

POLYMERIC RADIOTHERAPEUTIC PARTICLES, SUSPENSIONS AND METHODS FOR PRODUCING SAME

FIELD

The present invention relates to polymeric radiotherapeutic particles, compositions
5 and methods for producing same, wherein the radionuclide is an alpha and/or beta-emitting radionuclide. The invention also relates to the use of said particle and compositions, in the treatment of cancer and their use in loco-regional radiotherapy, brachytherapy and transarterial radioembolization.

10 BACKGROUND

Many previous attempts have been made to locally administer radioactive materials to patients with cancer as a form of therapy. In some of these the radioactive materials have been incorporated into particles and alike, which can be administered directly into e.g., cancerous tumours, where radioactive particles provided as a form of transarterial
15 radioembolization (TARE) (also known as selective internal radiation therapy (SIRT)). In TARE radioactive particles are administered via a catheter directly into the arterial blood supply of the target organ, such as the liver, to enable the delivery of the radioactive dosage directly at the tumour site, without systemic exposure of the radionuclide to the patient. This has the benefit that a higher amount of the desired radioactive dose is provided to the target
20 lesion, without exposing 'healthy' tissue to otherwise harmful radiation. Such stable radiotherapeutic particles e.g., non-degradable glass spheres (TheraSphere™) or resin based spheres (SIR-Spheres™) have been used for radioembolization for treating primary tumors and metastases to the liver.

For such radioactive exposure to be efficacious and restricted to the target tissue the
25 radiation emitted should be of high energy and short range, which is obtainable by alpha and/or beta radiation. Accordingly, radionuclides which have a high energy alpha and/or beta decay are highly preferred for such applications.

A clinical problem with the absorption of radionuclides on microparticles is the potential leaching of the radionuclides from the particles, which may cause inappropriate
30 radiation of non-target tissues, and accumulation of daughters in unfavourable locations and tissues.

To overcome the leaching problem phosphate precipitation has been utilized in e.g., WO2015168726, which immobilizes the radionuclide on polymeric particles, by the precipitation of ⁹⁰Y as an insoluble phosphate salt onto the particles, thereby forming

protrusions of phosphate salts on the surface of the particles, also leading to an inefficient utilization of the ^{90}Y raw materials.

- Alternatives which enable a more efficient absorption of ^{90}Y on particles, which does not form such protrusions is highly favourable, since it enables a more stable and robust
- 5 radioactive particle to be formed that in turn may reduce potential off-target unwanted radiation for the patient.

SUMMARY

- The present invention relates to an alpha- and/or beta-emitting radionuclide labelled particle, wherein said particle comprises or consists of a polymeric resin conjugated with sulfonic acid
- 10 and an ionically bound alpha- and/or beta-emitting radionuclide, such as e.g., alpha- and/or beta-emitting radionuclides selected from the group consisting of ^{90}Y , ^{225}Ac , ^{89}Sr , ^{153}Sm , ^{159}Gd , ^{18}F , ^{68}Cu , ^{69}Cu , ^{67}Ga , $^{99\text{m}}\text{Tc}$, ^{201}Ti , ^{111}In , ^{161}Tb and ^{177}Lu , preferably selected from the groups consisting of ^{90}Y , ^{68}Cu , ^{69}Cu , ^{225}Ac , ^{212}Pb , ^{177}Lu , more preferably the radionuclide is ^{90}Y . Accordingly, such radionuclide is preferably selected from radionuclides which emits no
- 15 more than 10 % gamma radiation of the total decay radiation, and thereby preferably the primary decay is alpha and/or beta decay. In addition, such radionuclides may have a decay half-life in the range of 1-300 hrs, such as between 30-100 hrs, or such as 50-70 hrs. In embodiments, the particle may e.g., have a size in the range of 5-400 μm , such as a size in the range of 10-300 μm , 25-250 μm , 50-200 μm , 100-150 μm , such above 50 μm and below
- 20 200 μm . Preferably, such particles are spherical, with a smooth surface, such as a surface which lacks protrusions, with a low roughness. It is also preferred that such particles are essentially free of phosphate. Particles described herein are preferably polymeric, formed from a polymeric resin, which e.g., comprises one or more elements selected from the group consisting of divinylbenzene, polystyrene, polyethylene glycol (PEG), Polycaprolactone
- 25 (PCL), polyurethane (PU), Polyvinylpyrrolidone (PVP), poly(2-hydroxyethyl-methacrylate), polyglycolide, polylactide, polyhydroxybutyrate, chitosan and hyaluronic acid, it may also be a co-polymer, where multiple polymers makes up the polymeric particle, preferably, the polymeric resin is a styrene divinylbenzene copolymer.
- 30 The present invention also relates to pharmaceutical compositions comprising a particle as disclosed herein. Such pharmaceutical compositions may e.g., further comprise a diluent, carrier, surfactant, and/or excipient, and/or a bioglue component, such as e.g., an albumin, such as e.g., bovine serum albumin, and/or glutaraldehyde, and/or a buffering agent, such as e.g., acetate, citrate, or glutamate. The pharmaceutical composition preferably comprises
- 35 with an amount of radionuclide that is 1kBq to 10GBq per dosing, or with an amount of

radionuclide that is 50 MBq to 1000 GBq suitable for multidose industrial scale production. In addition said pharmaceutical composition is suitable for intravenous, intratumor, and/or intracavitary injection.

5 The present invention also relates to the use of particles and/or pharmaceutical compositions disclosed herein, preferably as a medicament, such as for use in the treatment or amelioration of cancer, such as head and neck squamous cell carcinoma, metastatic melanoma, sarcoma, non-small cell lung cancer, colorectal cancer, primary and secondary hepatocellular carcinoma, pancreatic ductal adenocarcinoma, renal cell carcinoma, ovarian
10 cancer, muscle invasive bladder cancer, prostate cancer, and/or osteosarcoma, preferably, unresectable hepatocellular carcinoma and/or locally-advanced borderline-resectable pancreatic ductal adenocarcinoma, e.g., by single or repeated dosing. The particles and/or composition disclosed herein are also suitable for use in intracavitary therapy, or radiosynovectomy, radioembolization, such as transarterial radioembolization (TARE) and
15 intratumor injection, preferably in hepatocellular carcinoma, but also in other cancers disclosed herein. Particles as compositions as disclosed herein may also be suitable for radionuclide imaging. The present invention further relates to a method of treatment of cancer comprising administering an effective dose of an alpha- and/or beta-emitting radionuclide labelled particle as disclosed herein.

20

The present invention further relates to methods for producing an alpha- and/or beta-emitting radionuclide labelled particle or pharmaceutical composition as disclosed herein. Said method e.g., comprises; providing an alpha- and/or beta-emitting radionuclide or a salt thereof; providing a particle with a sulfonic acid conjugated polymeric resin; mixing said
25 alpha- and/or beta-emitting radionuclide and said particle in an aqueous solution at a pH in the range of pH 6.5-9.5; and isolating an alpha and/or a beta-emitting radionuclide labelled particle from said aqueous solution. Preferably, the mixing and isolation step is not separated by more than 60 minutes, such as no more than 30 minutes, such as no more than 15 minutes. Preferably, the aqueous solution has a pH in the range of pH 7-9,
30 preferably less than pH 8.5, such as less than pH 7.5, such as about pH 7.4. It is preferred that the method does not comprise immobilizing said radionuclide on said particle by phosphate precipitation.

The present invention also relates to a kit comprising; unlabelled particles comprising or
35 consisting of a polymeric resin conjugated with sulfonic acid; optionally reagents for preparing an alpha- and/or beta-emitting radionuclide labelled particle, or a pharmaceutical

composition as disclosed herein; and optionally instructions for preparing said particle or pharmaceutical composition. Said kit may further comprise, aqueous solution comprising an uncured curable biocompatible adhesive and/or non-polymerized hydrogel in an aqueous solution and an aqueous solution comprising a curing and/or polymerizing agent, preferably, 5 said curable biocompatible adhesive is an albumin, preferable bovine serum albumin, and said curing and/or polymerizing agent is an amide cross linking agent, preferably glutaraldehyde.

The present invention further relates to a method of preparing a cured ^{90}Y radiotherapeutic 10 composition comprising; providing a ^{90}Y labelled particle suspension, an uncured biocompatible adhesive and/or non-polymerized hydrogel in an aqueous solution, and a curing and/or polymerizing agent which upon mixture of said glue or hydrogel induces curing of said biocompatible adhesive and/or polymerization of said hydrogel; thereafter mixing said components to obtain a cured ^{90}Y radiotherapeutic composition, comprising biocompatible 15 adhesive and/or hydrogel embedded ^{90}Y radiotherapeutic particles.

BRIEF DESCRIPTION OF FIGURES

FIGURE 1

Energy-dispersive-X-ray-spectroscopy (EDS) analysis of polymeric particles prepared by ion 20 exchange absorption of ^{89}Y onto the polymeric particles.

FIGURE 2

Scanning electron microscopy (SEM) analysis of AG 50W-X4 resin with yttrium incorporated by absorption. 25

FIGURE 3

SEM analysis of already existing product on the market with Yttrium incorporated by precipitation.

FIGURE 4

SEM analysis (zoom) of already existing product on the market with Yttrium incorporated by precipitation.

FIGURE 5

35 SEM analysis of AG 50W-X4 resin with yttrium incorporated by absorption

FIGURE 6

EDS analysis showing yttrium (Y) and phosphorus (P) detected on the AG 50W-X4 with yttrium incorporated by precipitation of phosphate salts

5

FIGURE 7

SEM analysis of AG 50W-X4 with yttrium incorporated by precipitation of phosphate salts

FIGURE 8

10 SEM analysis of AG 50W-X4 resin with ⁹⁰Y incorporated by absorption

DETAILED DESCRIPTION

The present inventors have identified a treatment of cancer with less risk for off-target side effects based on a focal therapy using short-ranging alpha- and/or beta- emitters, especially
15 suitable for loco-regional radiotherapy such as brachytherapy and transarterial radioembolization.

A radionuclide labelled particle

One object of the present invention relates to a particle comprising a polymeric resin which
20 allows adhesion of an alpha and/or beta-emitting radionuclide, wherein the leaching of said radionuclide from the particle is sufficiently low to make the particle suitable for use in cancer therapy. Such a particle may be obtained by conjugating a polymeric particle with one or more suitable functional groups which enables strong binding of the radionuclide to the particle. Examples of strong cationic functional groups capable of strong binding of metal
25 ions are e.g., sulfonic acid and phosphoric acid, which are commonly used in cation exchange resins, such as the Lewatit® TP 260, AG® 50W and AG® MP-50 cation exchange resins.

Accordingly, an aspect of the invention relates to an alpha- and/or beta-emitting radionuclide
30 labelled particle, wherein said particle comprises or consists of a polymeric resin conjugated with sulfonic acid and an ionically bound alpha- and/or beta-emitting radionuclide.

Preferably, the particle is labelled by cation exchange, where the alpha- and/or beta-emitting radionuclide is provided as a soluble salt, preferably a chloride salt, but may also be
35 provided with an alternative anion such as e.g., bromide, iodide, sulphate, sulphite, nitrate,

acetate, phosphate, carbonate, or oxide, or other suitable alternative anions known to the skilled person.

Preferably, the cation exchange is enabled by the sulfonic acid group on the polymeric resin,
5 which may be pre-loaded with a counter ion, such as Na^+ , H^+ , Fe^{2+} or similar counter ion. To avoid e.g., acidification, the functional group is preferably loaded with Na^+ prior to the cation exchange with the salt of the alpha- and/or beta-emitting radionuclide.

Example 1 exemplifies the preparation of an yttrium (Y) labelled particle, wherein the cation
10 exchange is performed by exchanging the Na^+ ion bound by SO_3^- functional group with the Y^{3+} ion released from the Cl^- , thus producing NaCl and particle bound yttrium. Example 1, thus shows an example of how an alpha- and/or beta-emitting radionuclide may be absorbed on the surface of a suitable particle, in order to produce an alpha- and/or beta-emitting radionuclide labelled particle as described herein. In addition, the particles prepared as
15 described herein are generally smoother and have less protrusions than particles prepared using precipitation, as is respectively shown in figure 2 and in figure 3. Generally, a smooth surface is characterized by its even and regular texture, lacking any prominent irregularities or protruding features. Such surfaces are in context of the present invention generally spherical and provide a uniform and continuous contact area.

