Review: Radioactive Microspheres for Medical Applications

Urs Häfeli

Cleveland Clinic Foundation, Radiation Oncology Department T28
9500 Euclid Ave., Cleveland, OH 44195

e-mail: hafeliu@ccf.org

Keywords

radioactive microspheres, radioembolization, radiolabeling, brachytherapy, microspheres

Summary

This paper reviews the preparation and application of radioactive microspheres for medical purposes. It first discusses the properties of relevant radioisotopes and then explores the diagnostic uses of gamma-emitter labeled microspheres, such as blood flow measurement and imaging of the liver and other organs. The therapeutic uses of alpha- and beta-emitting microspheres, such as radioembolization, local tumor therapy and radiosynovectomy, are then described, and the recent developments in neutron capture therapy using gadolinium microspheres and boron liposomes discussed. The review concludes with some considerations in radiopharmaceutical kit preparations and radioisotope generator use, as well as with some radiobiological and dosimetric concerns.

Contents

Definition of microspheres

Many different kinds of microparticles are used for both diagnostic and therapeutic medical applications. In the broadest terms, as the name implies, microparticles or microspheres are defined as small spheres made of any material and sized from about 10 nm to about 2000 Φm. The term nanospheres is often applied to the smaller spheres (sized 10 to 500 nm) to distinguish them from larger microspheres. Ideally, microspheres are completely spherical and homogeneous in size (**Figure 1A**), although particles less homogeneous in size and shape are generally termed microspheres as well (**Figure 1B**). Depending on the preparation method and material used, microspheres show a typical size distribution which often deviates from the mono-sized ideal (**Figure 1C**). The category of microparticles also includes colloids which are crystallized, insoluble conglomerates of defined chemical composition, liposomes which are phospholipid vesicles, and naturally occurring particles such as red blood cells or leukocytes. When discussing general points in this review, the entire group of microparticles will simply be referred to as Amicrospheres≅. Larger molecules such as antibodies or peptides are also occasionally included in this group but will not be considered in this review. The therapy of tumors using radiolabeled antibodies, i.e. radioimmunotherapy, or radiolabeled peptides, has been nicely described by Wilder (1) and Papatheofanis (2).

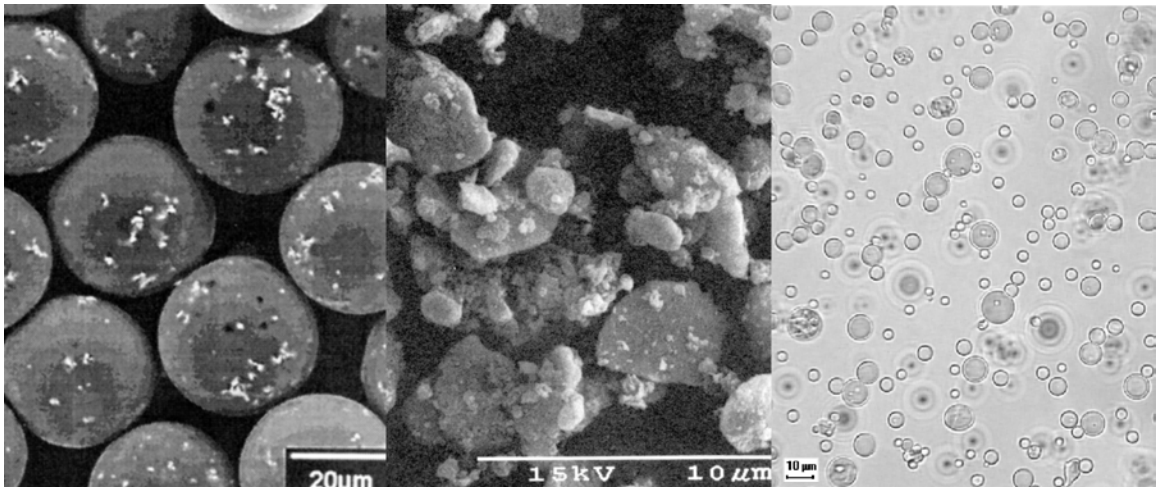

Figure 1: Microspheres for the delivery of radioactive isotopes. A) Spherical glass microspheres containing the two β-emitters ^{186}Re and ^{188}Re. B) Carbon-iron microspheres labeled with radioactive ^{99m}Tc. C) Poly(lactic acid) microspheres labeled with ^{188}Re.

Applications and in vivo fate of microspheres

The largest application for microspheres in medicine is drug delivery. Sales of advanced drug delivery systems in the U.S. alone exceeded $13 billion in 1997, and are expected to increase. The medical uses of particulate drug delivery systems cover all areas of medicine such as cardiology, endocrinology, gynecology, immunology, pain management and oncology. Most of the advanced drug delivery systems utilize microspheres or microcapsules for the encapsulation of drugs and proteins (see **Table 1**). The drug-loaded microspheres can be applied locally or delivered to the target area after intravenous injection by either passive means (e.g., trapping by size) or active means (e.g., magnetic targeting). From the target area, the encapsulated drug is slowly released over the desired time period, the length of which is determined mainly by the drug's biological half-life and its release kinetics from the microsphere matrix. This type of encapsulated drug delivery system has the advantage of protecting the encapsulated drug from the *in vivo* environment until time of release. Even very unstable substances such as growth hormones, interferon (3), or neuroactive peptides, (4) can be given in one daily dose instead of in several daily injections. Oral applications of very sensitive drugs such as insulin are also possible, as shown by Mathiowitz and others (5).

Table 1: Medical applications of microspheres

Mechanism	Application (Examples)	Microsphere Matrix (Examples)	Reference (Reviews)
Controlled drug delivery after local application	Release of proteins, hormones and peptides over extended times	PLA, PLGA, chitosan, polycyanoacrylate, polyanhydride	(6)
Oral drug delivery of easily degraded drugs	Gene therapy with DNA plasmids; delivery of insulin, LHRH	PLGA, styrene, polymethylmethacrylate	(7)
Vaccine delivery	Hepatitis, influenca, pertussis, ricin toxoid, diphteria toxoid; birth control	PLGA, chitosan	(8,9)
Drug targeting after intravenous / intraarterial application	Passive targeting of leaky tumor vessels, active targeting of tumor cell antigens, magnetic targeting with microspheres	Any biocompatible material; liposomes or erythrocytes	
Drug delivery without toxic side effects	Tumor targeting with doxorubicin, treatment of Leishmaniasis	PLA, PLGA, starch cyanoacrylates, etc.; (PEG-) liposomes	(10), (11)
Specific cell labeling	Stem cell extraction, bone marrow purging	Magnetic polystyrene microspheres	(12)
Affinity chromatography	Isolation of antibodies in immunology, cell separation, toxin extraction	Polymer resins such as Agarose-polyacrolein, Sephadex (polymer supports)	(13)
Adsorption of harmful substances in blood	Hemoperfusion	Agarose-PA, Sepharose, activated carbon, polyvinyl alcohol, polyacrylamide	(14)
Particle agglutination tests (qualitative and quantitative)	Diagnostic tests for infectious diseases (bacterial, viral, fungal Ψ); other tests in human diagnostics (growth hormones, FDP, Ψ)	Polystyrene (latex), silica, superparamagnetic particles	(15)

Endovascular embolization	Complex arteriovenous malformations in the brain; liver and other tumor treatment; management of life threatening hemoptysis and hematemesis	Poly(vinylalcohol), glass, polyurethane, poly(2- hydroxyethyl methacrylate)	(16)
Structure for cell growth	Cell culture of adherent cells in large amounts, 3D tissue structures possible	Gelatin, Sephadex, dextran, cellulose, collagen	(17)

Abbreviations: PLA = poly(lactic acid), PLGA = poly(lactide-co-glycolide), PEG = polyethylene glycol

The biodistribution and final fate of intravenously injected microspheres is highly dependent on their size and surface charge. Microspheres sized 10 to 30 Φm are larger than capillaries and will be trapped in the first capillary bed that they encounter. This effect is used for radioembolization therapy in which microspheres are injected into the artery that leads to the tumor of interest. Positively charged microspheres sized in the micrometer range are quickly taken out of the blood pool by the reticuloendothelial cells of the liver and spleen (18). Particles smaller than 0.1 Φm are able to pass the fenestration in the liver and may be able to target the hepatocytes, although most are still taken up by the liver's Kupffer cells. Negatively charged or neutral nanospheres such as small PEG-coated nanospheres or liposomes can evade this fast uptake and circulate in the blood system for up to several days (19). Over time, these long-circulating nanospheres will be concentrated in the tumor area because of the leaky capillary system of the newly growing tumor vasculature, which allows for extravasation of the nanospheres (20). A more active way of increasing the concentration of nano- or microspheres in the target tissue is to bind antibodies against the target cells on the nanospheres' surface (21). Alternatively, nanospheres, microspheres and colloids with a high affinity for white blood cells can be prepared. Such particles are rapidly taken up by the white blood cells and then concentrate in inflammatory regions because of chemotaxis and phagocytosis (22). Made radioactive, such nanospheres are useful for diagnostic (imaging) purposes, as well as for therapy.

General properties of radioactive microspheres

The subgroup of microspheres that is radioactive behaves and is generally used in a similar fashion to non-radioactive microspheres. But in addition to the matrix substance, which defines the microsphere and gives it its targeting properties in a desired tissue or organ, the radioactive microsphere also contains one or more radionuclide(s) that are intimately bound to it.

Even in small concentrations, radioactive microspheres are able to deliver high radiation doses to a target area without damaging the normal surrounding tissue. The radioactivity, unlike drugs, is never released from the microspheres but acts from within over a radioisotope-typical distance. The effective treatment range in tissue is up to about 90 Φm (10 cell layers) for α-emitters, never more than 12 mm for β-emitters and up to several centimeters for γ-emitters.

Alpha-emitters

Alpha particles are positively charged ions consisting of two protons and two neutrons, emitted during the radioactive decay of many nuclei with high atomic numbers. During decay, energy is released mainly as the kinetic energy of the α-particles. Since the path length of an α-particle with an energy of 5 to 8 MeV is on the order of 40 to 80 Φm, the effective treatment radius is limited to several cell diameters from the atom that emits the particle, and nonspecific irradiation of distant tissues is eliminated. (23) The high linear energy transfer (LET) of such energetic particles (~100 keV/Φm) and the limited ability of cells to repair the damage to DNA from α-particle irradiation contribute to their extraordinary cytotoxicity. At low doses in the range of 1 to 2 Gy, α-radiation is about 5 to 100 times more toxic than γ- or β-radiation. Furthermore, α-particle mediated cell killing is insensitive to conditions of hypoxia, which are often found in necrotic tumors and may compromise the clinical efficacy of β-, γ- or x-ray radiation.

The dosimetry of α-emitters is special since the dose deposition from the low-range α-particles must be considered on a cell by cell level. The normal approach of prescribing activity per gram of (tumor-)tissue will not lead to meaningful results, because it is very difficult to distribute radioactive microspheres absolutely homogeneously. Microdosimetry with α-emitters has been expertly described by Humm (24).

Most research with α-emitting radiopharmaceuticals and the first clinical trials in 1996 have involved antibodies labeled with ^{213}Bi, ^{211}At, ^{212}Bi, ^{225}Ac, ^{212}Pb, ^{255}Fm, ^{223}Ra, and ^{149}Tb (see **Table 2**). This work will not be covered here, but a comprehensive review of the so-called radioimmunotherapy with α-emitters is given by McDevitt (25).

Table 2. Alpha-emitters useful for delivery in particulate radiopharmaceuticals

Radioisotope	Half-life	α-yield	Other radiation (keV)	Range in tissue	Production
^{149}Tb	4.13 h	17%	β^- (400 max.), γ (165, 352, 511)	28 Φm	Accelerator
^{211}At	7.2 h	100%	γ (77-92, 500-900)	65 Φm	Accelerator
^{212}Bi	60 min	36%	γ (727, 12%), β (2246 max, 64%)	70, 42, 87 Φm	^{224}Ra-generator
^{213}Bi	45.6 min	100%	γ (440, 28%), β (1420 max, 98%)	43 Φm	^{225}Ra-generator
^{223}Ra	11.4 d	300%	γ (0.031-0.45)	43 Φm	^{227}Ac-generator
^{225}Ac	10.0 d	400%	γ (0.037-0.187)	48 Φm	^{225}Ra-generator
^{255}Fm	20.1 h	93.4%		63 Φm	^{255}Es-generator

Beta-emitters

In 1896, Henri Becquerel discovered β-decay, which is the commonly used name for β^-- or negatron-decay. During β-decay, a neutron in the unstable nucleus is transformed into a proton, an electron and a neutrino, which is an uncharged particle with undetectable small mass. Additionally free energy is produced and released in the form of kinetic energy and given to the electron and the neutrino. Since the free energy is distributed in an isotope-characteristic but random fashion to the β-electron and the neutrino, we will always measure a spectrum of electrons with different energies. An electron=s maximum energy E_{max} is measured when no energy transfer to the neutrino takes place (see **Table 3**). Each β-decay has its characteristic energy-spectrum, and the average energy is typically about a third of E_{max}. Passing through tissue, the ejected β-electrons interact with other (mainly water) atoms and lose energy, leading to excited and ionized atoms. These activated species (e.g., radicals) are responsible for therapeutic effects (e.g., DNA damage of cancer cells), but also for toxicity (damage to normal cells nearby).

One of the first β-emitters used in particulate form for the treatment of lung tumors was ^{198}Au-labeled microspheres (size 30-50 Φm) (26). Unfortunately, ^{198}Au also emits high energy γ-rays. This led to higher than necessary radiation doses to the other organs as well as to hospital personnel. To avoid this exposure, the pure β-emitters ^{32}P and ^{90}Y have been favored during the last decade and have become the predominant radioactive isotopes for many therapeutic applications. Recently, however, it has been shown that a certain amount of low-energy γ-radiation can actually be useful for imaging, either during or after the application of the radioactive microspheres (27). During infusion, with the help of a γ-camera or γ-detector, the surgeons are able to a) direct the radioactive microspheres and b) adjust the necessary amounts of radioactivity.

Short-lived radioisotopes (**Table 3**) can be used to optimize radiobiologic aspects of therapy. Specifically, it has been shown that not only the total dose, but the dose-rate is very important for the treatment outcome in radiotherapy (28). Short-lived radioisotopes such as ^{165}Dy or ^{188}Re pack the "punch" into a much shorter time-period, allowing less time for tumors to recover and grow back. Although much more research is required in this area, many of the radioactive β-emitting lanthanides are seen as promising candidates for local or directed radiotherapy, with microspheres serving as the delivery system.

Table 3. Beta-emitters useful in particulate radiopharmaceuticals

Radioisotope	Half-life	Average / max. beta-energy*	Max. range in tissue	X_{90}^	Gamma-lines	Production
^{3}H	12.3 y	5.7 / 18.0 keV			none	^{6}Li(n,α)^{3}H
^{14}C	5730 y	49.5 / 156.0 keV			none	^{14}N(n,p)^{14}C
^{32}P	14.3 d	694.9 / 1710.2 keV	8.7 mm	2.2 mm	none	^{32}S(n,p)^{32}P or ^{31}P(n,γ)^{32}P
^{90}Y	64.1 h	933.6 / 2280.0 keV	12.0 mm	2.8 mm	none	^{90}Sr/^{90}Y generator
^{131}I	8.0 d	181.7 / 806.9 keV	2.4 mm		364.5 keV (81.2%)	^{131}Te (β^-) ^{131}I
^{153}Sm	46.5 h	224.2 / 808.2 keV	3.0 mm	0.7 mm	103.2 keV (29.8%)	^{152}Sm(n,γ)^{153}Sm

^{165}Dy	2.3 h	440.2 / 1286.7 keV	6.4 mm	1.3 mm	94.7 keV (3.6%)	^{164}Dy(n,γ)^{165}Dy
^{166}Ho	26.8 h	665.1 / 1853.9 keV	10.2 mm	2.1 mm	80.6 keV (6.7%)	^{165}Ho(n,γ)^{166}Ho
^{169}Er	9.4 d	99.6 / 350.9 keV	1.0 mm		< 0.2%	^{168}Er(n,γ)^{169}Er
^{177}Lu	6.7 d	133.3 / 497.8 keV	1.7 mm		113.0 keV (6.4%) 208.4 keV (11.0%)	^{176}Lu(n,γ)^{177}Lu
^{186}Re	89.2 h	346.7 / 1069.5 keV	5.0 mm	1.0 mm	137.2 keV (9.42%)	^{185}Re(n,γ)^{186}Re
^{188}Re	17.0 h	764.3 / 2120.4 keV	11.0 mm	2.1 mm	155.0 keV (15.1%)	^{188}W/^{188}Re generator
^{198}Au	2.7 d	311.5 / 960.7 keV	4.4 mm	0.9 mm	411.8 keV (95.5%)	^{197}Au(n,γ)^{198}Au

*NuDat database (29); ^ Distance in tissue within which 90% of dose is deposited (30)

The dosimetry of β-emitting radioactive microspheres depends on the application. In the simplest case, when the radiopharmaceutical is distributed homogeneously throughout the target (tumor) area, the MIRD scheme is used (31,32). MIRD calculations can be done on a PC [using a program provided free to the interested user] (33). Harbert's calculations are used when the radiopharmaceutical is in a plane from which it irradiates the tissue (34) (Appendix K). This approach is, for example, appropriate in the treatment of cystic brain tumors or in radiosynovectomy (see below). Dosimetric modeling using Monte Carlo simulations can be used for microspheres of different sizes and different β-emitters (35-37).

Gamma-emitters

A large group of radioisotopes emits γ-rays during decay. Gamma rays represent excess energy that is given off as the unstable nucleus breaks up and decays in its efforts to reach a stable form. The energy is emitted in the form of electromagnetic radiation (photons), with a radioisotope-characteristic photon energy typically expressed in kiloelectron volts (keV). Photons are absorbed in biological material by both the photoelectric and Compton process, and then indirectly ionize the surrounding atoms, producing chemical and biological changes. Most γ-emitters are used primarily for diagnostic purposes and those used in nuclear medicine (**Table 4**) were chosen so that a) their γ-ray energy is not too high (radiation safety concerns) and matches the γ-camera, b) their half-life is practical and logistically feasible, c) they are easily available and inexpensive, and d) they can be bound to microspheres in an easy (kit) and stable fashion.

Table 4. Gamma-emitters used in particulate radiopharmaceuticals

Radioisotope	Half-life	Gamma lines (Efficiency)	Production
^{51}Cr	27.7 d	320 keV (10%)	^{50}Cr (n,γ) ^{51}Cr
^{67}Ga	78.2 h	93 keV (40%) 184 keV (20% 300 keV (17%) 393 keV (4%)	^{68}Zn (n,p) ^{67}Ga
^{99m}Tc	6.0 h	140 keV (89%)	^{99}Mo/^{99m}Tc-generator
^{111}In	2.8 d	171 keV (90%) 245 keV (94%)	^{111}Cd (p,n) ^{111}In
^{123}I	13.2 h	159 keV (83%) 528 keV (1%)	^{121}Sb (α,2n) ^{123}I
^{125}I	60 d	35 keV (7%) 27-32 keV x-ray (140%)	^{124}Xe (n,γ) ^{125}Xe ^{125}Xe _ ^{125}I

Preparation of radioactive microspheres

Microspheres can be made radioactive (= radiolabeled) either **during** or **after** their preparation. Although the former method is still more commonly used in medicine, the latter is preferred, especially for shorter-lived radioisotopes, because it is compatible with kit formulation. In this case, the microspheres can be stored for extended periods of time as part of a sterile nonradioactive kit and then be radiolabeled by the radiopharmacist in the nuclear medicine department shortly before use. Radiochemical stability problems are in this way minimized and logistical problems inherent to the use of radiopharmaceuticals avoided.

Depending on the particles, it is possible to enclose the activity, label throughout the entire volume, or label only certain structures, such as the surface, the outer or inner wall, the lipophilic or hydrophilic liposome compartment (**Figure 2**). The binding of radioactivity to particles can be done by covalent bonds, by chelation, by adsorption processes or by indirect means as, for example, avidin-biotin bonds which can bridge the microsphere and the radioisotope. In all these cases, *in vivo* biodegradation processes and reversible isotope exchange processes can lead to instability and release of the radioisotope into the immediate surrounding. Regarding biodegradation processes, the microsphere's building material might undergo rapid ester-bond cleavage depending on the target organ's enzymatic activity or the locally produced radiation. The cleavage of covalent bonds can be beneficial in drug targeting if it takes place in the target organ. For example, the lysosomal *in vivo* activation of an inactive prodrug into the effective drug as shown with polymers binding daunomycin or puromycin (38), is a precondition for pharmacological action. In the case of radioactive microspheres, however, absolutely no degradation is wanted until complete decay of the radioisotope.

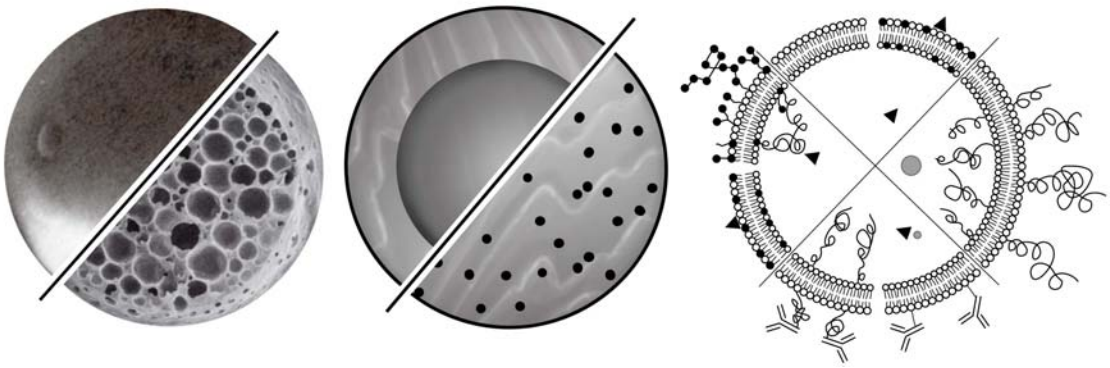

Figure 2. Depending on the particle type shown in this schematic drawing different compartments of the particles can be radiolabeled. A) Nonporous and porous microspheres or nanospheres. B) Reservoir and monolithic (matrix) microcapsules, C) Different types of liposomes.

Radiolabeling during the microsphere preparation

Colloids were the first microspheres to be radiolabeled during preparation. They are a unique class of radioactive microspheres (**Table 5**) that consist entirely of the defined inorganic compounds of a radioisotope which have precipitated into relatively homogeneous particles. The size range of colloids depends mostly on the preparation conditions such as temperature and pH and on the form of the precipitating agent. Sulfur colloids with ^{99m}Tc, for example, can be made in the size of 80 to 100 nm by treating a boiling acidic ^{99m}Tc pertechnetate solution with H_2S gas. Alternatively, sodium thiosulfate can be added to the radioactive solution, but the size distribution of the mixed colloid of Tc_2S_7 and sulfur is then much broader, from 80 to about 2000 nm (39). Other useful colloids are the hydroxides and oxides of ^{99m}Tc and ^{113m}In prepared by coprecipitation with ferric hydroxide (40) and the oxides of ^{99m}Tc prepared by coprecipitation with Sn(II) (41).

One method of microsphere preparation which facilitates the production of homogeneously sized albumin microspheres that incorporate many different kinds of radioactive colloids was first described by Zolle et al (42). The method consists of first transforming a radioactive substance into a precipitate, mixing it with an aqueous solution of albumin and then injecting it into a stirred solution of cotton seed oil. The fine dispersion of albumin droplets then forms spheric and stable albumin particles tightly enclosing the radioactive compounds after heating the mixture above 100 EC.

Microspheres made from or with proteins, such as the above albumin microspheres, always contain tyrosine and histidine. Their phenole- and imidazole-rings can be easily iodinated using methods such as the chloramine T, the iodogen, the Bolton-Hunter or the iodo-bead method, to name just a few. A very good review that describes these techniques in detail is available from Amersham (43). Another way of achieving iodinated microspheres is to radioiodinate the compound that will be incorporated into the microspheres during their formation. Yang et al. made radioactive PLA-microspheres by first labeling the contrast agents ethyliopanoate and ethyldiatrizoate, which were to be incorporated, with ^{131}I, dissolving them together with the polymer PLA in methylene chloride and then preparing the microspheres by a solvent evaporation method (44).

As with radioactive microspheres, radioactive liposomes can be made by adding radioactive compounds during their formation. Unilamellar liposomes of 70 nm diameter have been prepared by mixing the lipid-soluble radioactive complex oxodichloroethoxy-bis-(triphenylphosphine)^{186}rhenium(V) with phospholipids and the detergent sodium deoxycholate, followed by detergent removal on a small gel filtration column (45). Such biocompatible ^{186}Re-

liposomes can be used to deliver therapeutic radiation doses for radiosynovectomy (see below).

Table 5. Methods of preparing radioactive microspheres in which radiolabeling is done during formation of the microspheres.

Method of Labeling	Examples	References
Colloid precipitation	^{99m}Tc sulfur colloid	(39)
	^{113m}In ferric hydroxide colloids	(40)
	^{165}Dy-FHMA (~5 Φm)	(46)
	Chromic ^{32}Phosphate (1-2 Φm)	(47)
Inclusion of radiolabeled compound	^{99m}Tc-HSA-gelatin microcapsules	(48)
	^{131}I-ethyldiatrizoate-PLA microspheres	(44)
	^{125}I-iododeoxyuridine-PLGA microspheres	(49)
	^{125}I-HSA magnetic albumin microspheres	(50)
Isotope exchange	^{211}At-microspheres	(34)
	^{14}C-, ^{35}S- and ^{3}H-labeling	(51)
Lipophilic inclusion	^{186}Re/^{188}Re-triphenylphosphine-liposomes	(45)
In situ production	^{99m}Tc-Buckminster fullerenes (C_{60} or C_{80}) or aggregates thereof (Technegas)	(52)

Abbreviations: FHMA = ferric hydroxide macroaggregates, HSA = human serum albumin

A relatively recent development in the preparation of radioactive particles is the *in situ* production of ^{99m}Tc-particles in a Technegas generator (52). The ^{99m}Tc-pertechnetate is pyrolized together with carbon at 2500 EC and forms not only Buckminster fullerenes (C_{60} to C_{80}) each enclosing a technetium atom, but also agglomerates of graphite and technetium.

Radiolabeling after the microsphere preparation

Compared to radiolabeling during microsphere preparation, methods of radiolabeling already prepared microspheres are conceptually more straightforward. Spherical anion or cation exchange resins of different sizes are examples of microspheres which can be radiolabeled with ionic radionuclides (**Table 6**). The resins can be loaded with labeling efficiencies generally exceeding 95% by simple incubation in saline or aqueous buffer solutions containing the radioisotope. Their stability, however, has to be evaluated carefully, since not all resins have the capacity or the binding affinity necessary to bind radioisotopes such as $^{90}Y^{3+}$. Yttrium is a radioisotope that will, in its ionic form, be taken up easily by the bone marrow where it will remain until complete decay, leading to severe toxicity (myelosuppression). It is thus of utmost importance that bound ^{90}Y not be released *in vivo*. It has been found that of the cation-exchange resins Bio-Rex 70, Sephadex SP, Chelex 100, AG 50W-X8 or Cellex-P, only Bio-Rex 70 was able to provide the stability needed for ^{90}Y-radioembolization *in vivo* (53). Other ion-exchange resins have been used for the adsorption of negatively charged radioisotopes. Pertechnetate, $^{99m}TcO_4^-$, for example, has been adsorbed to 300 Φm-large Dowex 1-X4 beads (54). Even larger 1 mm Amberlite 410 resin pellets were labeled with pertechnetate in the same way (55) and have been used for GI transit studies. Chromate, $^{51}CrO_4^{3-}$, has been adsorbed to Dowex 1-X8 sized 10 to 50 Φm and used for the measurement of mucociliary functions (56).

Table 6. Methods of preparing radioactive microspheres from preformed, non-radioactive microspheres

Method of Labeling	Examples	References
Radiolabeling by ion exchange	Anion- and cation-exchange resins:	
	BioRex 70 loaded with ^{90}Y	(57)
	Dowex 1-X4 loaded with $^{99m}TcO_4^-$	(54), (55)
	Dowex I-X8 loaded with $^{51}CrO_4^{3-}$	(56)
Chelation (complex formation) of the radioisotope	^{111}In-DTPA-albumin microspheres	(58)
	^{68}Ga-DTPA-albumin microspheres	(59)
	^{99m}Tc-polystyrene latex microspheres	(60)
	^{186}Re-polycysteine/polylysine microspheres	(61)
Isotope exchange with ^{131}I, ^{125}I and ^{211}At	^{131}I-Mitomycin C gelatin microspheres	(62)
	^{131}I-albumin microspheres	(58)
	^{211}At-methacrylate microspheres	(63)
Neutron activation (typically n,γ-reaction)	^{90}Y-glass and ^{32}P-glass microspheres	(64)
	^{186}Re/^{188}Re-glass microspheres	(65)
	^{166}Ho-glass microspheres	(66)
	^{166}Ho-PLA microspheres	(67,68)
	^{186}Re/^{188}Re-PLA microspheres	(69)
Reduction to insoluble, colloidal compounds	^{99m}Tc-Sn PLA microspheres	(70)
Affinity to microsphere material	^{186}Re-HEDP bound to hydroxyapatite microspheres	(71)
	^{153}Sm-citrate bound to hydroxyapatite microspheres	(71)

Abbreviations: DTPA = Diethylenetriamine pentaacetic acid

Many different functional groups such as -OH, -NH_2, -SH and -COOH are used to bind specific drugs, radiolabeled chemicals, and chelators to microspheres, and to introduce other functional groups for further derivatization. These chemical modifications are possible before microsphere preparation, but are more commonly performed afterwards. For example, the chelator DTPA (**Figure 3**) has been bound via an amide bond to albumin microspheres using one of the DTPA's carboxyl groups (58). Such microspheres are quite versatile, since DTPA is able to chelate not only ^{111}In, but also ^{90}Y, ^{99m}Tc, ^{166}Ho and many other lanthanides. Currently, the two most stable and most often used chelators able to bind diagnostic and therapeutic radioisotopes are DOTA and MAG_3 (see **Figure 3**). DOTA (= 1,4,7,10-tetra-azacyclododecane N,N',N",N"'-tetraacetic acid) is able to complex ^{212}Bi (72) and has also been shown to chelate ^{90}Y and ^{111}In with better than 99% stability over 2 weeks (73). MAG_3 (= mercaptoacetylglycylglycylglycine) is able to complex the radioisotopes from group VIIB, ^{186}Re, ^{188}Re and ^{99m}Tc (74) at almost 100% stability in serum over 24 hours (75).

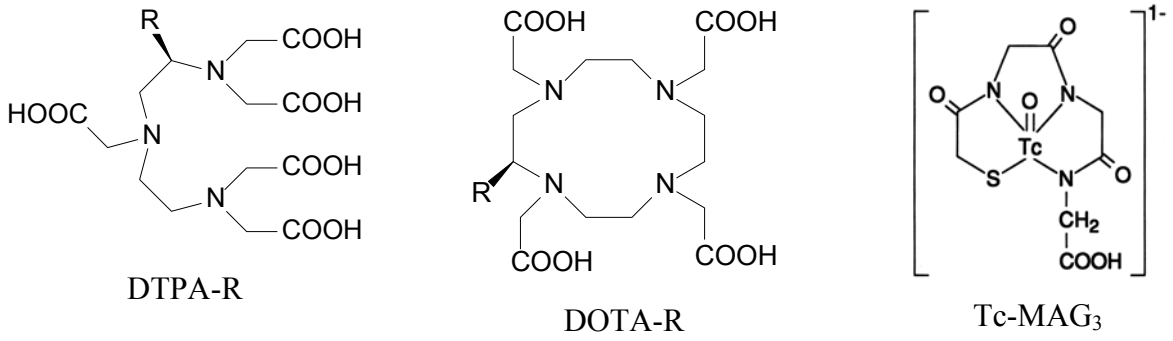

Figure 3. Typical chelators used to complex diagnostic and therapeutic radioisotopes ^{111}In, ^{90}Y, ^{212}Bi, ^{186}Re, ^{188}Re and ^{99m}Tc, among many others.

The radiolabeling of microspheres with chelator-groups on the surface typically involves an incubation with the radioisotope of between 5 and 60 minutes, at temperatures of 20 to 100 EC. The labeling of DOTA or DTPA with ^{90}Y, ^{111}In or many other $^{3+}$-charged ions occurs directly at the optimal pH. The complexation of the +VII pertechnetate or perrhenate, however, additionally involves the reduction of technetium and rhenium to the +V or +IV state. Many different reducing agents such as $NaBH_4$, $Na_2S_2O_4$, H_3PO_2, hydrazine, ascorbic acid or electric reduction have been used, but the most common method is the use of $SnCl_2$. The reduction and complexation of technetium, together with ways of developing it into kit form, has been well reviewed by Eckelman et al. and can be directly applied to many chelator-containing microspheres (76).

Microspheres made from appropriate materials can also be labeled using functional groups such as reduced sulfhydryl-groups, alone or in combination with nearby carboxyl- and amine-groups. This method has been termed the Adirect method≅ by chemists using it for the radioactive labeling of antibodies (77) and works especially well for microspheres made from proteins, such as the human serum albumin microspheres labeled with ^{188}Re after reduction using Sn(II) (78). Other microspheres that bind radioactivity with stabilities sufficient for therapy are $^{90}Y^{+3}$-labeled magnetic PLA microspheres with native carboxylic groups (79) and ^{99m}Tc-labeled polystyrene microspheres derivatized with poly(acryclic acid) in order to introduce carboxylic groups (60).

Radiolabeling by neutron activation of pre-made microspheres

A very effective way of preventing leakage of the radioactive isotope(s) from the microsphere is to seal the radioisotope into the microsphere matrix. The pre-made microspheres enclose the non-radioactive precursor of the radioisotope and are activated in a nuclear reactor by bombardment with thermal neutrons shortly before use (**Table 6**). The most stable matrix for this kind of microsphere activation is glass. Day and Ehrhardt pioneered such therapeutic radioactive microspheres (**Figure 4**) from aluminosilicate glass containing 17 mol% Y_2O_3 (80). The glass mixture was melted in a platinum crucible at 1600 EC, the annealed glass crushed and the splinters spheroidized by sprinkling them from above through an oxygen flame. During neutron-activation in the reactor, the non-radioactive ^{89}Y captured a neutron and became the radioactive β-emitter ^{90}Y. The leakage rate of the ^{90}Y enclosed in the glass matrix was extremely low. Not more than 92 Bq were released from 50 mg of microspheres when activated to therapeutic activities of 11.1 GBq. Very similar glass microspheres have also been prepared enclosing rhenium, resulting in $^{186}Re/^{188}Re$ microspheres after neutron activation (65). Advantages of glass microspheres are their excellent stability, radiation resistance, insolubility and non-toxicity. Disadvantages include their high density (3.3 g/ml) which makes the complete injection through syringes and intravenous lines difficult, and their non-biodegradability which can lead to immunologic reactions. Research is ongoing, however, in the preparation of glass microspheres from biodegradable glass material such as lithium boride (81).

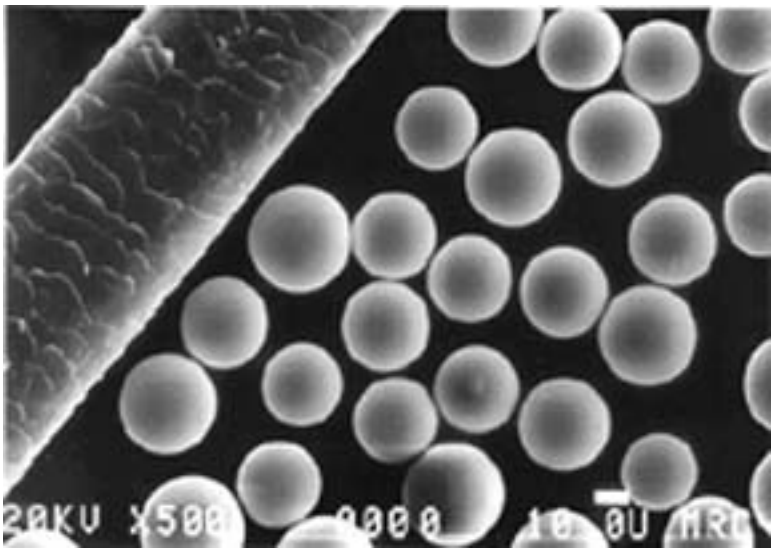

Figure 4. Yttrium glass microspheres for neutron activation in a nuclear reactor in comparison with the size of a hair.

The disadvantages of glass were overcome by the preparation of PLA-microspheres containing either an acetylacetone-complex of ^{165}Ho (67,68) or small particles of metallic rhenium in its native form, ^{185}Re and ^{187}Re (69) (**Table 6**). The stability of the activated ^{166}Ho and ^{186}Re/^{188}Re-microspheres was sufficient for therapy (less than 1% of activity released within a week). The activation time of these poly(lactic acid) microspheres, however, is limited due to the radiolytic breakdown of ester bonds and must be characterized for each polymer-microsphere composition. Specifically, it has been found that activation of rhenium microspheres made from PLA with a molecular weight of 2000 for 1 hour at a neutron-flux of 5H10^{12} n/cm^2/sec produced 450 MBq ^{188}Re and 78 MBq of ^{186}Re. Longer activation times led to melting and polymer breakdown (69). The therapy of liver tumors which requires high specific activities is thus not possible with these ^{188}Re/^{186}Re-PLA-microspheres, but they could be used in local treatment of brain metastases or applied to incompletely resected tumor tissue after surgery. Recently, ^{166}Ho-acetylacetonate-microspheres made from PLA with a molecular weight of 20,000 have been described (68). The authors reported that up to 1 hour of neutron-activation at a flux of 5H10^{13} n/cm^2/sec was possible, yielding an activity of 20 GBq in 400 mg of microspheres. This activity would be sufficient to allow for their transport to the hospital and use in liver tumor patients on the following day.

In situ neutron capture therapy using non-radioactive microspheres

Neutron capture therapy is an exciting bimodal tumor treatment concept originally proposed in 1936 by Locher (82). The first component of this therapy is the delivery to tumor cells of non-radioactive atoms or molecules either alone or packed into carriers such as liposomes or microspheres. The target nuclei have large thermal and/or epithermal neutron capture cross-sections and a resulting reaction having a large positive Q value. The aim is to attain a higher concentration of these nuclei in the tumor than in the surrounding normal tissue cells. The second component is the exposure of a selected patient volume to a neutron beam. During neutron capture *in situ*, excessive energy between the initial and final state of the reactive nuclei (the positive Q-value) is released either as the recoil energy of heavy particles (^{6}Li, ^{10}B) and α-particles, or as γ-rays (^{155}Gd, ^{157}Gd) (see **Table 7**). In the case of boron or lithium neutron capture, most of this energy is deposited in the tumor cell, since the range of the produced particles is less than 10 Φm. In the case of gadolinium neutron capture, energy is spread out more because of photonic interactions. Although the first clinical trials with neutron capture therapy were completed in the 1950=s, it took developments of the next 40 years to make this therapeutic approach very promising for cancer therapy (83). Developments included the synthesis of superior targeting compounds such as the sulfur containing boron compounds mercaptoundecahydrododecaborate (= BSH) or boronated porphyrins (= BOPP).

In neutron capture therapy, boron and gadolinium are generally delivered intravenously, although their delivery is also possible via microparticles. Tokumitsu et al developed Gd-DTPA loaded chitosan microspheres of 4.1 ∀ 1.3 Φm for intratumoral injection (84,85). Boron can be delivered in a similar way by packaging BSH into liposomes with anti-carcinoembryonic antigen antibodies on their surface (86). Different boron compounds have been encapsulated not only in the aqueous compartment of liposomes, but also in their bilayer and attached to their surface and work in this area is ongoing (87).

Table 7. Isotopes useful for neutron capture therapy. Their half-lives are zero.

Isotope	Reaction	Q [MeV]	Cross section σ_{th} [barn]	Mode of energy deposition	Range in tissue [Φm]	Delivery vehicles
^{6}Li	^{6}Li(n,α)^{3}H	4.784	940	α - 2.105 MeV ^{3}H - 2.734 MeV	< 10	
^{10}B	^{10}B(n,α)^{7}Li	2.790	3837	α_0 - 1.775 MeV α_1 - 1.47 MeV $^{7}Li_0$ - 1.015 MeV $^{7}Li_1$ - 0.84 MeV	7.2, 8.9	Liposomes containing BSH (86,88)
^{155}Gd	^{155}Gd(n,γ)^{156}Gd	11.452	61000			
^{157}Gd	^{157}Gd(n,γ)^{158}Gd	7.937	254000			Chitosan microspheres; microcapsules containing Gd-DTPA (84,85)

Diagnostic uses of radioactive microspheres

Diagnostic studies with radiopharmaceuticals include dynamic and static imaging and *in vivo* function tests. Dynamic imaging provides information about the biodistribution and pharmacokinetics of drugs in organs. Performed with a γ-camera, dynamic studies are generally carried out over a preset length of time and provide clues to the functioning of the organ being examined. Static imaging, on the other hand, provides morphological information about an organ such as its shape, location and size. Furthermore it allows the exact location of tumors to be determined. Static imaging, unlike dynamic imaging, is normally done at a single point in time, with the imaging time being dependent on the organ activity. In contrast to dynamic and static imaging, *in vivo* function tests do not require imaging. Instead they are evaluated by comparing an injected or swallowed amount of radioactivity to the measured radioactivity in urine or blood.

All three types of diagnostic studies can be performed with radioactive microspheres which contain one or several γ-emitters that can be detected by a γ-camera. The first such "microspheres" in clinical use were red and white blood cells, which were taken from a patient, labeled with ^{111}In or ^{51}Cr, and then re-injected. Red blood cells labeled with ^{51}Cr are commonly used for the measurement of red blood cell mass and for imaging of the spleen. For the latter purpose, the red blood cells are denatured by heating, which renders them spheroidal and nondeformable, and makes them easy to take up by the spleen. Another common application of radiolabeled red blood cells is the accurate determination of total systemic arterial blood flow or venous return, as well as, for blood flow determination within specific organs (89). These blood flow parameters are important when drugs for the treatment of cardiovascular diseases are evaluated. White blood cells labeled with ^{111}In-oxine are used for the detection of inflammatory diseases, abscesses or other infections. A less expensive method has been developed in which the neutral and lipophilic ^{99m}Tc-HMPAO complex is prepared from a kit and then incubated with the leukocytes (90). Platelets labeled with ^{111}In are also used to detect actively forming deep vein thrombi, to measure blood flow, and to detect regions of infection (91). Radiolabeled blood cells are still used today, although pre-made radioactive microspheres containing several different γ-emitters (see **Table 7**) are easier to use and do not require time-consuming labeling procedures (92). Unfortunately, the radioactive microspheres of homogenous size are made from polystyrene and thus are not biodegradable, making them inappropriate for clinical use.

Table 7: Radioactive microspheres for diagnostic applications

Application	Type of radioactive microspheres used	Particle size
Gated blood pool study	^{111}In- or ^{51}Cr-labeled red blood cells	6-8 Φm
Thrombus imaging in deep vein thrombosis	^{111}In-labeled platelets ^{99m}Tc-macro-aggregated human serum albumin (MAA) ^{99m}Tc-sulfur colloid	0.5-1 Φm 10-90 Φm 0.05-0.6 Φm
Blood flow measurements	Polystyrene-microspheres labeled with the γ-emitters ^{141}Ce, ^{57}Co, ^{114m}In, ^{85}Sr, ^{51}Cr, and others (animal experiments)	10 Φm, 15 Φm (other sizes)
Investigation of biodistribution and fate of (drug-loaded) microspheres	^{3}H, ^{14}C-labeled microspheres (animal experiments) ^{141}Ce-polystyrene microspheres	all sizes 11.4 Φm
Lung scintigraphy	^{99m}Tc-impregnated carbon particles (= Technegas) ^{99m}Tc-macro-aggregated human serum albumin (MAA)	50 nm 10-90 Φm
Diagnostic radioembolization	^{99m}Tc-macro-aggregated human serum albumin (MAA)	10-90 Φm
Liver and spleen imaging	^{99m}Tc-macro-aggregated human serum albumin (MAA) ^{99m}Tc-sulfur colloid ^{99m}Tc-tin colloid	10-90 Φm 0.05-0.6 Φm 0.05-0.6 Φm
Bone marrow imaging	^{99m}Tc-sulfur colloid ^{99m}Tc-antimony sulfide colloid	0.05-0.6 Φm 0.05-0.6 Φm
Infection localization	^{111}In-labeled leukocytes ^{111}In-labeled liposomes ^{99m}Tc-labeled liposomes ^{99m}Tc-albumin nanocolloid	12-20 Φm 20 nm B 1 Φm 20 nm B 1 Φm <80 nm
Tumor imaging	^{99m}Tc-labeled liposomes ^{67}Ga-NTA- or ^{111}In-NTA-labeled liposomes	20 nm B 1 Φm 65 nm
Gastrointestinal transit studies	^{99m}Tc-sulfur colloid ^{111}In-labeled ion exchange resins	0.05-0.6 Φm
Local restenosis prevention in coronary arteries	^{141}Ce microspheres (preliminary imaging tests)	11.4 Φm

For the diagnosis of pulmonary embolism, both the inhalation of small, radioactive ^{99m}Tc-carbon particles (Technegas) and the perfusion of the lung with ^{99m}Tc-labeled albumin particles are used. In the first case, Technegas behaves, due to the small particle size of less than 100 nm, much more like a gas than a radioaerosol and diffuses into the entire accessible lung volume. In the second case, macroaggregated albumin is mainly used for the quantification of shunts associated with intrapulmonary arteriovenous malformations and the diagnosis of pulmonary diseases such as cancer and hypertension (**Figure 5**). The diagnostic determination of shunts within an organ is generally done prior to using radioactive microspheres in radioembolization therapy (see below) (93) in order to prevent radiotoxicity to the lungs (94). The biological half-life of the albumin particles is only 1 to 3 hours, so any therapeutic interventions can easily be performed afterwards.

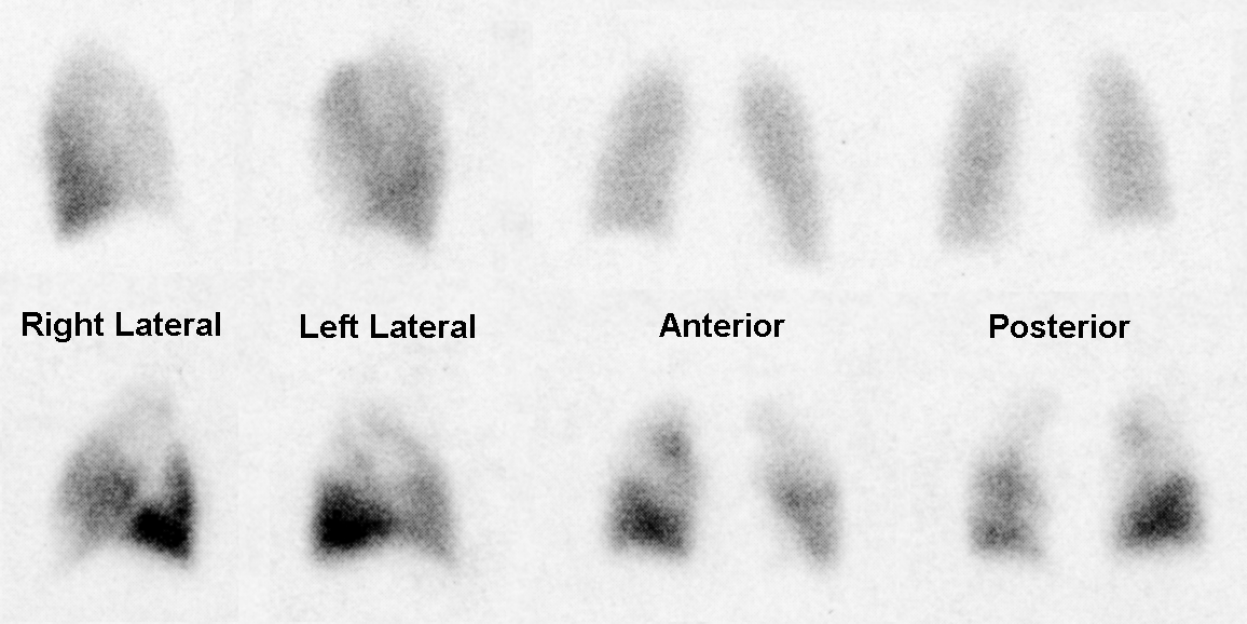

Figure 5. Diagnostic lung imaging obtained after the injection of ^{99m}Tc-labeled macroaggregated albumin in different projections. The top row shows a normal lung and the bottom row the lung of a patient with multiple pulmonary emboli in both lobes of the lungs.

For liver, spleen, bone marrow and lymphatic system imaging, colloidal microparticles, such as ^{99m}Tc-sulfur colloids, are most useful (**Table 7**). To illustrate, **Figure 6** shows the changes in a cirrhotic patient made visible by ^{99m}Tc-sulfur colloid. The lymphatic system can also be imaged or targeted with drugs through the use of the poly(lysine) nanospheres (95). The ideal nanospheres for this purpose are 10-30 nm, contain carbohydrate groups on the surface, and are able to bind the γ-emitter ^{111}In via the covalently bound chelator DTPA.

The radiopharmaceutical ^{99m}Tc-sulfur colloid is also used for gastrointestinal blood loss studies, for the preparation of a ^{99m}Tc-labeled egg sandwich for gastric emptying studies (39), and for the determination of esophageal transit and gastro-esophageal reflux. For colonic transit studies, radioisotopes with a half-life longer than ^{99m}Tc are more appropriate, and ^{111}In-labeled ion exchange resins, but also ^{131}I-cellulose are utilized (96). Latex-particles of 2.5 Φm size and labeled with ^{99m}Tc have also been shown to give excellent abdominal images (97). In all these gastrointestinal transit time studies, the size of the radiolabeled microspheres does not influence the measured times.

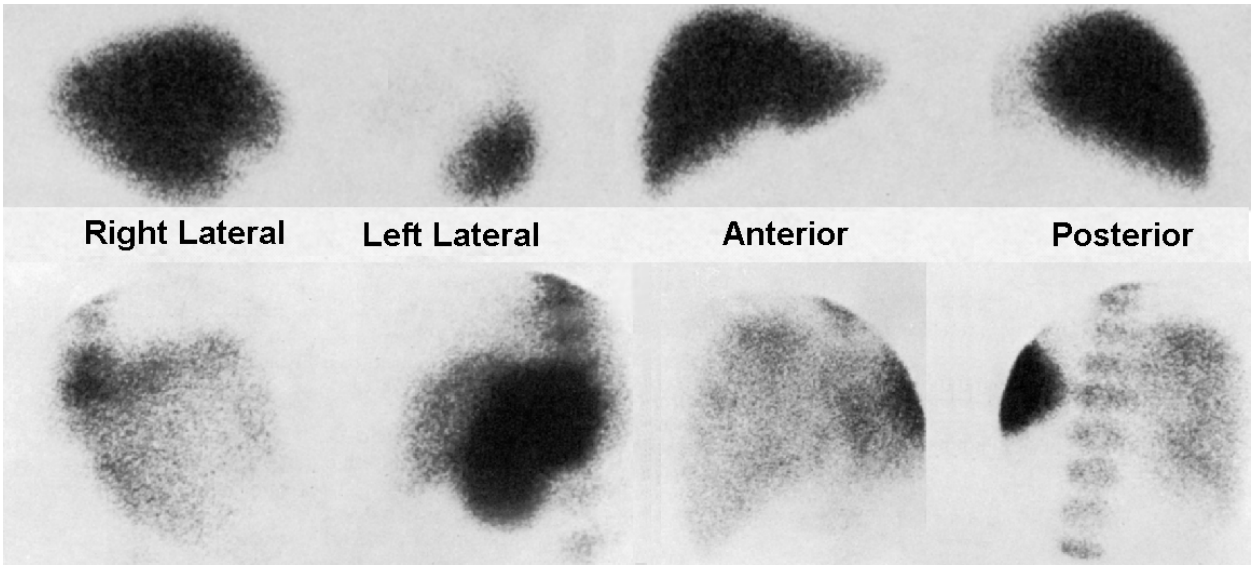

Figure 6. Liver scintigraphy performed with ^{99m}Tc-sulfur colloid in different projections. The top row shows a normal liver, the bottom row the corresponding views of a liver from a patient with cirrhosis.

Radiolabeled liposomes, another diagnostic class of radioactive particles, has been used for tumor imaging since 1977. In order to prolong the blood residence time and maximize tumor uptake, neutral, positively and negatively charged small unilamellar vesicles (= SUV=s) of 65 nm encapsulating ^{111}In were made and their biodistribution measured in mice (98). The highest uptake of 18.5% of injected dose per gram of tumor was measured with the neutral liposomes. A further attempt to minimize the high blood-background radioactivity levels was to inject ^{67}Ga- or ^{111}In-labeled liposomes containing biotin groups on their surface and then chasing the non-tumor bound liposomes 2 hours later with avidin (99). This chase removed the unbound liposomes effectively from the circulation and the blood concentration of the radioactive liposomes dropped to a tumor-to-blood ratio of about 15 to 1 shortly after the avidin chase. The avidin-biotin-liposome conglomerates accumulated in the liver and increased the liver activity about 2.5 fold.

Radiolabeled microspheres can also be used to image cancer lesions. An interesting application is the use of PLA-microspheres labeled with ^{131}I-iopanoic acid derivatives for the imaging of liver tumors (44). The normal liver parenchyma lined with Kupffer cells takes up the microspheres, but the cancer lesions do not possess fixed macrophages and therefore exclude the radioactive microspheres, showing the focal lesions as defects. The recently introduced ^{99m}Tc-PLA microspheres which were radiolabeled in a $SnCl_2$-containing kit could be used for the same application (100), although the stability described as "more than 80% bound after 6 hours" is not optimal yet.

The *in vivo* faith of microspheres after intra-arterial catheter-mediated delivery through a porous balloon to a rabbit's femoral artery has been investigated with radioactive ^{141}Ce-microspheres (101). