biodegradable linkages include polymers, copolymers and oligomers of glycolide, lactide, caprolactone, dioxanone, and trimethylene carbonate. Illustrative enzymatically-hydrolysable biodegradable linkages include peptidic linkages cleavable by metalloproteinases and collagenases. Additional illustrative biodegradable linkages include polymers and copolymers of poly(hydroxy acid)s, poly(orthocarbonate)s, poly(anhydride)s, poly(lactone)s, poly(aminoacid)s, poly(carbonate)s, and poly(phosphonate)s.

**[0145]** The chemical hydrolysis of lactide in the invention results in the increase of lower critical solution temperature (LCST) of the polymer (by decreasing the overall hydrophobicity of the polymer) and thus its bioresorptive capacity.

#### *Preferred polymers*

**[0146]** The polymer preferably comprises the first monomer in an amount of from about 1 to about 15 mol%. In various embodiments, the first monomer may be present in about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, or about 15 mol%. Preferably, the first monomer is OEGMA.

**[0147]** The polymer preferably comprises the second monomer in an amount of from about 5 to about 50 mol%. In various embodiments, the second monomer may be present in about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46,

about 47, about 48, about 49, or about 50 mol%. Preferably, the second monomer is PLA/HEMA.

**[0148]** The polymer preferably comprises the third monomer in an amount of up to 15 mol%. In various embodiments, the third monomer may be present in about 0, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, or about 15 mol%. Preferably, the third monomer is NAS.

**[0149]** The polymer preferably comprises the fourth monomer in an amount which makes up the remainder to 100% of the polymer composition, for example, from about 50 and about 85 mol%. In an embodiment, the mol% of the fourth monomer can be up to about 85%, preferably, about 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84 or 85 mol%. Preferably, the fourth monomer is NIPAAm.

**[0150]** The percentages recited herein relate to the composition of the final polymer and not the feed amounts utilised when forming the polymer.

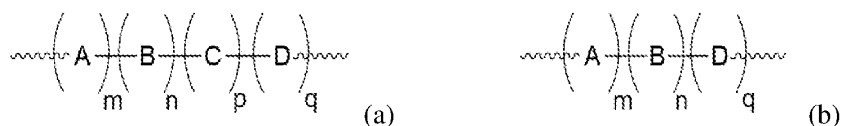
**[0151]** In one embodiment, the polymer preferably comprises: the first monomer in an amount of from about 1 to about 15 mol%; the second monomer in an amount of from about 5 to about 50 mol%; the third monomer in an amount of up to 15 mol%; and the fourth monomer in an amount of up to about 85 mol%.

**[0152]** Preferably, the first monomer is OEGMA, the second monomer is PLA/HEMA, the third monomer is NAS and the fourth monomer is NIPAAm.

**[0153]** In another embodiment, the polymer preferably comprises: the first monomer in an amount of about 7 mol%; the second monomer in an amount of about 30 mol%; the third monomer in an amount of about 7 mol%; and the fourth monomer in an amount of about 53 mol%.

**[0154]** Preferably, the first monomer is OEGMA, the second monomer is PLA/HEMA, the third monomer is NAS and the fourth monomer is NIPAAm.

**[0155]** In one embodiment, the polymer of the present invention is a polymer of Formula (a) or Formula (b):



**[0156]** wherein

**[0157]** A is the first monomer (a water-binding monomer), for example, OEGMA;

**[0158]** B is the second monomer (a monomer that is capable of imparting mechanical

properties to a polymer), for example, PLA/HEMA;

**[0159]** C, if present, is the third monomer (a monomer that has a functional group for binding to an NSPP), for example, NAS; and

**[0160]** D is the fourth monomer (a monomer that is capable of imparting phase transition characteristics to the polymer), for example, NIPAAm.

**[0161]** In various embodiments, m is an integer from 1 to 20; n is an integer from 1 to 20; p is an integer from 0 to 20; and q is an integer from 1 to 20.

**[0162]** Exemplary polymers of the present invention are represented by Formula (a) and/or Formula (b), as shown above, wherein A is the water-binding monomer OEGMA, B is the strengthening monomer PLA/HEMA, C, if present, is the crosslinker NAS, D is the phase transition monomer NIPAAm, and m, n and p, q, x and y are as defined above.

**[0163]** A person skilled in the art will be aware that the monomers A, B, C and D may be present in the polymer in any order, provided that the required water-binding, strengthening and/or cross-linking capabilities are achieved.

**[0164]** It has also been discovered that some monomers, such as PLA/HEMA, polyesters such as poly(lactic acid), poly(caprolactone), poly(glycolide), and their random copolymers (*e.g.*, poly(glycolide-co-lactide) and poly(glycolide-co-caprolactone) and other biodegradable and biocompatible polymers, can elevate the LCST of the preferred polymer used in the present invention during degradation of biodegradable segments (*e.g.*, PLA) *in vivo*, leading to bioresorption of the polymer. This provides the additional advantage that the polymers used in the present invention may be designed so as to be biodegradable *in vivo*.

**[0165]** The overall size of the preferred polymer used in the present invention may differ, depending on factors such as the types of monomers that are incorporated into the polymer, the type of NSPP that is sought to be used to form the polymer, and the conditions under which the protein is to be coupled to the polymer. However, in general, the preferred polymer used in the present invention may be a molecule of about 1 to about 100 kDa, about 5 to about 60 kDa, or about 30 kDa. In various embodiments, the polymer of the present invention may be a molecule of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or about 100 kDa.

*PNPHO – Formula (I)*

[0166] A preferred polymer of the present invention is Poly(NIPAAm-*co*-NAS-*co*-(PLA/HEMA)-*co*-OEGMA), *i.e.*, “PNPHO”. The polymer PNPHO preferably comprises OEGMA in an amount of from about 1 and about 15 mol%, PLA/HEMA in an amount of from about 5 and about 50 mol%, NAS in an amount of up to 15 mol%, and NIPAAm in an amount which makes up the remainder to 100% of the polymer composition, for example, from about 50 to about 85 mol%.

[0167] The percentages recited herein relate to the composition of the final polymer and not the feed amounts utilised when forming the polymer.

[0168] In one embodiment, preferably the polymer comprises:

[0169] OEGMA in an amount of from about 3 to about 8 mol% (for example from about 4 to about 6 mol%);

[0170] HEMA-PLA in an amount of from about 5 to about 9 mol% (for example from about 6 to about 8 mol%);

[0171] NAS in an amount of at least about 7 mol%; and

[0172] NIPAAm in an amount of up to about 85 mol% (for example up to about 81 mol%).

[0173] In another embodiment, the polymer comprises:

[0174] OEGMA in an amount of about 5 mol%;

[0175] HEMA-PLA in an amount of about 7 mol%;

[0176] NAS in an amount of about 7 mol%; and

[0177] NIPAAm in an amount of about 81 mol%.

[0178] A preferred form of the polymer PNPHO for use in the present application is a polymer of Formula (I), as drawn above.

[0179] Based on Formula I, defined previously:

[0180] A is oligo (ethylene) glycol monomethyl ether methacrylate OEGMA;

[0181] B is hydroxyethyl methacrylate poly(lactic acid) (HEMA-PLA);

[0182] C is N-acryloxysuccinimide (NAS); and

[0183] D is N-isopropylacrylamide (NIPAAm).

[0184] A preferred form of the polymer PNPHO for use in the present application is a polymer of Formula (I), as drawn above. In addition, x is in the range of 1-1000 and y is in the range of 1-1000 and m, n, p, and q are in the range of 1-20.

[0185] A person skilled in the art will be aware that the monomers A, B, C and D may be present in the polymer in any order, provided that the required water-binding,

strengthening and/or cross-linking capabilities are achieved.

*PPHO – Formula (II)*

**[0186]** Another preferred polymer of the present invention is Poly(NIPAAm-*co*-(PLA/HEMA)-*co*-OEGMA), *i.e.*, “PPHO”. The polymer PPHO preferably comprises OEGMA in an amount of from about 1 and about 15 mol%, PLA/HEMA in an amount of from about 5 and about 50 mol%, and NIPAAm in an amount which makes up the remainder to 100% of the polymer composition, for example, from about 50 to about 85 mol%. In preferred embodiments, PPHO comprises OEGMA in about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or about 15 mol% and/or PLA/HEMA in about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or about 50 mol% and/or NIPAAm in an amount of about 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84 or about 85 mol%.

**[0187]** The percentages recited herein relate to the composition of the final polymer and not the feed amounts utilised when forming the polymer.

**[0188]** A preferred form of the polymer PPHO for use in the present application is a polymer of Formula (II), as drawn below. In addition, x is in the range of 1-1000 and y is in the range of 1-1000 and m, n, and q are in the range of 1-20.

**[0189]** A person skilled in the art will be aware that the monomers A, B, and D may be present in the polymer in any order, provided that the required water-binding, strengthening and/or cross-linking capabilities are achieved.

*Synthesis of polymers*

**[0190]** A person skilled in the art will be aware of suitable methods of synthesising the preferred polymers used in the present invention. These include methods such as ring-opening polymerisation, addition polymerisation (including free radical polymerisation) and condensation polymerisation.

**[0191]** The formation of the preferred polymers, PNPPO and PPHO, is described in the examples below.

*Excipients and biologically-active agents*

**[0192]** Pharmaceutically-acceptable excipients may be included in the compositions and/or polymer of the present invention, and include any and all solvents, dispersion media, inert

diluents, or other liquid vehicles, dispersion or suspension aids, granulating agents, surface active agents, disintegrating agents, isotonic agents, thickening or emulsifying agents, preservatives, binding agents, lubricants, buffering agents, oils, and the like, as suited to the particular dosage form desired. Remington (Gennaro, A. R., Remington: The Science and Practice of Pharmacy, 21st Ed (2006) Lippincott Williams & Wilkins) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

**[0193]** Excipients such as colouring agents, coating agents, sweetening, flavouring, and perfuming agents can be present in the composition, according to the judgment of the formulator.

**[0194]** Biologically active agents or drug compounds that may be added to the composition and/or polymer of the present invention include proteins, glycosaminoglycans, carbohydrates, nucleic acids and inorganic and organic biologically active compounds, such as enzymes, antibiotics, anti-neoplastic agents, local anaesthetics, hormones, angiogenic agents, anti-angiogenic agents, growth factors (*e.g.*, insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF) and transforming growth factor- $\beta$  (TGF $\beta$ )), antibodies, neurotransmitters, psychoactive drugs, anticancer drugs, chemotherapeutic drugs, drugs affecting reproductive organs, genes, and oligonucleotides.

**[0195]** A composition containing components such excipients and/or biologically active agents can be produced by combining a polymer of the present invention with an NSPP, combining this with one or more other components and then freeze-drying the resulting composition. This leads to a ready to use polymer.

**[0196]** The amount of polymer, NSPP and biologically active agent present in the composition will necessarily depend upon the particular drug and the condition to be treated. A person skilled in the art will be aware of appropriate agents and amounts to use to treat the condition.

#### *Compositions for forming hydrogels*

**[0197]** The present invention also relates to a preferred composition useful for forming a hydrogel for use in the invention.

[0198] The composition of the present invention comprises a polymer and optionally an NSPP, the polymer comprising:

[0199] a first water-binding monomer; and

[0200] a second monomer that imparts mechanical properties;

[0201] optionally, a third monomer that is an NSPP-binding monomer, comprising a functional group that is capable of binding to the NSPP;

[0202] a fourth monomer capable of imparting phase transition characteristics to the hydrogel;

[0203] wherein the natural or synthetic peptide or protein (NSPP) may be Thymosin beta-4 or a functional homolog thereof;

[0204] and wherein the binding of the NSPP to the second monomer crosslinks the polymer, thereby enabling formation of a hydrogel when the composition is contacted with water.

[0205] The term “composition”, as used herein, refers to a solid or liquid composition containing the components mentioned above. In some embodiments, other components such as pharmaceutically-acceptable excipients and biologically active agents (*e.g.*, drugs, vitamins and minerals), to assist in repair and/or re-generation of the target bone tissue, and/or to provide a method of achieving targeted delivery of biologically active compounds, may also be included in the preferred compositions used in the present invention.

[0206] In general, the amount of polymer in the composition used in the present invention is an amount that allows for the formation of hydrogels.

[0207] In some embodiments, the amount of polymer in the composition ranges: from about 1% w/w to about 90% w/w, from about 2% w/w to about 80% w/w, from about 4% w/w to about 70% w/w, from about 5% w/w from about 60% w/w, from about 5% w/w to about 50% w/w, from about 6% w/w to about 40% w/w, from about 7% w/w to about 30% w/w or from about 8% w/w to about 20% w/w.

[0208] In some embodiments, the amount of polymer is: about 1% w/w, about 2% w/w, about 3% w/w, about 4% w/w, about 5% w/w, about 6% w/w, about 7% w/w, about 8% w/w, about 9% w/w, about 10% w/w, about 15% w/w, about 20% w/w, about 25% w/w, about 30% w/w, about 35% w/w, about 40% w/w, about 45% w/w, about 50% w/w, about 55% w/w, about 60% w/w, about 65% w/w, about 70% w/w, about 75% w/w, about 80% w/w or more. In some embodiments, the amount of polymer is approximately 85% w/w.

[0209] As a general rule, the solidity of the hydrogel increases with higher polymer

concentrations in the composition.

[0210] In general, the amount of NSPP in the composition of the present invention is an amount that allows for the formation of hydrogels.

[0211] In some embodiments, the amount of NSPP in the composition ranges: from about 0.01% w/w to about 60% w/w, from about 1% w/w to about 50% w/w, from about 1% w/w to about 40% w/w, from about 5% w/w to about 30% w/w, from about 5% w/w to about 20% w/w, or from about 5% w/w to about 10% w/w.

[0212] In some embodiments, the percent of NSPP is about 1% w/w, about 2% w/w, about 3% w/w, about 4% w/w, about 5% w/w, about 6% w/w, about 7% w/w, about 8% w/w, about 9% w/w, about 10% w/w, about 20% w/w, about 30% w/w, about 40% w/w, about 50% w/w, or more.

[0213] The % w/w is based on the total weight of the composition before the composition is contacted with water.

#### **Detailed Description of a Preferred Embodiment**

[0214] Preferred embodiments of the invention will now be described, by way of example only, as follows:

##### *Example 1 – PPHO dissolution in iohexol solution (Omnipaque 350)*

[0215] Lyophilised PPHO polymer was collected and added directly to iohexol solution with 350 mg iodine/mL to achieve PPHO final concentrations of 50 mg/mL, 100 mg/mL and 200 mg/mL. The resulting solutions were stored at 2 to 8 °C for dissolution under constant shaking. The results after nearly 4 days of mixing showed the formation of a two phase mixture, involving an organic-like swollen gel layer and a separate aqueous supernatant.

[0216] The results suggest that PPHO polymer is not directly soluble in Omnipaque 350.

##### *Examples 2 to 4 – PPHO dissolution in iohexol solution (300 I mg/mL (Example 2), 240 mg I/mL (Example 3) and 175 mg I/mL (Example 4)*

[0217] Example 1 was repeated with weaker/less concentrated iohexol solutions. The hypothesis was this that by decreasing the iohexol concentration, PPHO becomes soluble in the solutions. The results showed very similar outcome to that achieved in Example 1.

[0218] Therefore, it was concluded that the PPHO polymer is not soluble directly in iohexol solutions.

*Table 1. Summary of results from Examples 1 to 4*

| PPHO<br>(mg/mL) | Iohexol<br>(mg I/mL) | Soluble?<br>(Y/N) |
|-----------------|----------------------|-------------------|
| 50              | 350                  | N                 |
| 100             | 350                  | N                 |
| 200             | 350                  | N                 |
| 50              | 300                  | N                 |
| 100             | 300                  | N                 |
| 200             | 300                  | N                 |
| 50              | 240                  | N                 |
| 100             | 240                  | N                 |
| 200             | 240                  | N                 |
| 50              | 175                  | N                 |
| 100             | 175                  | N                 |
| 200             | 175                  | N                 |

*Example 5 – PPHO dissolution and mixing with iohexol solution (350 mg I/mL)*

**[0219]** In this example, PPHO was dissolved in either distilled water or in phosphate buffered saline solution (PBS) to achieve the final polymer concentration of 200 mg/mL. Within 24 hours under constant mixing, a uniform single phase solution of PPHO polymer was achieved. This stock solution was decoded as TL200. The resulting solution was then mixed with different volume ratios with iohexol 350 mg I/mL, per details provided in Table 2.

**[0220]** The results showed that surprisingly, the dissolution of PPHO in an aqueous solution, followed by the addition of iohexol solution to the dissolved polymer solution resulted in the formation of a single-phase final solution. The resulting solutions were then placed inside an incubator at 37 °C and the thermoresponsive nature of the solutions was verified by using a vial tilting method after 1 minute of incubation.

*Table 2. Formulation of different PPHO solutions with a range of polymer concentration and iohexol content by mixing a pre-made PPHO solution (200 mg/mL) with iohexol 350 mg I/mL solution*

| TL200<br>(mL) | Iohexol<br>350 (mL) | PPHO<br>(mg/mL) | Product Code | Soluble?<br>(Y/N) | Thermoresponsive?<br>(Y/N) |
|---------------|---------------------|-----------------|--------------|-------------------|----------------------------|
| 3             | 0.5                 | 171             | TL170-IOP050 | Y                 | Y                          |
| 2.5           | 1                   | 143             | TL145-IOP100 | Y                 | Y                          |
| 2             | 1.5                 | 114             | TL115-IOP150 | Y                 | Y                          |
| 1.5           | 2                   | 86              | TL085-IOP200 | Y                 | Y                          |
| 1             | 2.5                 | 57              | TL060-IOP250 | Y                 | Y                          |
| 0.5           | 3                   | 29              | TL030-IOP300 | Y                 | N                          |

*Example 6 – Iohexol dilution with distilled water and dissolution of PPHO*

**[0221]** In this example, iohexol 350 mg I/mL was diluted with different amounts of distilled water. The resulting solutions were then used to dissolve PPHO polymer at the concentration of 200 mg/mL.

**Table 3.** Formulation of different PPHO solutions with a range of polymer concentration and iohexol content by mixing a pre-made PPHO solution (200 mg/mL) with iohexol 350 mg I/mL solution and water

| Iohexol 350 (mL) | Water (mL) | PPHO (mg/mL) | Product Code | Soluble? (Y/N) | Thermoresponsive? (Y/N) |
|------------------|------------|--------------|--------------|----------------|-------------------------|
| 0.25             | 0.75       | 200          | TL200-IOP90  | Y              | Y                       |
| 0.5              | 0.5        | 200          | TL200-IOP175 | Y              | Y                       |
| 0.75             | 0.25       | 200          | TL200-IOP265 | Y              | Y                       |
| 0.8              | 0.2        | 200          | TL200-IOP280 | Y              | Y                       |
| 0.85             | 0.15       | 200          | TL200-IOP298 | N              | N/A                     |
| 0.9              | 0.1        | 200          | TL200-IOP315 | N              | N/A                     |

**[0222]** The results showed that multiple formulations with 200 mg/mL of PPHO and a relatively high iohexol content can be formulated. These single-phase solutions were thermoresponsive and formed hydrogel upon incubation at 37 °C.

*Example 7 – Formulation of TL150-IOP90 and test of radiopaque properties of the formulation*

**[0223]** In this example, 22.5 mL of TL200 was diluted with 7.5 mL of 350 mg I/mL to achieve a PPHO concentration of 150 mg/mL and 90 mg I/mL. The resulting solution was then injected subcutaneously in a warm cadaveric porcine model. In total nearly 5 mL of the product was injected. After administration, a CT-scan of the site was used to verify the presence of the hydrogel and its thermoresponsive and radiopaque properties.

**[0224]** Having regard to *Figure 1* of the accompanying drawings, it can be seen that injection of 5 mL of TL150-IOP090 in a cadaveric subcutaneous model and the formation of a hydrogel (white gel, shown in black arrows) at the administration site (a). A CT-scan of the injected site showed the presence of a radiopaque white structures at the administration site, shown by white arrows (b).

*Example 8 – Product Hounsfield units*

**[0225]** Four formulations were formed, and their Hounsfield units were measured. The results are shown in *Table 4*.

**[0226]** The results confirmed that it is feasible to form injectable/flowable formulations with different Hounsfield units to match the required clinical needs. In addition, a linear regression with  $R^2 = 0.973$  is achieved (*see, Figure 2*), allowing further product formulations.

*Table 4. Hounsfield units of different TL formulations, formed with a wide range of iohexol contents*

| Product Code | Iohexol (mg I/mL) | HU      |
|--------------|-------------------|---------|
| TL200-IOP000 | 0                 | 80.01   |
| TL150-IOP90  | 88                | 1588.72 |
| TL050-IOP175 | 175               | 2615.90 |
| TL100-IOP225 | 225               | 2921.60 |

*Example 9 – PNPPO/PPHO (1:1) Formulation*

**[0227]** In this example, 50 mg/mL of PNPPO polymer and 50 mg/mL of PPHO polymer were dissolved in 1 mL of a buffered solution containing, ~100  $\mu$ L 350 mg I/mL and 900  $\mu$ L PBS. The buffered solutions were prepared in advance by dissolution of 350 mg I/mL in PBS with 1:9 volume ratio. This configuration is denoted as “TP050/TL50-IOP45”. Results showed that a uniform, single phase solution was formed upon the dissolution of two polymers in the buffered solution at 2 to 8 °C. Thereafter the solutions were added to a pre-warmed PBS at 37 °C to simulate the gelation process in the physiological condition. The results confirmed that upon the increase of temperature, TP050/TL50-IOP45 solution transitions into a cohesive and structurally stable hydrogel.

*TP050/TL50-IOP45 application through catheter*

**[0228]** The TP050/TL50-IOP45 solution formed above was used to test the flowability of the formulation through medical catheters. For this study, 150 cm x 6 cm 2.7F catheter (Excelsior XT-27) was used. To simulate the physiological condition, the catheter was submerged in a pre-warmed PBS for 5 minutes. Thereafter, the catheter was primed with warmed water for injection. TP050/TL50-IOP45 solution was delivered through the catheter, using a standard syringe for injection. The result, shown in *Figure 3*, confirmed that the solution flows through the catheter, forming a cohesive string of hydrogel once reaching the physiological temperature.

*Example 10 – PNPFO/PPHO (1:2) Formulation*

**[0229]** In this example, 25 mg/mL of PNPFO polymer and 50 mg/mL of PPHO polymer were dissolved in 1 mL of a buffered solution, containing ~100  $\mu$ L 350 mg I/mL and 900  $\mu$ L PBS. The buffered solutions were prepared in advance, by dissolution of 350 mg I/mL in PBS with 1:9 volume ratio. The product configuration is denoted as “TP025/TL50-IOP45”. Results showed that a uniform, single phase solution was formed upon the dissolution of two polymers in the buffered solution at 2 to 8 °C. Thereafter the solutions were added to a pre-warmed PBS at 37 °C to simulate the gelation process in the physiological condition. The results confirmed that upon the increase of temperature, TP025/TL50-IOP45 solution transitions into a cohesive and structurally stable hydrogel.

*Example 11 – PNPFO Formulation*

**[0230]** In this example, 100 mg/mL of PNPFO polymer was dissolved in 1 mL of a buffered solution containing, ~100  $\mu$ L 350 mg I/mL and 900  $\mu$ L PBS. The buffered solutions were prepared in advance by dissolution of 350 mg I/mL in PBS with 1:9 volume ratio. This configuration is denoted as “TP100-IOP45”. Results showed that a uniform, single phase solution was formed upon the dissolution of two polymers in the buffered solution at 2 to 8 °C. Thereafter the solutions were added to a pre-warmed PBS at 37 °C to simulate the gelation process in the physiological condition. The results confirmed that upon the increase of temperature, TP100-IOP45 solution transitions into a cohesive and structurally stable hydrogel (*Figure 4*).

*Example 12 – Flowability and capability of TL050/TP050 with IOP240 and TL050/TP050 with IOP225 to flow through microcatheter within an in vitro vessel model*

**[0231]** In this example, the TP050/TL50-IOP240 solution formed above was used to test the flowability of the formulation through catheters and an *in vitro* vessel model under fluoroscopy imaging. Phosphate buffer solution (PBS) was pumped at 37 °C into the simulated *in vitro* vessel model through a pump. For this study, a 2F Trevo catheter was used. TP050/TL50-IOP240 solution was delivered through the catheter, using a standard syringe for injection. The result, shown in *Figure 5(a)*, confirmed that the solution flows through the catheter and forms a solid white coil-like gel at the tip of the catheter upon entering the flow within the vessel model. Also, the formation of the gel was observable through live fluoroscopy. Similar results were observed by passing TL050/TP050-IOP225 through a 2.9F Maestro catheter, as shown in *Figure 5(b)*.

*Example 13 – Flowability and capability of TL050/TP050 with IOP240 to flow through microcatheter within an ex vivo porcine model*

[0232] In this example, the TL050/TP050-IOP240 solution formed above was used to test the flowability of the formulation through a 2F Trevo catheter (as an example) within microvessels in an *ex vivo* renal porcine model. The results, shown in *Figure 6(a)* and *Figure 6(b)*, confirmed that the formulation was flowable and can flow within the targeted microvessels, visible under live fluoroscopy imaging.

*Example 14 – Formation of TL050-IOP1000*

[0233] In this example, Histodenz<sup>TM</sup> (a non-ionic X-ray contrast compound) was dissolved in phosphate buffered saline solution at the concentration of 1000 mg/mL. The solution was then used to dissolve PPHO polymer at different concentrations. The formed final solution with 50 mg/mL of PPHO was coded TL050-IOP1000. This solution was a single phase and formed a hydrogel upon the increase of temperature to 37 °C. The measured Hounsfield numbers from these hydrogels were 3,994.90.

### **Industrial Applicability**

[0234] It will be appreciated that the present invention finds ready applicability in the biomedical, surgical and therapeutic fields. The biocompatible PNPPO or PPHO polymers in aqueous solution provide for administration via catheter as advanced thermoresponsive biomaterials for intravascular embolisation and carriers for radioisotopes.

[0235] In respect of the invention the key findings of the present investigation were:

- i. The inventive biomaterials can be combined with any contrast agent, including but not limited to iodinated radiographic media and heavy-metal MRI chelates.
- ii. PNPPO and PPHO solutions in iohexol have been prepared in concentrations such as 225, 175 and lower mg/mL.
- iii. The inventive biomaterials can be applied in delivering radioisotopes through catheters for targeted therapeutic radioisotopes.
- iv. The final hydrogel-based system is flowable (liquid) throughout a catheter/microcatheter within the body and once applied to the target site forms a gel.
- v. The inventive solutions form stable hydrogels upon extrusion from a catheter in a simulated physiological condition (*i.e.*, catheter submerged in pre-warmed water at 37 °C

for at least 5 minutes to simulate physiological condition) and clinical application of the catheter.

vi. The developed system is radiopaque due to its ability to conjugate with a contrast agent, thus can be seen under X-ray.

vii. A PNPFO or PPHO-based system can be applied for treating diseases or conditions including but not limited to gastrointestinal tract haemorrhage, bleeding from vascular malformations, neoplasms, redistribution of preoperative blood flow, varicoceles, or endoleaks, and tumours.

**[0236]** Although the invention has been described with reference to specific examples it will be appreciated by those skilled in the art that the invention may be embodied in many other forms.

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**THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-**

1. A flowable polymer solution for intravascular (arterial and venous) administration comprising the polymer and at least one solvent, the polymer comprising:
  - 5 a first monomer for binding water;
  - a second monomer for imparting mechanical properties;
  - optionally, a third monomer for binding to a natural or synthetic peptide or protein (NSPP); and
  - a fourth monomer for imparting thermoresponsive phase-transition
  - 10 behaviour.
2. A polymer solution according to claim 1, further comprising at least one radiographic intravenous contrast agent.
- 15 3. A polymer solution according to claim 2, wherein the at least one radiographic intravenous contrast agent is selected from contrast media for digital subtraction angiography (DSA), computer tomography (CT) and fluoroscopy such as iodinated contrast media, tantalum, and bismuth-based materials (*e.g.*, bismuth chelate), heavy metal chelates contrast media for MRI including gadolinium agents (*e.g.*, gadobutrol) or MRI nanoparticles.
- 20 4. A polymer solution according to claim 3, wherein the at least one contrast agent is an iodine-based contrast agent.
- 25 5. A polymer solution according to claim 4, wherein the iodine-based contrast agent is iohexol (1-N,3-N-bis(2,3-dihydroxypropyl)-5-[N-(2,3-dihydroxypropyl)acetamido]-2,4,6-triiodobenzene-1,3-dicarboxamide).
6. A polymer solution according to any one of the preceding claims, wherein the at
- 30 least one solvent comprises phosphate buffered saline (PBS) and/or water.
7. A polymer solution according to any one of the preceding claims, wherein the first monomer is selected from: polyethers, polyvinyl alcohol (PVA); poly(vinyl

pyrrolidone) (PVP); poly(amino acids) and dextran.

8. A polymer solution according to claim 7, wherein the polyethers are selected from:  
polyethylene glycol (PEG), oligo(ethylene glycol) (OEG), polyethylene oxide  
5 (PEO), polyethylene oxide-co-propylene oxide (PPO), co-polyethylene oxide block  
or random copolymers thereof, preferably the first monomer is oligo (ethylene)  
glycol monomethyl ether methacrylate (OEGMA).
9. A polymer solution according to any one of the preceding claims, wherein the  
10 second monomer is a methacrylate, or a random co-polymer comprising a  
methacrylate.
10. A polymer solution according to claim 9, wherein the second monomer is selected  
from: hydroxyethyl methacrylate (HEMA), a hydroxyethyl methacrylate poly(lactic  
15 acid) copolymer (PLA/HEMA), poly(lactic acid), poly(caprolactone),  
poly(glycolide), poly(glycolide-colactide) or poly(glycolide-co-caprolactone),  
preferably the second monomer is hydroxyethyl methacrylate poly(lactic acid)  
(PLA/HEMA).
- 20 11. A polymer solution according to any one of the preceding claims, wherein the third  
monomer has electrophilic functional groups for binding to the NSPP.
12. A polymer solution according to claim 11, wherein the third monomer is selected  
from: N-hydroxysulfosuccinimide (SNHS), N-hydroxyethoxylated succinimide  
25 (ENHS), and N-acryloxysuccinimide (NAS), preferably the third monomer is N-  
acryloxysuccinimide (NAS).
13. A polymer solution according to any one of the preceding claims, wherein the fourth  
monomer has a lower critical solution temperature (LCST) less than about 37 °C.
- 30 14. A polymer solution according to claim 13, wherein the fourth monomer is selected  
from: poly(ethylene oxide)/poly(propylene oxide) and poly(N-isopropylacrylamide)  
(PNIPAAm) homopolymers and copolymers, preferably the fourth monomer is (N-

isopropylacrylamide) (NIPAAm).

15. A polymer solution according to any one of the preceding claims, wherein the polymer comprises: the first monomer in an amount of from about 1 to about 15 mol%; the second monomer in an amount of from about 5 to about 50 mol%; the third monomer in an amount of 0 to about 15 mol%; and the fourth monomer in an amount which makes up the remainder to 100% of the polymer.
16. A polymer solution according to any one of the preceding claims, wherein: the first monomer is OEGMA; the second monomer is PLA/HEMA; the third monomer is NAS; and the fourth monomer is NIPAAm,  
wherein the polymer comprises: OEGMA in an amount of from about 1 to about 15 mol%; PLA/HEMA in an amount of from 5 to about 50 mol%; NAS in an amount of from 0 to about 15 mol%; and NIPAAm in an amount of up to about 85 mol%.
17. A polymer solution according to any one of the preceding claims, wherein the polymer solution can be mixed *in situ* with one or more cargo compounds selected from therapeutics, drugs or isotopes, or a combination thereof prior to administration to dilute the polymer solution accordingly.
18. A polymer solution according to claim 17, wherein the one or more cargo compounds are mixed with the polymer solution via direct syringe-syringe mixing.
19. A polymer solution according to claim 18, wherein the syringes for mixing cool down the polymer solution and one or more cargo compounds during mixing.
20. A polymer solution according to claim 18, wherein the syringes for mixing protect the operator against radiation exposure.
21. A polymer solution according to any one of the preceding claims, wherein the third monomer is present and the polymer solution has a concentration of polymer between about 10 mg/mL and about 120 mg/mL.

22. A polymer solution according to any one of claims 1 to 20, wherein the third monomer is absent and the polymer solution has a concentration of polymer between about 5 mg/mL and about 200 mg/mL.
- 5
23. A polymer solution according to any one of the preceding claims, wherein administering the polymer at a treatment site is performed by injection through a catheter.
- 10
24. A polymer solution according to claim 23, wherein the catheter is a fine gauge interventional vascular catheter/microcatheter (1F-6F), such as a French 3.3 mm catheter.
- 15
25. A polymer solution according to any one of the preceding claims, wherein the intra-vascular administration is part of a transcatheter arterial embolisation (TEA) procedure.
- 20
26. A method of performing a transcatheter arterial embolisation (TEA) procedure, the method comprising administering to a subject in need of such treatment a polymer solution according to any one of claims 1 to 25.
27. A method according to claim 26, wherein the administration is performed at, or substantially at body temperature.
- 25
28. A method according to claim 27, wherein the thermoresponsive polymer solution forms an adhesive hydrogel network upon extrusion from a catheter.
- 30
29. Use of a polymer solution according to any one of claims 1 to 25 in the manufacture of a medicament or delivery vehicle for administration via a transcatheter arterial embolisation (TEA) procedure.
30. Use according to claim 29, wherein the administration is performed at body temperature.

31. Use according to claim 30, wherein the thermoresponsive polymer solution forms an adhesive hydrogel network upon extrusion from a catheter.
- 5 32. A kit for forming a flowable polymer solution for intravascular (arterial and venous) administration, the kit comprising a polymer comprising:
- a first monomer for binding water;
  - a second monomer for imparting mechanical properties;
  - optionally, a third monomer for binding to a natural or synthetic peptide or
  - 10 protein (NSPP);
  - a fourth monomer for imparting thermoresponsive phase-transition behaviour; and
  - at least one solvent for forming a solution of the polymer; and
  - means for administering the polymer solution; and
  - 15 optionally instructions for administering the polymer solution via a transcatheter arterial embolisation (TEA) procedure.
33. A kit according to claim 32, further comprising at least one radiographic intravenous contrast agent selected from contrast media for digital subtraction angiography
- 20 (DSA), computer tomography (CT) and fluoroscopy such as iodinated contrast media, tantalum, and bismuth-based materials (*e.g.*, bismuth chelate), heavy metal chelates contrast media for MRI including gadolinium agents (*e.g.*, gadobutrol) or MRI nanoparticles.