20

In contrast, a surface with protrusions features raised or uneven elements, creating variations in height and texture. These protrusions can take various forms, from small bumps to more complex structures. Surfaces with protrusions are often designed for specific purposes like providing grip, such as in the arteries of patients.

25

The protrusions of a surface can be quantified. Surface protrusions (or roughness) is a function of the length scale it is measured at and the frequency response function of the instrument used to acquire data. Scanning or measuring the surface of the particle using two- and three-dimensional probes identify the protrusions with various tip widths and radii.

30

These can be quantified and compared. Thus, in the present context, a particle with a smooth surface with little or no protrusions is defined as a particle with less than ten protrusions of minimum 1 μm height as determined by a scanning electron microscope (SEM). In one or more exemplary embodiments of the present invention, a particle with a smooth surface with little or no protrusions is defined as a particle with less than ten

35

protrusions of minimum 1 μm height as determined by a scanning electron microscope (SEM). In one or more exemplary embodiments of the present invention, a particle with a

smooth surface with little or no protrusions is defined as a particle with less than ten protrusions of minimum 2 μ m height as determined by a scanning electron microscope (SEM). In one or more exemplary embodiments of the present invention, a particle with a smooth surface with little or no protrusions is defined as a particle with less than 50 protrusions of minimum 1 μ m height as determined by a scanning electron microscope (SEM). In one or more exemplary embodiments of the present invention, a particle with a smooth surface with little or no protrusions is defined as a particle with less than 5 protrusions of minimum 2 μ m height as determined by a scanning electron microscope (SEM).

In one or more exemplary embodiments of the present invention, a particle with a smooth surface with little or no protrusions is a particle of the present invention, wherein the smooth surface with little or no protrusions is defined by a particle surface that has less protrusion than the same particle which has been labelled using phosphate precipitation. Phosphate precipitation of radionuclides onto particles, as described herein, are well-known in the art.

This is especially evident from Example 2, which shows the process wherein 100-times the amount of Y was used in the preparation of the particles, using both the new process disclosed herein and the previous phosphate precipitation method. As can be seen from the comparison of figure 5 (particles prepared by the novel process) and figure 7 (particles prepared by the previous phosphate precipitation process), the high amount of Y used in the preparation of the particles leads to formation of multiple salt precipitate layers on the particles (Figure 7), which may lead to flaking from the particles of the Y containing phosphate salts, leading to unwanted migration of potentially radioactive material from the particles.

Example 3 further shows that the amount of ^{90}Y absorbed on the particles exceeds 85%, in particular exceeds 96% of the initial radioactivity amount provided initially in the solution, thereby greatly optimizing the amount of Y transferred to the particles, thereby immensely reducing the radioactive waste from the production of the particles. Example 4 further shows that the particles only have a very limited leakage of less than 1% following various temperature stresses.

In principle the polymeric particle may be made up of any suitable polymer or co-polymer known to the skilled person which enables its use as a resin for the functional group capable of binding the radionuclide, as described herein.

- 5 The particle presented in examples 1-3 are produced with a styrene divinylbenzene copolymer resin, which is activated by conjugation of sulphate, as described above.

Alternative polymeric materials are e.g., Cellulose based resins, Sepharose, polyacrylate resins, polystyrene and silica gels. In embodiments, the particles comprise styrene. In other
10 embodiments, the particles comprise divinylbenzene.

Preferably, the polymeric resin making up the particle is a styrene divinylbenzene copolymer.

In the context of the present disclosure, "lattice" refers to a three-dimensional arrangement
15 of polymeric components within the particle, which is preferably a spherical particle comprising crosslinked polymeric molecules, as disclosed herein. This arrangement may involve formation of a lattice structure from a single type of polymeric molecules or formation of a lattice structure from multiple different types of polymeric molecules by e.g., crosslinking or covalent inter- or intra- molecular binding of the polymers to provide spherical or
20 substantially spherical particles. The components making up the lattice structure will impact the properties of the particle. In the present disclosure, the particle preferably comprises a crosslinked styrene divinylbenzene copolymer lattice, which is produced as a spherical particle, with a specific size profile and properties, as disclosed herein. The lattice structure is preferably conjugated, with functional groups, such as e.g., sulphonic or phosphoric acid,
25 which allows for binding of the radionuclides as disclosed herein.

Accordingly, in the present disclosure, polymeric molecules of the particle may comprise a crosslinked polymeric lattice. In embodiments, the crosslinking degree of the crosslinked polymeric lattice is about 2%-10%, such as 3-7%, such as 4%, 6% or 8% preferably about
30 4%.

In the context of the present disclosure "resin" refer to a polymerized structure of polymers, formulated as a particle or a bead to achieve desired properties such as e.g., hardness, chemical resistance, thermal stability, pore size and particle size. In the present disclosure,
35 the resin is preferably formulated as spherical particles, with a specific size profile, as disclosed herein, combined with particular functional groups, such as sulphonic or

phosphoric acid, which allows for binding of the radionuclides as disclosed herein. Examples of commercially available resins are e.g., Lewatit® TP 260, AG® 50W and AG® MP-50 resins. In embodiments, the resin is a polystyrene-divinylbenzene sulfonic acid resin.

- 5 The resins of the present invention may have lattice structure. Thus, the polymeric particle lattice of the present invention may refer to polymeric resin. These terms can be used interchangeably. When resin making up the particle is a styrene divinylbenzene copolymer, the resin may be styrene divinylbenzene copolymer lattice. Thus, one example of these types of resins is a polystyrene-divinylbenzene sulfonic acid resin.

10

In embodiments, the particle has a size in the range of 5-400 µm, such as 25-250 µm, such as 50-200 µm, such as 100-150 µm, such above 60 µm and below 150 µm. In embodiments, the particle is having a size in the range of 70-150 µm. In preferred embodiments, the particle has a size in the range of 20-50 µm, more preferably 30-35 µm. In embodiments, the
15 particle has a size in the range of 5-400 µm, with a mean particle size of 20-50 µm, preferably a mean particle size of 30-35 µm.

20

In preferred embodiments, the particle is essentially phosphate free. By essentially phosphate free is meant that the preparation of the particles preferably does not involve a step of phosphate precipitation of the radionuclide in order to immobilize the particle on the particle. The omission of the phosphate precipitation step is essential in order to obtain a smooth particle, with little or no protrusions, otherwise resulting from the phosphate precipitation. In the present context, the term “essentially” means that the amount of phosphate is less than for particles that have been prepared using phosphate precipitation.

25

Thus, one or more embodiments of the present invention relates to the particle of the present invention where the amount of phosphate is less than 100 ppm. Thus, one or more embodiments of the present invention relates to the particle of the present invention where the amount of phosphate is less than 10 ppm. Thus, one or more embodiments of the present invention relates to the particle of the present invention where the amount of
30 phosphate is less than 1 ppm. The new method of absorbing Y-90 via ionic binding rather than phosphate precipitation allow the product to be more stable over Temperature stress conditions reducing the percentage of Y-90 leaching.

35

The particle is in particular embodiments composed of a divinylbenzene copolymer. In further embodiments, the divinylbenzene copolymer has a crosslinking degree of 2%-10%, such as 3-7%, such as 4%, 6% or 8% preferably about 4%.

5 **A radionuclide**

The radionuclides of the present invention can be any alpha- and/or beta-emitting radionuclide.

10 The main advantages of alpha and/or beta particle emitting compounds in local therapy in e.g., the liver is the shorter range, typically less than 0.1 mm for alpha emitters such as ^{212}Bi and ^{212}Pb and mm to cm ranges for beta-particles from medical beta-emitters such as ^{90}Y , ^{82}Br and ^{201}Tl , compared to gamma emitting particles such as $^{99\text{m}}\text{Tc}$, ^{111}In and ^{18}F .

15 Use of alpha-emitters and beta-emitters would in an intratumoral setting reduce risk for toxicity due to irradiation of non-target regions such as adjacent healthy tissue including blood vessels or even other proximal internal organs. Thus, in preferred embodiments, the decay of said alpha-, and/or beta-emitting radionuclide is primarily alpha and/or beta decay.

20 In embodiments, the alpha- and/or beta-emitting radionuclide is selected from the group consisting of ^{90}Y , ^{225}Ac , ^{89}Sr , ^{153}Sm , ^{159}Gd , ^{18}F , ^{68}Cu , ^{69}Cu , ^{67}Ga , $^{99\text{m}}\text{Tc}$, ^{201}Tl , ^{111}In , ^{161}Tb , ^{212}Pb and ^{177}Lu . More preferably, the alpha- and/or beta-emitting radionuclide is selected from the group consisting of ^{90}Y , ^{68}Cu , ^{69}Cu , ^{225}Ac , ^{177}Lu . Most preferable, the beta-emitting radionuclide is ^{90}Y . ^{90}Y is preferred due to its release of high amounts of beta radiation (2.2 MeV) and low tissue penetration length (up to 11 mm) (*Barrio et. al.*), making it especially
25 suitable for tumour directed radiotherapy, such as local-regional radiotherapy, brachytherapy or radioembolization. ^{90}Y is a radioactive isotope of yttrium with 39 protons and 51 neutrons, giving it an atomic mass of 90. ^{90}Y is a beta-emitter, that primarily decays by emission of beta radiation and decays into stable ^{90}Zr , with a half-life of 64.4 hrs. ^{90}Y is the result of decay from ^{90}Sr , and accordingly, ^{90}Y is a progeny of ^{90}Sr , which decays to ^{90}Y with a half-life
30 of 29 years, making it a good ^{90}Y generator, where the different ionic properties of the two materials may be used to separate the ^{90}Y from ^{90}Sr . ^{90}Sr is generally obtained from ^{235}U fission processes.

35 The emission of gamma radiation is critical for the clinical application of the particles as mentioned herein since a high dose of gamma radiation would irradiate both the patient and the person preparing and/or providing the therapy. Accordingly, it is preferred that the alpha,

and/or beta-emitting radionuclide emits no more than 10%, such as less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less than 0.1 % gamma radiation of the total decay radiation.

For the nuclide to be suitable for therapy, the half-life of the radionuclide is also of great importance, and it is preferred that the decay of the particle is in a range which allows for fast administration of the desired dosage of radiation, while also allowing the practitioner to prepare and administer the treatment. Accordingly, in embodiments, the alpha- and/or beta-emitting radionuclide has a decay half-life in the range of 1-300 hrs, such as between 10-100 hrs, or such as 50-70 hrs.

The amount of ^{90}Y used per patient dosage may be in the range of 1 kBq to 10 GBq, depending on the type of cancer to treat. In examples for liver cancer, such as hepatocellular carcinoma a dose in the range of 20 MBq to 1 GBq. Accordingly, the particle as disclosed herein, may be prepared with a dose in the range of 1 kBq to 10 GBq, such as between 100-500 MBq, such as about 10 MBq, 20 MBq, 30 MBq, 40 MBq, 50 MBq, 60 MBq, 70 MBq, 80 MBq, 90 MBq, 100 MBq, 110 MBq, 120 MBq, 130 MBq, 140 MBq, 150 MBq, 160 MBq, 170 MBq, 180 MBq, 190 MBq, 200 MBq, 210 MBq, 220 MBq, 230 MBq, 240 MBq, 250 MBq, 260 MBq, 270 MBq, 280 MBq, 290 MBq, 300 MBq, 310 MBq, 320 MBq, 330 MBq, 340 MBq, 350 MBq, 360 MBq, 370 MBq, 380 MBq, 390 MBq, 400 MBq, 410 MBq, 420 MBq, 430 MBq, 440 MBq, 450 MBq, 460 MBq, 470 MBq, 480 MBq, 490 MBq or such as about 500 MBq may be considered suitable. Dosages generally depends on the size of the tumour.

A pharmaceutical composition

Another aspect of the present invention relates to a pharmaceutical composition comprising a particle as disclosed herein.

The composition may be a particle suspension comprising monodisperse or polydisperse particles labelled with an alpha- and/or beta-emitting radionuclide.

The composition is preferably an aqueous composition.

In embodiments, said composition comprises particles as disclosed herein and a diluent, carrier, surfactant, deflocculant and/or excipient.

Acceptable carriers and pharmaceutical carriers include but are not limited to non-toxic buffers, fillers, isotonic solutions, solvents and co-solvents, anti-microbial preservatives, anti-

oxidants, wetting agents, antifoaming agents and thickening agents etc. More specifically, the pharmaceutical carrier can be but are not limited to normal saline (0.9 %), half-normal saline, Ringer's lactate, dissolved sucrose, dextrose, e.g. 3.3 % Dextrose/0.3 % Saline, water for injection (WFI). The physiologically acceptable carrier can contain a radiolytic
5 stabilizer, e.g. ascorbic acid, human serum albumin, which protect the integrity of the radiopharmaceutical during storage and shipment.

The pharmaceutical compositions can comprise a multitude of particles. These can be the same or different.
10

Thus, in another embodiment of the present invention is the pharmaceutical composition a particle suspension comprising monodisperse or polydisperse particles labelled with an alpha- and/or beta-emitting radionuclide.

15 In additional aspects, a composition comprising the particles as disclosed herein comprises a curable adhesive and/or a polymerizable hydrogel. It is preferred that said adhesive or hydrogel is biocompatible, such that the adhesive or hydrogel component in the composition does not induce any adverse effects upon administration. In the context of the present disclosure, a hydrogel is a three-dimensional network of hydrophilic polymer chains that has
20 the ability to absorb and retain water or biological fluids while maintaining its structural integrity. These materials are highly biocompatible, meaning they are well-tolerated by living organisms and can interact with biological systems without causing harm or adverse reactions to the tissue into which they are applied or administered. Within the framework of the present invention, the hydrogels may be polymerized to form an adhesive structure, in
25 which the radioactive particles are embedded. Generally polymerized hydrogels are formed through cross-linking polymer chains, resulting in a stable, three-dimensional network structure. These hydrogels are solid or gel-like materials, maintaining their shape and integrity in the presence of moisture or biological fluids. In contrast, unpolymerized hydrogels are in a liquid or semi-liquid state and lack the cross-linked structure of their polymerized
30 counterparts. Such hydrogels are often used as injectable biomaterials, allowing for in-situ polymerization at the application site, making them suitable for minimally invasive medical procedures, such as intratumorally administration via image assisted administration.

Biocompatible curable adhesives/glue designs are specifically formulated adhesives that
35 bond biological materials together while safeguarding their integrity and biocompatibility, the curing process, typically initiated by UV light, controlled heat, or by addition of a curing

agent, offers precise control over bonding strength and speed, minimizing damage to sensitive biological tissue. Examples of such biocompatible adhesives are e.g., BIOGLUE®, composed of bovine serum albumin which is cured using glutaraldehyde crosslinking; and fibrin glue comprising the protein fibrinogen and the protein curing agent thrombin. Various
5 suitable alternatives are known to the skilled person, such as collagen-based adhesives, alginate adhesives and gelatine adhesives. In the context of the present invention, a biocompatible adhesive is to be understood as an adhesive which is a specialized bonding material designed to adhere to biological tissues or medical devices while minimizing adverse reactions or harm to living organisms. This type of adhesive is formulated to be
10 substantially non-toxic, non-irritating, and compatible with the human body or other biological systems. Biocompatible adhesives are commonly used in medical applications such as wound closure, tissue grafting, and the assembly of implantable devices, where it provides a secure and durable bond without causing inflammation, toxicity, or other negative biological responses. Accordingly, it is preferred that the biocompatible adhesive as disclosed herein is
15 minimally toxic or irritating to the surrounding tissue, when such tissue is non-cancerous.

In embodiments, the composition comprises a one part of a two-component adhesive, such as e.g., BIOGLUE® (Artivion Inc.; Atlanta, GA), which comprises a first component, being bovine serum albumin, and a second component being glutaraldehyde.

20

In embodiments, the composition comprises an ⁹⁰Y labelled particle with a divinylbenzene copolymer resin. In embodiments, the composition further comprises bovine serum albumin and/or glutaraldehyde.

25 In additional embodiments, the composition of as disclosed herein is a pharmaceutical composition, injectable composition, or a pharmaceutical formulation.

In embodiments the pharmaceutical composition prepared with an amount of radionuclide that is 1 kBq to 10 GBq per dosing.

30

For instance, if 100 patient doses are produced in one batch per day this could be made up of a total of 1-10 GBq per each of the 100 doses manufactured.

In another embodiment of the present invention is the pharmaceutical composition prepared
35 with an amount of radionuclide that is suitable for multidose industrial scale production e.g., 50 MBq to 1000 GBq.

A method for producing a radionuclide labelled particle

The present invention also relates to a method for producing an alpha- and/or beta-emitting radionuclide labelled particle.

5

Firstly, an alpha- and/or beta-emitter is provided e.g., as a salt or in an aqueous solution, such as e.g., an aqueous solution comprising ^{90}Y or a powder comprising $^{90}\text{YCl}_3$, or another alpha- and/or beta-emitting radionuclide, or a salt thereof as disclosed herein.

10 Thereafter the alpha- and/or beta-emitter is mixed with a particle suspension, comprising particles which are functionalized to bind to e.g., the ^{90}Y ions in the solution. Such functional groups may e.g., be phosphoric acid or sulphonic acid or a combination of such. Essential is that the functional group should be capable of binding the alpha- and/or beta-emitter in an aqueous solution with a pH in a physiological range.

15

Following mixture of the radionuclide and the particles, the suspension is preferable left for a period of time i.e., labelling time, which allows for the radionuclide to be bound by the particles, e.g., by cation exchange of an ion bound functional group on the particles, and the cationic radionuclide, this may e.g., be for a duration of about 60 minutes, such as no more
20 than 30 minutes, such as no more than 15 minutes after step. The mixing of the particles and the alpha- and/or beta-emitter is often followed by an isolation step, which may also comprise several washing steps. For instance, as shown in example 1 and 2, the mixing may e.g., be done in a reaction vessel, such as a chromatographic column, or a tube containing a filter suitable for isolating the particles and the aqueous solution containing unbound
25 radionuclide, such as e.g., a polypropylene fitted disc, column or similar. Alternatively, the isolation and washing may e.g., be done via centrifugation, where the particles and the aqueous solution are separated into a liquid-liquid and a particle-liquid phase. Further alternative isolation methods for isolating the labelled particle and the unconjugated radionuclide are well known to the skilled person.

30

However, the optimal time to allow binding will depend on the specific radionuclide, and its binding kinetics with the particles. The half-life of the specific radionuclide is essential, where it follows that the shorter the half-life of the radionuclide, the shorter the process step should be, in order to maintain as high a radioactivity dose as possible.

35

What the inventors herein found was that the methods as provided herein provides for a faster and more efficient labelling method of the particles, compare to the available methods, which otherwise also includes a step of ion immobilization by precipitation, a process which is both time consuming and reduces the overall recovery of the alpha- and/or beta-emitting radionuclide, since more ions will detach from the particles in the precipitation step.

Accordingly, the methods presented herein for producing a radioactive particle, produces less waste and consumes less raw materials, than particles produced according to the current available methods. Furthermore, the precipitation, such as e.g., performed by rapid pH adjustments by addition of phosphate salts, such as sodium phosphate, creates protrusions on the surface of the particles, which may lead to unintended flaking of the alpha- and/or beta-emitting radionuclide phosphate salts from the particles. Accordingly, by the use of phosphate precipitation, the amount of radioactivity per particle becomes restricted to an amount which does not result in flaking. Accordingly, in the preparation of the particles of the present invention it is preferred that the method does not comprise immobilizing said radionuclide on said particle by phosphate precipitation. Accordingly, the present method enables the preparation of alpha- and/or beta-emitting radionuclide labelled particles which are essentially phosphate free.

Thus, as disclosed herein the inventors found that the amount of alpha- and/or beta-emitting radionuclide that may be bound to the particle using the methods disclosed herein is higher than what is possible by conventional means. This results in the potential benefits, that a lower number of particles is needed to produce the same or similar dose, due to the higher loading capacity on each particle, in addition, it also opens for the use of higher dosages since the effective amount of particles that can be delivered is essentially the same, while the radioactivity per particle might be higher compared to the particles comprising precipitation immobilized alpha- and/or beta-emitting radionuclide.

Accordingly, in embodiments, the invention also relates to a method for producing an alpha- and/or beta-emitting radionuclide labelled particle as disclosed herein and/or or a pharmaceutical composition disclosed herein.

The method comprises:

- Providing an alpha- and/or beta-emitting radionuclide or a salt thereof,
- Providing a particle with a sulfonic acid and/or phosphonic acid, preferably a sulfonic acid conjugated polymeric resin,
- Mixing said alpha- and/or beta-emitting radionuclide and said particle in an aqueous solution at a pH in the range of pH 6.5-9.5,

- Isolating an alpha and/or a beta-emitting radionuclide labelled particle from said aqueous solution.

Accordingly, the method disclosed herein may comprise providing a salt of a nuclide, preferable ^{90}Y , such as $^{90}\text{YCl}_3$ or $^{90}\text{Y}_2(\text{SO}_4)_3$, $^{90}\text{Y}_3(\text{CH}_3\text{CO}_2)_3$, $\text{Y}(\text{NO}_3)_3$ or other salts. Additionally, the particles provided for said method are preferably conjugated with sulphuric acid, and preferably comprises of a co-polymeric styrene divinylbenzene. Examples of particles suitable for preparing the alpha- and/or beta-emitting radionuclide labelled particle are e.g., AG® 50W or AP® MP-50 or Aminex 50W- X4, X6resins (BioRad), Sulfopropyl Sepharose™ resins (GELifescience), and other sulphopropyl or sulphate conjugated agarose, dextran or similar resins.

In further embodiments, the invention relates to a method for producing an alpha- and/or beta-emitting radionuclide labelled particle as disclosed herein and/or or a pharmaceutical composition disclosed herein, comprising:

- Providing an ^{90}Y salt, preferably $^{90}\text{YCl}_3$,
- Providing a styrene divinylbenzene copolymer particle functionalized with a sulfonic acid with a size of 5-400 μm , preferably a size in a range of 20-125 μm , more preferably a size in the range of 20-50 μm , most preferably about 30-35 μm ,
- Mixing said salt and said particle in an aqueous solution at a pH in the range of pH 6.5-9.5, and
- Isolating a ^{90}Y labelled particle from said aqueous solution.

In embodiments, the mixing and isolation step is performed no more than 60 minutes, such as no more than 30 minutes, such as no more than 15 minutes within each other.

In the solution the pH is may be essential for some applications for several reasons, such as suitability for injection, where pH ranges far beyond physiological pH might induce unwanted tissue irritation, for example, if the pH is greater than 9, this may result in irritation of the blood vessels when the suspension is injected into the artery during TARE (SIRT) procedure. For these reasons, the pH is preferably in the range of pH 7-9, preferably less than pH 8.5, such as less than pH 7.5, such as about pH 7.4.

As shown in examples 1 and 2, the methods disclosed herein enables the labelling of particles with different ratios between the radioactive nuclides and the particles, and also at

radionuclide: particle ratios which introduces flaking from the particles when phosphate precipitation is used.

Accordingly, in embodiments, the weight ratio between the radionuclide and the particle is in the range of 1:40-1:6000 (mg/mg), such as in the range of 1:100-1:6000, such as in the range of 1:1000-1:6000, such as about 1:5000. The weight ratio of radionuclide to particle may be in the range of 1:40 to 1:6000, such as in the range of 1:50 to 1:5000, or such as in the range of 1:60 to 1:4000. Suitable sub-ranges within the broad range include, but are not limited to 1:50 to 1:3000, 1:75 to 1:2500, 1:100 to 1:2000, 1:150 to 1:1500, and 1:200 to 1:1000, with further exemplary sub-ranges being 1:45 to 1:100, 1:100 to 1:1000, and 1:1000 to 1:6000. In embodiments, the weight ratio between the radionuclide and the particle is about 1:5800. In embodiments, the weight ratio between the radionuclide and the particle is more than 1:5800, such as more than 1:5000, 1:4000, 1:3000, 1:2000, 1:1000, 1:500, 1:250, 1:100 or such as more than 1:75.

15

Accordingly, in additional embodiments, the weight ratio between the radionuclide and the particle is in the range of 1:40-1:1000, such as in the range of 1:50-1:100, 1:45-1:75, such as about 1:50. In embodiments, the weight ratio between the radionuclide and the particle is about 1:53.

20

To prepare the labelled particles for administration, it might be preferred to prepare the labelled particles in an aqueous suspension, wherein such an aqueous suspension may e.g., in addition to water for injection (WFI), comprise a buffering agent, such as e.g., acetate, phosphate, citrate, or glutamate. Alternatively, the particle suspension consists essentially of the alpha- and/or beta-emitting radionuclide labelled particles and water for injection (WFI).

25

Furthermore, for several ions, the potential for formation of water-soluble anionic hydroxides, such as e.g., $[Y(OH)_4]^-$, which could lead to removal of the bound alpha- and/or beta-emitting radionuclide from the particles.

30

In addition, said method may also comprise combining the alpha- and/or beta-emitting radionuclide labelled particle suspension with a biocompatible adhesive. The adhesive may e.g., be a single component adhesive or two- or more component adhesive, wherein said adhesive is curable once administered to the intended site, such as e.g., a tumour. Curing of a single component adhesive may occur by addition of heat, oxygen, UV-light, or similar methods. Curing of a two-component adhesive generally involves mixing of a first

35

component and a second component, whereafter the mixture of the two initiates the curing of the adhesive. Examples of such curable two-component adhesives are e.g., BIOGLUE®, comprising the two components bovine serum albumin and glutaraldehyde, VISTASEAL™ comprising the components fibrinogen and thrombin, hyaluronic acid-based adhesives and
5 gelatine-based adhesives.

Accordingly, the method provided herein may be used to prepare a composition, pharmaceutical composition and/or injectable composition comprising an alpha- and/or beta-emitting radionuclide labelled particle.
10

Additionally, as shown in example 8, the radionuclide labelled particle suspensions prepared as described herein have a low leaching rate leading of as low as 0,005% of the radionuclide following heat treatment up to 150°C for 30 minutes.

15 In particular, this low leaching rate further enables the use of autoclave and/or treatment by other heat intensive sterilisation methods after preparation of the radionuclide particle suspensions as disclosed herein.

Moreover, the low leaching rate under high temperatures enables the radionuclide particle
20 suspensions and bioglues comprising radionuclide particle suspensions produced according to the method disclosed herein to be used with procedures that have a heat intensive element, e.g. laser surgery, without experiencing any substantially increased leaching of the radionuclide from the site of insertion.

25 In additional embodiments, the present invention relates to a method of preparing a cured ⁹⁰Y radiotherapeutic composition comprising,

a) Providing,

- i. a ⁹⁰Y labelled particle suspension,
- ii. an uncured biocompatible adhesive and/or non-polymerized hydrogel
30 in an aqueous solution,
- iii. a curing and/or polymerizing agent which upon mixture with ii), or i) and ii) induces curing of said biocompatible adhesive and/or polymerization of said hydrogel, and

b) Mixing i., ii. and iii. to obtain a cured ⁹⁰Y radiotherapeutic composition,
35 comprising biocompatible adhesive and/or hydrogel embedded ⁹⁰Y radiotherapeutic particles.

In the context of the present disclosure, "cured" denotes the state where the adhesive has completed its transformation from a liquid or malleable form into a solid or semi-solid state.

This transformation occurs through chemical reactions, exposure to specific catalysts,
5 ultraviolet (UV) light, or elevated temperatures, depending on the type of adhesive. Once cured, the adhesive forms a strong, permanent bond between surfaces, exhibiting properties like strength, stability, and resistance to external factors.

In embodiments, the uncured biocompatible adhesive is an albumin. In embodiments, the
10 non-polymerized hydrogel comprises one or more components selected from the list consisting of chitosan, hyaluronic acid, collagen, gelatin, elastin, alginate, cellulose, and glycosaminoglycan. In embodiments, the curing agent may e.g., be glutaraldehyde or similar amide crosslinking agents, such as e.g., NHS-based or imidoester-based cross linkers, or hydroxy targeting crosslinkers such as diglycidyl ether (DDE) based crosslinkers.

15

A kit

In additional aspects the present invention also relates to kits for producing the particles and/or compositions as disclosed herein.

20 In particular the present invention also relates to a kit comprising; unlabelled particles comprising, or consisting of a polymeric resin conjugated with sulfonic acid; optionally reagents for preparing an alpha- and/or beta-emitting radionuclide labelled particle, or a pharmaceutical composition as disclosed herein; and optionally instructions for preparing said particle or pharmaceutical composition.

25

Said kit may further comprise, aqueous solution comprising an uncured curable biocompatible adhesive and/or non-polymerized hydrogel in an aqueous solution.

The kit may further comprise an aqueous solution comprising a curing and/or polymerizing
30 agent.

In embodiments, said curable biocompatible adhesive is preferably an albumin, preferable bovine serum albumin in additional embodiments, said curing and/or polymerizing agent is an amide cross linking agent, preferably glutaraldehyde.

35 The kit enables the production of said particles and/or compositions as disclosed herein, directly at the practitioner, thus greatly simplifying the process of providing the proper

treatment dose, without the need for extensive and laborious preparation of the radioactive particle.

Accordingly, the invention also relates to a method of preparing a cured ^{90}Y radiotherapeutic composition comprising, providing a ^{90}Y labelled particle suspension and an uncured biocompatible adhesive and/or non-polymerized hydrogel in an aqueous solution. Said method may further comprise providing a curing and/or polymerizing agent which upon mixture with said biocompatible adhesive and/or non-polymerized hydrogel induces curing of said biocompatible adhesive and/or polymerization of said hydrogel. Curing or polymerization of said biocompatible adhesive and/or non-polymerized hydrogel, may also be initiated by other external factors such as but not limited to exposure to UV-radiation, heat and/or air. Said method may thus comprise curing and/or polymerizing said ^{90}Y radiotherapeutic composition using a curing and/or polymerizing agent which upon mixture with said uncured biocompatible adhesive and/or non-polymerized hydrogel induces curing of said biocompatible adhesive and/or polymerization of said hydrogel, or initiating curing via exposure to UV-radiation, heat and/or air, thus obtaining a cured/polymerized ^{90}Y radiotherapeutic composition.

A cured or polymerized composition, may for instance be exceptionally suitable for cancer therapy where it is essential that the radioactive component of the treatment is kept in place in the indented surroundings, in order to reduce potential risks of particle mitigation, where the particles may migrate to unintended tissues and result in unwanted and harmful irradiation.

25 **A medical device**

In an aspect of the present invention is the particle according to the present invention a medical device or is comprised in a medical device.

A medical device is any instrument, apparatus, appliance, software, material or other article, whether used alone or in combination, including the software intended by its manufacturer to be used specifically for diagnostic and/or therapeutic purposes and necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of: Diagnosis, prevention, monitoring, treatment or alleviation of disease; Diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap; Investigation, replacement or modification of the anatomy or of a physiological process; Control of conception; and which does not achieve its principal intended action in or on the human

body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means.

Medical devices vary according to their intended use and indications. Examples range from simple devices such as tongue depressors, medical thermometers, and disposable gloves to advanced devices such as computers which assist in the conduct of medical testing, implants, and prostheses.

According to the FDA a medical device is “an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including a component part, or accessory which is: recognized in the official National Formulary, or the United States Pharmacopoeia, or any supplement to them, intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or intended to affect the structure or any function of the body of man or other animals, and which does not achieve any of its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of any of its primary intended purposes.”

The particles are carriers of radioactivity that are designed to have a limited, if any, chemical action within the body, and this allows for radiotherapy with very limited unwanted side-effects, such as toxicity.

Thus, in one embodiment is the term “medical device” understood as FDA’s definition above.

Medical uses

In particular embodiments, the particles or compositions as disclosed herein are for use as a medicament.

In particular medical uses of the particles or compositions as disclosed herein includes human or veterinary use in (1) loco-regional radiotherapy such as brachytherapy, (2) radioembolization, in particular in transarterial radioembolization, (3) Intracavitary therapy and (4) radiosynovectomy.

In preferred embodiments, the particles or pharmaceutical composition disclosed herein are for use in loco-regional radiotherapy, such as brachytherapy and transarterial

radioembolization (TARE). Transarterial radioembolization (TARE) is also known as selective internal radiation therapy (SIRT). Thus, one embodiment of the present invention relates to the particles or pharmaceutical composition disclosed herein are for use in transarterial radioembolization (TARE). One embodiment of the present invention relates to
5 the particles or pharmaceutical composition disclosed herein are for use in brachytherapy.

Loco-regional radiotherapy is a procedure where ionizing radiation is precisely directed to eliminate or damage cancer cells in specific local and regional areas, avoiding off-target and/or systemic exposure to the radioactive source. This modality primarily targets the tumor
10 and its adjacent tissues, reducing the probability of local and regional recurrence. It can be administered externally or internally and is often used in conjunction with other cancer therapies like surgery, chemotherapy, target therapies (such as but not limited to immunotherapy), aiming to provide optimal therapeutic benefits with minimized adverse effects to the surrounding healthy tissues and organs. Accordingly, in preferred
15 embodiments the particle as disclosed herein is for use in loco-regional radiotherapy.

Brachytherapy is a specialized and precise form of loco-regional radiotherapy. Brachytherapy involves placing a fixed radioactive source directly inside or very close to the
20 tumor, enabling the delivery of high doses of radiation to more localized areas. This helps to minimize damage to surrounding healthy tissues. Brachytherapy is particularly effective for cancers of the prostate, cervix, liver, and breast, allowing targeted treatment with fewer side effects. The precise nature of brachytherapy makes it a viable option for tumors located in critical body structures where accuracy is paramount.
25 Thus, a composition as disclosed herein which comprises the radioactive particles, and a biocompatible adhesive, is especially suited for brachytherapy as it enables the administration of high radioactivity doses, to specific areas, such as a tumour, which are fixed in place due to the curing of the adhesive once administered. As mentioned the curing reduces the side effects otherwise associated with classical radiation therapy.
30 Accordingly, in preferred embodiments the particle as disclosed herein is for use in brachytherapy.

TARE is a medical procedure used to treat liver tumours, such as primary hepatocellular carcinoma or secondary liver cancers. During TARE, radioactive microspheres are injected
35 directly into the blood vessels that supply the tumour. These microspheres emit radiation that targets and destroys cancer cells while sparing healthy tissue. TARE can help shrink

tumours, alleviate symptoms, and improve the quality of life for patients who are not suitable candidates for surgery including liver resection and orthotopic liver transplantation or other treatments, offering a targeted and well-tolerated option for managing certain liver malignancies and other types of malignancies.

5

In embodiments the particles of the present invention or compositions comprising same are also suitable for use in transarterial radioembolization.

10 In further embodiments, the particles of the present invention or compositions comprising same are for use in the treatment or amelioration of cancer.

Accordingly, uses of the particles or compositions as disclosed herein may also include use in the treatment or amelioration of cancer, such as e.g., in the treatment of head and neck squamous cell carcinoma, metastatic melanoma, sarcoma, non-small cell lung cancer, colorectal cancer, primary and secondary hepatocellular carcinoma, pancreatic ductal adenocarcinoma, renal cell carcinoma, ovarian cancer, muscle invasive bladder cancer, prostate cancer, and/or osteosarcoma, preferably, unresectable hepatocellular carcinoma and/or locally-advanced borderline-resectable pancreatic ductal adenocarcinoma. Preferably the particles or compositions as disclosed herein are used in the treatment of unresectable hepatocellular carcinoma.

20

Transarterial radioembolization may include treatment of primary or metastatic cancer in an organ e.g., the liver by administering the particles of the present invention to a blood vessel leading to a tumor in the liver or another solid organ infiltrated by tumor tissue.

25

In addition, different dosages of ^{90}Y to be delivered may be preferable for different types of metastatic cancer, depending on the tumour size, density, position and surrounding tissue.

In radionuclide therapy, the Gray (Gy) stands as a fundamental unit for quantifying radiation dose. This measurement signifies the amount of ionizing radiation energy deposited within biological tissues. One Gray is equivalent to one joule of energy absorption per kilogram of tissue ($1 \text{ Gy} = 1 \text{ J/kg}$). Gray is essential for determining the optimal radiation dose delivered to the targeted tissue or tumor while minimizing harm to healthy surrounding tissues.

30 The choice of the appropriate radiation dose in Grays hinges on factors such as the disease type, its stage, the specific radionuclide used, the affected tissue or organ, and individual

35

patient characteristics. Before treatment, the practitioner often calculates the required dosage to be administered tailored to each patient's unique medical situation.

Radionuclide therapy is a specialized field within nuclear medicine, where the Gray (Gy) plays a vital role in ensuring safe and effective radiation treatment. By maintaining this

5 balance, medical professionals can optimize therapeutic outcomes while minimizing potential side effects. For example, for HCC, 120 Gy is typically considered a reasonable minimum target dose, and the more precise the targeting of the treatment is, the higher doses may be administered. For other indications, lower or higher doses may be favored. Accordingly, in
10 embodiments, the target-absorbed dose is in the range of 1 Gy to 500 Gy, such as between 50 Gy and 400 Gy, such as about 50Gy, 60Gy, 70Gy, 80Gy, 90Gy, 100Gy, 110Gy, 120Gy, 130Gy, 140Gy, 150Gy, 160Gy, 170Gy, 180Gy, 190Gy, 200Gy, 210Gy, 220Gy, 230Gy, 240Gy, 250Gy, 260Gy, 270Gy, 280Gy, 290Gy, 300Gy, 310Gy, 320Gy, 330Gy, 340Gy, 350Gy, 360Gy, 370Gy, 380Gy, 390Gy, or such as about 400Gy. Preferably the target-absorbed dose is in the range of 40-300 Gy, such as about 40, 50, 60, 70, 80, 100, 150,
15 200, 250, or about 300 Gy.

In addition, MBq (megabecquerel) is often also used to measure the actual dose delivered to a patient, where the dose in the syringe can be readily measured and compared to the amount of radioactivity present in the syringe after injection, the active dose delivered to the
20 patient may be readily calculated.

In embodiments, the active dose delivered to the patients is in the range of 1 kBq to 10 GBq, such as in the range of 10-50 MBq, such as in the range of 50-500 MBq, such as in the range of 500 MBq to 1 GBq, or such as in the range of 1 GBq to 3 GBq, or such as in the
25 range of 3-10 GBq.

In embodiments the particles are administered directly into a tumor.

Another aspect of the present invention relates to a method of treatment or amelioration
30 comprising administration of the particles or the pharmaceutical composition as disclosed herein to an individual in need thereof.

In embodiments, the particles and/or compositions described herein are administered by through the hepatic artery.

In particular embodiments, the particles and/or compositions are used to treat hepatocellular carcinoma, including but not limited to injection or administration into a lesion, or to a treatment site following resection of a lesion. In addition, treatment may include intra-tumoral administration or distal metastasis site.

5

In embodiments, the composition as disclosed herein is for single treatment or repeated dosing.

Imaging

10 Radionuclide imaging leverages beta-emitting radionuclides to visualize internal structures and assess physiological functions within the human body. Beta-emitters, such as ^{99m}Tc and ^{131}I , are commonly employed in this imaging modality due to their ability to emit beta particles, which are high-energy electrons or positrons. The particles of the present invention are preferably administered via intratumor administration, allowing for direct targeting to
15 target specific tissues and/or organs of interest. Once administered, the beta-emitting radionuclides undergo radioactive decay, emitting beta particles that can be detected by a gamma camera or positron emission tomography (PET) scanner. In addition, radionuclide imaging may also be used to evaluate the site of injection of particles and/or compositions described herein.

20

Accordingly, in embodiments particles and compositions as described herein may be for use in radionuclide imaging.

It should be understood that any feature and/or aspect discussed above in connection with
25 the compounds according to the invention apply by analogy to the methods described herein.

The following figures and examples are provided below to illustrate the present invention. They are intended to be illustrative and are not to be construed as limiting in any way.

30 EXAMPLES

Example 1 - Incorporation of Yttrium on cation exchange resin

Aim

The present example aims to show that it is possible to incorporate the Yttrium onto microspheres by absorption at a neutral pH, without precipitating it as an insoluble
35 phosphate salt.

Materials

Raw Materials

YCl₃ anhydrous from Sigma-Aldrich

- 5 AG 50W-X4 resin 200-400 from BioRad Laboratories, Inc

Method

Yttrium (90Y) chloride in dilute HCl 0.04 M contains 0.1-300 GBq Yttrium (90Y) pr. 1 ml, on the reference date and time corresponding to 0.005-15 micrograms of Yttrium [90Y] (as

- 10 Yttrium [90Y] chloride).

In the present example 89Y was used instead of 90Y, and to mimic the intended amount of radiation required in the final vials, the amount of yttrium was calculated from the intended dose/mL.

Accordingly, to reach a dose of 1.5 GBq in 1 mL of sterile solution of ⁹⁰Y in 0.04M HCl (1

- 15 $\mu\text{mol } ^{90}\text{Y}$ to reach 1.5 Gbq ⁹⁰Y), it was calculated that about 90 μg of Yttrium (89Y or 90Y) pr. 530mg of dry resin was required.

The procedure has been tested through 4 main steps:

- titration of the resin;
- 20 - conditioning of the resin as sodium salt;
- preparation of YCl₃ solution;
- absorption of Yttrium onto the resin.

- Titration of the resin: 1.6 g of AG 50W-X4 cation exchange resins (supplier: Bio-Rad
- 25 Laboratories, Inc Code # 1421351, sulfonic acid functional group) was added to a 5 ml syringe fitted with fitted with a polypropylene fritted disc and washed with NaOH 1.0 M to perform the cation exchange until the pH of the collected water phase was above pH 7 (basic), which indicates a termination of the ion exchange. The collected water phase was titrated with HCl 1.0 M until the pH was neutral (about pH 7). 3.5 mmol of NaOH was needed
- 30 to perform cation exchange, suggesting that the loading of the dry resin corresponds to 6.5 mmol of active sites for 1.0 g of resin.

- Conditioning of the resin as sodium salt: the step is designed to generate 530 mg of phosphate free resin with 1.5 GBq of 90-Y solution. Between 1.5 and 1.7 g of AG 50W-X4
- 35 cation exchange resin was added to a 5 ml syringe fitted with a polypropylene disc. The

resin was washed; firstly with 3.5 ml of NaOH 1.0 M to perform the cation exchange and then four times with 2 ml of water for injection (WFI) to complete the conditioning of the resin as sodium salt.

5 Preparation of YCl₃ solution and absorption of Yttrium onto the resin:

Firstly, 90 µg of YCl₃ anhydrous was dissolved in 1 ml of 0.04M HCl to obtain a homogenous solution. Secondly, 0.4 ml NaOH 0.1 M was added to the Yttrium Chloride solution to reach neutral pH.

- 10 The neutralized YCl₃ solution was added to the syringe containing the resin and 1 ml of water was used to wash the Yttrium vial and the syringe. The mixture was then gently shaken for 15 min, to allow absorption of yttrium into the activated resin microspheres.

The final solution was filtered, and the resin was washed multiple times with WFI.

15

Energy-dispersive X-ray spectroscopy (EDS) was conducted to validate the incorporation of Yttrium on the particles.

- 20 In addition, the Yttrium labelled particles were imaged by scanning electron microscopy and compared to particles prepared by phosphate precipitation of Yttrium to the particles.

Results

- As is shown in figure 1 Energy-dispersive X-ray spectroscopy (EDS) analysis of the produced particles shows that this procedure allows Yttrium to be absorbed on the resin, verifying the absorption of Yttrium onto the cation exchange resin at neutral pH without the use of phosphate salts and without precipitation of the metal. The stoichiometry presented for Yttrium absorption, could be replicated for other metals such as e.g., Holmium, Lutetium or Actinium.
- 25

- 30 The Scanning Electron Microscopy (SEM) of the product presented in figure 2 shows a homogeneous and thin surface of the microspheres compare to the particles prepared according to the methods of the prior art, utilizing phosphate precipitation, (See figure 3 and figure 4), which shows a denser structure with rough surfaces due to the precipitate. Such rough structure may be more susceptible of leaching in case of alterations in the environmental conditions, such as e.g., changes in pH.
- 35

Accordingly, the Yttrium-90 bound on biodegradable microspheres produced according to the described process, provides an improved and simplified labelling process.

5 Example 2 - Incorporation of high concentration of YCl_3 on cation exchange resin

Aim

The aim of the present example is to test the feasibility of incorporation of high amounts of Yttrium on the particles. The experiments have been carried out using the novel method and the phosphate-salt precipitation method.

10

Materials

Raw Materials

YCl_3 anhydrous from Sigma-Aldrich

AG 50W-X4 resin 200-400 from BioRad Laboratories, Inc

15

Na_3PO_4 solution 1.25% w/v (weight/volume) (mm = 163, 941 g/mol)

Phosphate buffer solution 0.1 M, pH 7.5

Methods

Novel Method:

20

Titration of the resin: The resin was titrated as described in example 1.

Conditioning of the resin as sodium salt: Between 1.5 and 1.7 g of AG 50W-X4 cation exchange resin is added to a 5 ml syringe fitted with a polypropylene fritted disc. The resin is washed firstly with 3.5 ml of NaOH 1.0 M to perform the cation exchange and then with 2 ml of WFI for four times to complete the conditioning of the resin as sodium salt.

25

Preparation of YCl_3 solution and absorption of Yttrium onto the resin: 10 mg (10 milligrams) of YCl_3 anhydrous was dissolved in 0.04M HCl to obtain a homogenous solution. 0.4 ml NaOH 0.1 M was added to the Yttrium Chloride solution to reach neutral pH.

30

The neutralized YCl_3 solution was added to the syringe containing the resin and 1 ml of water was used to wash the Yttrium vial and the syringe. The mixture is then gently shaken for 15 min, to allow absorption of yttrium into the activated resin microspheres. The final solution is filtered and resin washed multiple times with WFI.

35

Phosphate salt precipitation method:

In brief, preparation of the phosphate precipitated particles was conducted according to the following the steps:

- 10 mg of YCl_3 were dissolved in 2.0 mL of H_2SO_4 0.1 M, and then the Yttrium sulphate solution was added to the syringe containing the resin.
- 2.0 mL of Na_3PO_4 solution (1.25% w/v) added to the reaction vessel to precipitate the Yttrium phosphate on the resin (AG 50W-X4, as in example 1).
- The particles were washed multiple times with 2.0 mL of phosphate buffer solution 0.1 M pH 7.5 until the pH is stabilized. Microspheres washed with 2.0 mL of water for injection.

Results

To overstress the process and to show that binding more Yttrium was possible using the process as disclosed in example 1, an amount of Yttrium 100-times greater than the one typically used in previous methods, was absorbed onto the AG 50W-X4 cation exchange resin using the current process. The resulting particles were compared to particles prepared according to the current available methods using phosphate precipitation, also with a 100-times higher Yttrium amount.

Scanning Electron Microscopy (SEM) of the product shows an homogeneous and thin surface of the microspheres analysis of the product (figure 5) shows that this procedure allows Yttrium to be absorbed on the resin, concluding that the incorporation of Yttrium onto the resin at neutral pH without the use of phosphate salts and without precipitation of the metal, is possible even using over 100-times more Yttrium.

Energy-dispersive X-ray spectroscopy (EDS) analysis of the phosphate precipitated particles (figure 6) shows that this procedure allows Yttrium to be precipitated as phosphate salt onto the resin.

As can be seen in figure 7 the particles prepared according to the phosphate precipitation method using the high Yttrium amounts, was not able to assure a stable structure when high amount of Yttrium is used, due to deposition of multiple layers of the phosphate salts, which will lead to a leaching and flaking of free Yttrium from the particles. If the pH is kept at 12.5, there is a risk of formation of water-soluble anionic hydroxides $[\text{Y}(\text{OH})_4]^-$ that could lead to the removal of yttrium-90 from the surface of the microspheres. Moreover, if the pH is greater than 9, this may result in irritation of the blood vessels when the suspension is

injected into the artery. For these reasons, the pH is preferably less than 8.5 and more preferably less than 7.5, but more preferably about 7.4.

The present example thus demonstrates that stressing the process using over 100 times more Yttrium compared to conventional process still allows the absorption of high quantity of Yttrium onto microspheres and that the absorbed Yttrium does not substantially leach from the particulate material under physiological conditions.

Example 3 - Measurement and production of radioactive particles

10 *Aim*

The present example aims to show that it is possible to measure and produce radioactive particles using the new process described in example 1 incorporating radioactive Yttrium (90Yttrium) onto microspheres by absorption at a neutral pH, without precipitating it as an insoluble phosphate salt.

15

Materials

Raw Materials

90YCl₃ from Eckert & Ziegler

AG 50W-X4 resin 200-400 from BioRad Laboratories, Inc

20

Method

The procedure has been tested through 6 main steps:

- titration of the resin;
- conditioning of the resin as sodium salt;
- 25 - preparation of 90YCl₃ solution;
- absorption of 90Yttrium onto the resin;
- extraction and elution phase by washing the Y-90 resin;
- process yield calculation.

30 Titration of the resin: The resin was titrated as described in example 1.

Conditioning of the resin as sodium salt:

Between 1.5 and 1.7 g of AG 50W-X4 cation exchange resin (AG 50W-X4 resin 200-400 from BioRad Laboratories, Inc) was added to a vacuum filtration unit. The resin was washed with 4.0 ml of NaOH 1.0 M to perform the cation exchange. Vacuum was applied until resin became dry. pH of collected fraction was checked (acceptance criteria pH 11-13). Resin was

then washed 4 times with 50 mL of water for injection (WFI) for a total of 200 mL to complete the conditioning of the resin as sodium salt. Vacuum was applied until resin became dry. pH of collected fraction was checked (Acceptance criteria pH = 6.00-8.00).

5 Preparation of 90YCl₃ solution and absorption of 90Yttrium onto the resin:

Resin was collected from vacuum filtration unit and transferred to 50 mL polypropylene tube. The activity 0.1 mL of 90YCl₃ (from Eckert & Ziegler) original sample was measured in the dose calibrator.

10 The 0.1mL 90YCl₃ sample was diluted by adding 0.90 mL of 0.04 N HCl to obtain a homogenous solution. 0.4 ml NaOH 0.1 M was added to the Yttrium Chloride solution to reach neutral pH (Acceptance Criteria: 6.00-8.00). The activity of the diluted 90Y sample was measured in the dose calibrator.

The neutralized 90YCl₃ solution was transferred, using a syringe, and was added to the 50 mL propylene tube containing the resin. Using the same syringe 1 ml of water was used to wash all the 90Yttrium solution from the syringe and was pushed onto the resin tube. The mixture was then gently shaken for 15 min, to allow absorption of 90Yttrium into the activated resin microspheres.

After the transfer have been completed, the leftover activity in the 90Yttrium vial was measured using a dose calibrator.

20

Extraction and elution phase by washing the Y-90 resin:

A funnel with polyethylene frit was put on ring stand and a new 50 mL polypropylene tube was placed underneath (elution fraction tube). The 90Y-resin was transferred from 50 mL polypropylene tube to funnel. The leftover activity in the 50 mL polypropylene tube was measured using a dose calibrator.

Using a 10 mL syringe, the product wash pushed through the funnel and the fraction was collected in the 50 mL polypropylene tube.

The resin fraction was washed 4 times with 1.6 mL of water: using the plunger of a 10 mL syringe, the water was pushed through the funnel until resin was dry.

30 The activity of the resin in the funnel was measured using a dose calibrator as well as the activity of the elution fraction.

8 mL of WFI are added to the funnel to form a suspension, that is placed in a 10 ml syringe which was made empty into the final 10 mL vial. The activity of the vial, leftover activity of the syringe and leftover activity in the funnel were measured using a dose calibrator:

Process yield calculation:

The yield of the process was calculated by applying the following formula:

$$\text{Yield} = (\text{final activity} / \text{Initial } ^{90}\text{Y Fraction}) * 100$$

The losses have been calculated as well. Losses = elution fraction + unwashed 50 mL tube

5 + empty polyethylene funnel/frit.

Results

As is shown in Table1 the yield obtained with the described process is very high (97%).

10

Sample	Activity
Initial ^{90}Y Sample	25.7 mCi
Diluted ^{90}Y Sample	23.9 mCi
Initial ^{90}Y Fraction (1.0 mL)	16.75 mCi @ 14:14
Activity remaining in vial	7.15 mCi @ 14:15
Elution Fraction	88.6 μCi @ 14:36
Unwashed 50mL tube	223 μCi @ 14:35
Final Activity (Resin Suspension Final Product Vial)	16.25 mCi @14:43
Empty Polyethylene Funnel/Frit	252 μCi @14:42
Calculated yield	97% $(16.25 / 16.75) * 100$
Calculated losses	563.6 μCi $(88.6 \mu\text{Ci} + 223 \mu\text{Ci} + 252 \mu\text{Ci})$

Table 1 - Radioactivity measurements and yield and losses calculation.

In addition, the Yttrium labelled particles were imaged by scanning electron microscopy.

15

Scanning Electron Microscopy (SEM) of the product shows an homogeneous and thin surface of the microspheres analysis of the product (figure 8) shows that this procedure allows Yttrium to be absorbed on the resin, concluding that the incorporation of Yttrium onto the resin at neutral pH without the use of phosphate salts and without precipitation of the metal, is possible.

20

Yttrium-90 decays with a half-life of 64 hours, while emitting a high energy pure beta radiation. However, the process is also applicable to all the others metals/radionuclides which may also be used in place of Yttrium-90 (i.e. but not limited to holmium, lutetium, actinium, rhenium) applying same stoichiometry described above.

25

Example 4 - In vitro stability of radiolabelled microparticles**AIM**

The present example aims to show that 90-Yttrium microspheres produced with the novel method are stable at high temperature so they can be terminal sterilized using autoclave.

5

Approved and well adopted autoclave cycles, among the Industries, are:

- 121 °C for 30 minutes;
- 132 °C for 7 minutes.

A further stress-test was performed using higher temperature: 150 °C for 30 minutes.

10

A control group at ambient temperature (25 °C) was used as well.

Materials

Same materials described and used in Experiment 3.

Method15

The starting activity of 90Yttrium was different but the production and measurements steps of radioactive microspheres are the same as described in example 3:

- titration of the resin;
- conditioning of the resin as sodium salt;
- preparation of 90YCl3 solution;
- 20 - absorption of 90Yttrium onto the resin;
- extraction and elution phase by washing the Y-90 resin;
- process yield calculation.

25

After calculating the process yield the collected resin was resuspended with 8 mL of water for injection.

The resulting suspension was spitted into 4 equal parts (2 mL each sample) into 10 mL glass vials with crimped septum.

The following activity of each vial was measured:

- 30
- a. Activity of Vial A: 42.9 mCi @ 14:41
 - b. Activity of Vial B: 5.90 mCi @ 14:41
 - c. Activity of Vial C: 1.579 mCi @ 14:42
 - d. Activity of Vial D: 830 µCi @ 14:42

The vials were then subjected to the respective stress conditions as described in the Table 2 below:

	Vial # A	Vial # B	Vial # C	Vial # D
Stress Test Conditions	121°C/ 30 min	132°C/ 7 min	150°C/ 30 min	Ambient temperature

Table 2 - Stress Temperature conditions

- 5 The activity of each vial was checked using a dose calibrator after equilibration at room temperature according to what is reported in Table 3.

	Vial # A	Vial # B	Vial # C	Vial # D
Activity of the suspension after equilibration to room temperature	42.3 mCi @ 16:54	5.81 mCi @ 17:00	1.55 mCi @ 17:08	821 µCi @ 17:12

Table 3 - Activity measurements of each vial after T cycle

- 10 Each vial was resuspended by gentle swirling, decrimped and transferred onto the funnel with polyethylene frit and filtered individually. The elution fraction and the activity left in the decrimped vial was checked using a dose calibrator, results reported in Table 4.

	Vial # A	Vial # B	Vial # C	Vial # D
				15
Elution fraction	2 µCi	1.7 µCi	8.4 µCi	5.25 µCi
Activity left in decrimped vial	2.96 mCi	1.067 mCi	413.0 µCi	90.0 µCi

Table 4 - Activity measurements of elution fraction and vial leftover

20 Results

As is shown in Table 5 the measured % of loss of 90Y after Temperature stress test was very low (0.005%-0.7%). Moreover, for the 2 approved autoclave cycle (121 °C for 30 minutes and 132 for 7 minutes) the measured % of loss of 90Y after Temperature stress test was 0.005% and 0.04% respectively.

25

	Vial # A	Vial # B	Vial # C	Vial # D
--	----------	----------	----------	----------

*Initial Activity of the suspension	42.9 mCi @ 14:41	5.90 mCi @ 14:41	1.579 mCi @ 14:42	830 µCi @ 14:42
Stress Test Conditions	121°C/30 min	132°C/7 min	150°C/30 min	Ambient
Activity of the suspension after equilibration to room temperature	42.3 mCi @ 16:54	5.81 mCi @ 17:00	1.55 mCi @ 17:08	821 µCi @ 17:12
Vials resuspended, decrimped, and transferred onto the funnel to check elution activity				
Elution fraction	2 µCi	1.7 µCi	8.4 µCi	5.25 µCi
Activity left in decrimped vial	2.96 mCi	1.067 mCi	413.0 µCi	90.0 µCi
% Loss	$\frac{0.002mci}{(42.3 - 2.96)mci} * 100 = 0.005 \%$	$\frac{0.0017mci}{(5.81 - 1.067)mci} * 100 = 0.04 \%$	$\frac{0.0084 mci}{(1.55 - 0.413)mci} * 100 = 0.7 \%$	$\frac{0.0052 mci}{(0.821 - 0.09)mci} * 100 = 0.7 \%$

Table 5 - Summary of results

During this experiment, the goal was to mimic a terminal sterilization process, heating the final product and determining the amount of activity remaining attached to the microspheres and the % of 90 Yttrium loss after the heating process.

Additionally, the microspheres were overstressed by subjecting the composition to a temperature of 150 °C for 30 minutes, which is above the normal conditions of autoclavation.

As a conclusion, the simulation of the terminal sterilization of the 90Y-resin using high temperature showed that the 90Y remained attached to the resin when exposed to various temperatures for different time durations. The % of loss Y90 measured was very low (0.005% and 0.04% i.e., less than 1% (0.7%) even under over-stressing condition.

Example 5 - Measurement and production of radioactive particles using a resin conditioned with NaOH

Aim

The present example aims to show that it is possible to measure and produce radioactive particles using the new process described in the present disclosure, incorporating

radioactive Yttrium (^{90}Y trium) onto microspheres by absorption at a neutral pH, without precipitating it as an insoluble phosphate salt, using microspheres Aminex 50W-X4 resin, 25-37 μm . Despite the resin comes already in sodium form, it has been decided to evaluate the conditioning step with NaOH anyway and seek for results.

5

Materials

Raw Materials

 $^{90}\text{YCl}_3$ from Eckert & ZieglerAminex 50W-X4 resin, 25-37 μm from BioRad Laboratories, Inc

10

Method

The procedure has been tested through 6 main steps:

- titration of the resin;
- conditioning of the resin as sodium salt;
- 15 - preparation of $^{90}\text{YCl}_3$ solution;
- absorption of ^{90}Y trium onto the resin;
- extraction and elution phase by washing the Y-90 resin;
- process yield calculation.

20 Titration of the resin: The resin was titrated as described in example 1.

Conditioning of the resin as sodium salt:

1.16 g of AG 50W-X4 cation exchange resin (Aminex 50W-X4 resin, 25-37 μm from BioRad Laboratories, Inc) was added to a vacuum filtration unit. The resin was washed with 4.0 ml of
25 NaOH 1.0 M to perform the cation exchange. Vacuum was applied until resin became dry. pH of collected fraction was checked (acceptance criteria pH 11-13). Resin was then washed 4 times with 50 mL of water for injection (WFI) for a total of 200 mL to complete the conditioning of the resin as sodium salt. Vacuum was applied until resin became dry. pH of collected fraction was checked (Acceptance criteria pH = 6.00-8.00).

30

Preparation of $^{90}\text{YCl}_3$ solution and absorption of ^{90}Y trium onto the resin:

Resin was collected from vacuum filtration unit and transferred to 50 mL polypropylene tube. The activity 0.1 mL of $^{90}\text{YCl}_3$ (from Eckert & Ziegler) original sample was measured in the dose calibrator.

The 0.1mL $^{90}\text{YCl}_3$ sample was diluted by adding 0.90 mL of 0.04 N HCl to obtain a homogenous solution. 0.4 ml NaOH 0.1 M was added to the Yttrium Chloride solution to reach neutral pH (Acceptance Criteria: 6.00-8.00). The activity of the diluted ^{90}Y sample was measured in the dose calibrator.

- 5 The neutralized $^{90}\text{YCl}_3$ solution was transferred, using a syringe, and was added to the 50 mL propylene tube containing the resin. Using the same syringe 1 ml of water was used to wash all the ^{90}Y solution from the syringe and was pushed onto the resin tube. The mixture was then gently shaken for 15 min, to allow absorption of ^{90}Y into the activated resin microspheres.
- 10 After the transfer have been completed, the leftover activity in the ^{90}Y vial was measured using a dose calibrator.

Extraction and elution phase by washing the Y-90 resin:

- 15 A funnel with polyethylene frit was put on ring stand and a new 50 mL polypropylene tube was placed underneath (elution fraction tube). The ^{90}Y -resin was transferred from 50 mL polypropylene tube to funnel. The leftover activity in the 50 mL polypropylene tube was measured using a dose calibrator.

Using a 10 mL syringe, the product wash pushed through the funnel and the fraction was collected in the 50 mL polypropylene tube.

- 20 The resin fraction was washed 4 times with 1.6 mL of water: using the plunger of a 10 mL syringe, the water was pushed through the funnel until resin was dry.
The activity of the resin in the funnel was measured using a dose calibrator as well as the activity of the elution fraction.

- 25 8 mL of WFI are added to the funnel to form a suspension, that is placed in a 10 ml syringe which was made empty into the final 10 mL vial. The activity of the vial, leftover activity of the syringe and leftover activity in the funnel were measured using a dose calibrator:

Process yield calculation:

The yield of the process was calculated by applying the following formula:

- 30 $\text{Yield} = (\text{final activity}/\text{Initial } ^{90}\text{Y Fraction}) * 100$

The losses have been calculated as well. Losses = elution fraction + unwashed 50 mL tube + empty polyethylene funnel/frit.

Results

- 35 As is shown in Table 6 the yield obtained with the described process is good (84%).

Sample	Activity
Initial ⁹⁰ Y Fraction (1.0 mL)	8.2 mCi @ 19:44
Elution Fraction	3.09 µCi @ 20:12
Unwashed 50mL tube	112.5 µCi @ 20:14
Final Activity (Resin Suspension Final Product Vial)	6.90 mCi @ 20:20 (small volume not recoverable from funnel)
Calculated yield	84% (6.90 / 8.2) * 100
Calculated losses	1.3 mCi

Table 6 - Radioactivity measurements and yield and losses calculation.

Yttrium-90 decays with a half-life of 64 hours, while emitting a high energy pure beta radiation. However, the process is also applicable to all the others metals/radionuclides which may also be used in place of Yttrium-90 (i.e. but not limited to holmium, lutetium, actinium, rhenium) applying same stoichiometry described above.

Example 6 - Measurement and production of radioactive particles using resin without NaOH conditioning

Aim

The present example aims to show that it is possible to measure and produce radioactive particles using the new process as described in the present disclosure, incorporating radioactive Yttrium (90Yttrium) onto microspheres by absorption at a neutral pH, without precipitating it as an insoluble phosphate salt, using microspheres Aminex 50W-X4 resin, 25-37 µm.

Materials

Raw Materials
90YCl3 from Eckert & Ziegler
Aminex 50W-X4 resin, 25-37 µm from BioRad Laboratories, Inc

Method

The procedure has been tested through 5 main steps:

- washing with water for injection;
- preparation of 90YCl3 solution;
- absorption of 90Yttrium onto the resin;

- extraction and elution phase by washing the Y-90 resin;
- process yield calculation.

Conditioning of the resin by washing with water:

- 5 1.13 g of AG 50W-X4 cation exchange resin (Aminex 50W-X4 resin, 25-37 μm from BioRad Laboratories, Inc) was added to a vacuum filtration unit. Because the resin is already in sodium form the Resin was only washed with 4 mL of water for injection (WFI) without carrying on the step with NaOH. Vacuum was applied until resin became dry. pH of collected fraction was checked (Acceptance criteria pH = 6.00-8.00).

10

Preparation of $^{90}\text{YCl}_3$ solution and absorption of $^{90}\text{Yttrium}$ onto the resin:

Resin was collected from vacuum filtration unit and transferred to 50 mL polypropylene tube. The activity 0.1 mL of $^{90}\text{YCl}_3$ (from Eckert & Ziegler) original sample was measured in the dose calibrator.

- 15 The 0.1mL $^{90}\text{YCl}_3$ sample was diluted by adding 0.90 mL of 0.04 N HCl to obtain a homogenous solution. 0.4 ml NaOH 0.1 M was added to the Yttrium Chloride solution to reach neutral pH (Acceptance Criteria: 6.00-8.00). The activity of the diluted ^{90}Y sample was measured in the dose calibrator.

- 20 The neutralized $^{90}\text{YCl}_3$ solution was transferred, using a syringe, and was added to the 50 mL propylene tube containing the resin. Using the same syringe 1 ml of water was used to wash all the $^{90}\text{Yttrium}$ solution from the syringe and was pushed onto the resin tube. The mixture was then gently shaken for 15 min, to allow absorption of $^{90}\text{Yttrium}$ into the activated resin microspheres.

- 25 After the transfer have been completed, the leftover activity in the $^{90}\text{Yttrium}$ vial was measured using a dose calibrator.

Extraction and elution phase by washing the Y-90 resin:

- 30 A funnel with polyethylene frit was put on ring stand and a new 50 mL polypropylene tube was placed underneath (elution fraction tube). The ^{90}Y -resin was transferred from 50 mL polypropylene tube to funnel. The leftover activity in the 50 mL polypropylene tube was measured using a dose calibrator.

Using a 10 mL syringe, the product wash pushed through the funnel and the fraction was collected in the 50 mL polypropylene tube.

- 35 The resin fraction was washed 4 times with 1.6 mL of water: using the plunger of a 10 mL syringe, the water was pushed through the funnel until resin was dry.

The activity of the resin in the funnel was measured using a dose calibrator as well as the activity of the elution fraction.

- 8 mL of WFI are added to the funnel to form a suspension, that is placed in a 10 ml syringe
5 which was made empty into the final 10 mL vial. The activity of the vial, leftover activity of the syringe and leftover activity in the funnel were measured using a dose calibrator:

Process yield calculation:

The yield of the process was calculated by applying the following formula:

- 10 Yield = (final activity/Initial ⁹⁰Y Fraction) * 100

The losses have been calculated as well. Losses = elution fraction + unwashed 50 mL tube + empty polyethylene funnel/frit.

Results

- 15 As is shown in Table 7 the yield obtained with the described process is very high (> 95%).

Sample	Activity
Initial ⁹⁰ Y Fraction (1.0 mL)	9.85 mCi @ 19:30
Elution Fraction	1.40 µCi @ 20:11
Unwashed 50mL tube	112.5 µCi @ 20:14
Final Activity (Resin Suspension Final Product Vial)	9.40 mCi @ 20:15
Calculated yield	95.4% (9.40 / 9.85) * 100
Calculated losses	450 µCi

Table 7 - Radioactivity measurements and yield and losses calculation

- 20 Yttrium-90 decays with a half-life of 64 hours, while emitting a high energy pure beta radiation. However, the process is also applicable to all the other metals/radionuclides which may also be used in place of Yttrium-90 (i.e. but not limited to holmium, lutetium, actinium, rhenium) applying same stoichiometry described above.

Example 7 - Measurement and production of radioactive particles no conditioning with NaOH – scale up activity***Aim***

- 5 The present example aims to show that it is possible to measure and produce radioactive particles using the new process as described in the present disclosure, incorporating radioactive Yttrium (⁹⁰Yttrium) onto microspheres by absorption at a neutral pH, without precipitating it as an insoluble phosphate salt, using microspheres Aminex 50W-X4 resin, 25-37 µm.

10 *Materials*

Raw Materials

⁹⁰YCl₃ from Eckert & Ziegler

Aminex 50W-X4 resin, 25-37 µm from BioRad Laboratories, Inc

15 *Method*

The procedure has been tested through 5 main steps:

- washing of the resin with water for injection;
- preparation of ⁹⁰YCl₃ solution;
- absorption of ⁹⁰Yttrium onto the resin;
- 20 - extraction and elution phase by washing the Y-90 resin;
- process yield calculation.

Conditioning of the resin by washing with water:

- 1.14 g of AG 50W-X4 cation exchange resin (Aminex 50W-X4 resin, 25-37 µm from BioRad Laboratories, Inc) was added to a vacuum filtration unit. Because the resin is already in sodium form the Resin was only washed with 4 mL of water for injection (WFI) without carrying on the step with NaOH. Vacuum was applied until resin became dry. pH of collected fraction was checked (Acceptance criteria pH = 6.00-8.00).

30 Preparation of ⁹⁰YCl₃ solution and absorption of ⁹⁰Yttrium onto the resin:

Resin was collected from vacuum filtration unit and transferred to 50 mL polypropylene tube. The activity 0.1 mL of ⁹⁰YCl₃ (from Eckert & Ziegler) original sample was measured in the dose calibrator.

- The 0.1mL ⁹⁰YCl₃ sample was diluted by adding 0.90 mL of 0.04 N HCl to obtain a
35 homogenous solution. 0.4 ml NaOH 0.1 M was added to the Yttrium Chloride solution to

reach neutral pH (Acceptance Criteria: 6.00-8.00). The activity of the diluted 90Y sample was measured in the dose calibrator.

The neutralized 90YCl₃ solution was transferred, using a syringe, and was added to the 50 mL propylene tube containing the resin. Using the same syringe 1 ml of water was used to wash all the 90Yttrium solution from the syringe and was pushed onto the resin tube. The mixture was then gently shaken for 15 min, to allow absorption of 90Yttrium into the activated resin microspheres.

After the transfer have been completed, the leftover activity in the 90Yttrium vial was measured using a dose calibrator.

Extraction and elution phase by washing the Y-90 resin:

A funnel with polyethylene frit was put on ring stand and a new 50 mL polypropylene tube was placed underneath (elution fraction tube). The 90Y-resin was transferred from 50 mL polypropylene tube to funnel. The leftover activity in the 50 mL polypropylene tube was measured using a dose calibrator.

Using a 10 mL syringe, the product wash pushed through the funnel and the fraction was collected in the 50 mL polypropylene tube.

The resin fraction was washed 4 times with 1.6 mL of water: using the plunger of a 10 mL syringe, the water was pushed through the funnel until resin was dry.

The activity of the resin in the funnel was measured using a dose calibrator as well as the activity of the elution fraction.

8 mL of WFI are added to the funnel to form a suspension, that is placed in a 10 ml syringe which was made empty into the final 10 mL vial. The activity of the vial, leftover activity of the syringe and leftover activity in the funnel were measured using a dose calibrator:

Process yield calculation:

The yield of the process was calculated by applying the following formula:

$$\text{Yield} = (\text{final activity}/\text{Initial 90Y Fraction}) * 100$$

The losses have been calculated as well. Losses = elution fraction + unwashed 50 mL tube + empty polyethylene funnel/frit.

Results

As is shown in Table 8 the yield obtained with the described process is outstanding (> 99%).

Sample	Activity
--------	----------

Initial ⁹⁰ Y Fraction (1.0 mL)	38.03 mCi @ 16:36
Elution Fraction	89.8 µCi @ 20:11
Unwashed 50mL tube	86,6 µCi @ 17:04
Final Activity (Resin Suspension Final Product Vial)	37.94 mCi @ 20:17
Calculated yield	99.8% (37.94 / 38.03) * 100
Calculated losses	230 µCi

Table 8 - Radioactivity measurements and yield and losses calculation

- 5 In addition, the Yttrium labelled particles were imaged by scanning electron microscopy.

Scanning Electron Microscopy (SEM) of the product shows an homogeneous and thin surface of the microspheres analysis of the product. The SEM shows that this procedure allows Yttrium to be absorbed on the resin, concluding that the incorporation of Yttrium onto the resin at neutral pH without the use of phosphate salts and without precipitation of the metal, is possible.

Yttrium-90 decays with a half-life of 64 hours, while emitting a high energy pure beta radiation. However, the process is also applicable to all the other metals/radionuclides which may also be used in place of Yttrium-90 (i.e. but not limited to holmium, lutetium, actinium, rhenium) applying same stoichiometry described above.

Example 8 - In vitro stability of radiolabelled microparticles

AIM

- 20 The present example aims to show that 90-Yttrium microspheres produced with the novel method are stable at high temperature so they can be terminal sterilized using autoclave.

Approved and well adopted autoclave cycles, among the Industries, are:

- 121 °C for 30 minutes;
- 25 132 °C for 7 minutes.;
- a further stress-test was performed using higher temperature: 150 °C for 30 minutes.

Materials

Same materials described and used in example 7.

Method

The starting activity of ^{90}Y trium was different but the production and measurements steps of radioactive microspheres are the same as described in example 7:

- washing of the resin with water for injection;
 - 5 - preparation of $^{90}\text{YCl}_3$ solution;
 - absorption of ^{90}Y trium onto the resin;
 - extraction and elution phase by washing the Y-90 resin;
 - process yield calculation.
- 10 After calculating the process yield the collected resin was resuspended with 8 mL of water for injection.
- The resulting suspension was spitted into 4 equal parts (2 mL each sample) into 10 mL glass vials with crimped septum.
- The following activity of each vial was measured:
- 15 a. Activity of Vial A: 10.41 mCi @ 17:12
- b. Activity of Vial B: 14.28 mCi @ 17:12
- c. Activity of Vial C: 13.42 mCi @ 17:13

The vials were then subjected to the respective stress conditions as described in the Table 9 below:

	Vial # A	Vial # B	Vial # C
Stress Test Conditions	121°C/ 30 min	132°C/ 7 min	150°C/ 30 min

Table 9 - Stress Temperature conditions

The activity of each vial was checked using a dose calibrator after equilibration at room temperature according to what is reported in Table 10.

	Vial # A	Vial # B	Vial # C
Activity of the suspension after equilibration to room temperature	9.25 mCi @ 19:09	12.70 mCi @ 19:00	12.10 mCi @ 18:51

25 Table 10 - Activity measurements of each vial after T cycle

Each vial was resuspended by gentle swirling, decrimped and transferred onto the funnel with polyethylene frit and filtered individually. The elution fraction and the activity left in the decrimped vial was checked using a dose calibrator, results reported in Table 11.

	Vial # A	Vial # B	Vial # C ₅
Elution fraction	0,34 μ Ci	0.40 μ Ci	0.62 μ Ci
Activity left in decrimped vial	361 μ Ci	362 μ Ci	206 μ Ci

Table 11 - Activity measurements of elution fraction and vial leftover

10

Results

As is shown in Table 12 the measured % of loss of ⁹⁰Y after Temperature stress test was very low (0.003%-0.005%). Moreover, for the 2 approved autoclave cycle (121 °C for 30 minutes and 132 for 7 minutes) the measured % of loss of ⁹⁰Y after Temperature stress test was 0.004% and 0.003% respectively.

15

	Vial # A	Vial # B	Vial # C
*Initial Activity of the suspension	10.41 mCi @ 17:12	14.28 mCi @ 17:12	13.42 mCi @ 17:13
Stress Test Conditions	121°C/30 min	132°C/7 min	150°C/30 min
Activity of the suspension after equilibration to room temperature	9.25 mCi @ 19:09	12.70 mCi @ 19:00	12.10 mCi @ 18:51
Vials resuspended, decrimped, and transferred onto the funnel to check elution activity			
Elution fraction	0,34 μ Ci	0.40 μ Ci	0.62 μ Ci
Activity left in decrimped vial	361 μ Ci	362 μ Ci	206 μ Ci
% Loss	$\frac{0.00034 \text{ mCi}}{(9.25 - 0.361) \text{ mCi}} * 100 = 0.004 \%$	$\frac{0.0004 \text{ mCi}}{(12.70 - 0.362) \text{ mCi}} * 100 = 0.003 \%$	$\frac{0.00062 \text{ mCi}}{(12.10 - 0.206) \text{ mCi}} * 100 = 0.005 \%$

Table 12 - Summary of results

During this experiment, the goal was to mimic a terminal sterilization process, heating the final product and determining the amount of activity remaining attached to the microspheres and the % of 90 Yttrium loss after the heating process.

- 5 Additionally, the microspheres were overstressed by subjecting the composition to a temperature of 150 °C for 30 minutes, which is above the normal conditions of autoclavation.

- As a conclusion, the simulation of the terminal sterilization of the 90Y-resin using high temperature showed that the 90Y remained attached to the resin when exposed to various
- 10 temperatures for different time durations. The % of loss Y90 measured was very low (0.003% and 0.005% i.e., much less than 1% (0.005%) even under over-stressing condition.

CLAIMS

1. An alpha- and/or beta-emitting radionuclide labelled particle, wherein said particle comprises or consists of a polymeric resin conjugated with sulfonic acid and an ionically bound alpha- and/or beta-emitting radionuclide, wherein the particle has a size in the range of 5-400 μm , and wherein said particle has a smooth surface with little or no protrusions.
2. The alpha- and/or beta-emitting radionuclide labelled particle according to claim 1, wherein the smooth surface with little or no protrusions is defined by a particle surface that has less protrusion than the same particle which has been labelled using phosphate precipitation.
3. The alpha- and/or beta-emitting radionuclide labelled particle according to claims 1-2, wherein the smooth surface with little or no protrusions is defined by a particle with a surface that has less than 10 protrusions each with a minimum height of 1 μm .
4. The alpha- and/or beta-emitting radionuclide labelled particle according to any of the preceding claims, wherein said particle is essentially phosphate free.
5. The alpha- and/or beta-emitting radionuclide labelled particle according to any of the preceding claims, wherein the amount of phosphate in the particle is less than 1 ppm.
6. The alpha- and/or beta-emitting radionuclide labelled particle according to any of the preceding claims, wherein the polymeric resin is a styrene divinylbenzene copolymer.
7. The alpha- and/or beta-emitting radionuclide labelled particle according to any of the preceding claims, wherein the polymeric resin is a polystyrene-divinylbenzene sulfonic acid resin.
8. The alpha- and/or beta-emitting radionuclide labelled particle according to any of the preceding claims, wherein the alpha- and/or beta-emitting radionuclide is selected from the group consisting of ^{90}Y , ^{225}Ac , ^{89}Sr , ^{153}Sm , ^{159}Gd , ^{18}F , ^{68}Cu , ^{69}Cu , ^{67}Ga , $^{99\text{m}}\text{Tc}$, ^{201}Ti , ^{111}In , ^{161}Tb , ^{212}Pb and ^{177}Lu .

9. The alpha- and/or beta-emitting radionuclide labelled particle according to any of the preceding claims, wherein the beta-emitting radionuclide is ^{90}Y .
- 5 10. The alpha- and/or beta-emitting radionuclide labelled particle according to any of the preceding claims, wherein the decay of said alpha, and/or beta-emitting radionuclide emits no more than 10 % gamma radiation of the total decay radiation.
- 10 11. The alpha- and/or beta-emitting radionuclide labelled particle according to any of the preceding claims, wherein the alpha- and/or beta-emitting radionuclide has a decay half-life in the range of 1-300 hrs, such as between 10-100 hrs, or such as 50-70 hrs.
- 15 12. The alpha- and/or beta-emitting radionuclide labelled particle according to any of the preceding claims, wherein the decay of said alpha, and/or beta-emitting radionuclide is primarily alpha and/or beta decay.
13. A pharmaceutical composition comprising a particle according claim 1-12.
14. The pharmaceutical composition according to claim 13, comprising a diluent, carrier, surfactant, deflocculant and/or excipient.
- 20 15. The pharmaceutical composition according to claim 13 or 14, further comprising a biocompatible adhesive or a component thereof.
- 25 16. The pharmaceutical composition according to any of claims 13-15, further comprising an albumin, such as e.g., bovine serum albumin.
- 30 17. The pharmaceutical composition according to any of claims 13-16, prepared with an amount of radionuclide that is 1kBq to 10GBq per dosing, or with an amount of radionuclide that is 50 MBq to 1000 GBq suitable for multidose industrial scale production.
18. The pharmaceutical composition according to any of claims 13-17, which is suitable for intratumor, intracavitary, and/or intra-arterial injection.

19. An alpha- and/or beta-emitting radionuclide labelled particle according to any of claims 1-12, or a pharmaceutical composition according to any of claims 13-18, for use as a medicament.
- 5 20. An alpha- and/or beta-emitting radionuclide labelled particle according to any of claims 1-12, or a pharmaceutical composition according to any of claims 13-18, for use in the treatment or amelioration of cancer.
- 10 21. An alpha- and/or beta-emitting radionuclide labelled particle according to any of claims 1-12, or a pharmaceutical composition according to any of claims 13-18, for use in the treatment of head and neck squamous cell carcinoma, metastatic melanoma, sarcoma, non-small cell lung cancer, colorectal cancer, primary and secondary hepatocellular carcinoma, pancreatic ductal adenocarcinoma, renal cell carcinoma, ovarian cancer, muscle invasive bladder cancer, prostate cancer, and/or
- 15 osteosarcoma, preferably, unresectable hepatocellular carcinoma and/or locally-advanced borderline-resectable pancreatic ductal adenocarcinoma.
22. An alpha- and/or beta-emitting radionuclide labelled particle according to any of claims 1-12, or a pharmaceutical composition according to any of claims 13-18, for
- 20 use in the treatment of unresectable hepatocellular carcinoma.
23. An alpha- and/or beta-emitting radionuclide labelled particle according to any of claims 1-12, or a pharmaceutical composition according to any of claims 13-18, for use in locoregional radiotherapy.
- 25 24. An alpha- and/or beta-emitting radionuclide labelled particle according to any of claims 1-12, or a pharmaceutical composition according to any of claims 13-18, for use in transarterial radioembolization.
- 30 25. An alpha- and/or beta-emitting radionuclide labelled particle according to any of claims 1-12, or a pharmaceutical composition according to any of claims 13-18, for use in brachytherapy.
- 35 26. An alpha- and/or beta-emitting radionuclide labelled particle according to any of claims 1-12, or a pharmaceutical composition according to any of claims 13-18, for use in radionuclide imaging.

27. An alpha- and/or beta-emitting radionuclide labelled particle according to any of claims 1-12, or a pharmaceutical composition according to any of claims 13-18, for single treatment or repeated dosing.
- 5
28. A method for producing an alpha- and/or beta-emitting radionuclide labelled particle according to any of claims 1-12, or a pharmaceutical composition according to any of claims 13-18, comprising
- 10
- a) Providing an alpha- and/or beta-emitting radionuclide or a salt thereof,
 - b) Providing a particle with a sulfonic acid conjugated polymeric resin,
 - c) Mixing said alpha- and/or beta-emitting radionuclide and said particle in an aqueous solution at a pH in the range of pH 6.5-9.5, and
 - d) Isolating an alpha and/or a beta-emitting radionuclide labelled particle from said aqueous solution.
- 15
29. The method according to claim 28, wherein pH of the aqueous solution is in the range of pH 7-9, preferably less than pH 8.5, such as less than pH 7.5, such as about pH 7.4.
- 20
30. The method according to claim 28 or 29, wherein the weight ratio between said radionuclide and said particle is in the range of 1:40-1:6000 (mg/mg), such as in the range of 1:100-1:6000, such as in the range of 1:1000-1:6000, such as about 1:5000.
- 25
31. The method according to any of claims 28-30, wherein the weight ratio between said radionuclide and said particle is in the range of 1:40-1:1000, such as in the range of 1:50-1:100, 1:45-1:75, such as about 1:50.
- 30
32. The method according to any of claims 28-31, wherein said method does not comprise immobilizing said radionuclide on said particle by phosphate precipitation.
33. The method according to any of claims 28-32, wherein the aqueous suspension comprises a buffering agent, such as e.g., acetate, phosphate, citrate, or glutamate.
- 35
34. The method according to any of claims 28-33, wherein in step d) is performed within no more than 60 minutes, such as no more than 30 minutes, such as no more than 15 minutes after step c).

35. A kit comprising;

- a) An unlabelled particle comprising or consisting of a polymeric resin conjugated with sulfonic acid, optionally
- 5 b) Reagents for preparing an alpha- and/or beta-emitting radionuclide labelled particle according to any of claims 1-12, or a pharmaceutical composition according to any of claims 13-18, and optionally
- 10 c) Instructions for preparing an alpha- and/or beta-emitting radionuclide labelled particle according to any of claims 1-12, or a pharmaceutical composition according to any of claims 13-18.

36. A kit according to claim 35, further comprising;

- d) An aqueous solution comprising an uncured curable biocompatible adhesive and/or non-polymerized hydrogel in an aqueous solution, and
- 15 e) An aqueous solution comprising a curing and/or polymerizing agent.

37. A kit according to claim 36, wherein said curable biocompatible adhesive is an albumin, preferable bovine serum albumin, and said curing and/or polymerizing agent is an amide cross linking agent, preferably glutaraldehyde.

20

38. A method of preparing a cured ⁹⁰Y radiotherapeutic composition comprising,

- a) Providing,
 - 25 i. a ⁹⁰Y labelled particle suspension comprising particles according to any one of claims 1-13,
 - ii. an uncured biocompatible adhesive and/or non-polymerized hydrogel in an aqueous solution,
 - iii. a curing and/or polymerizing agent which upon mixture with ii), or i) and ii) induces curing of said biocompatible adhesive and/or
 - 30 polymerization of said hydrogel, and
- b) Mixing i., ii. and iii. to obtain a cured ⁹⁰Y radiotherapeutic composition, comprising biocompatible adhesive and/or hydrogel embedded ⁹⁰Y radiotherapeutic particles.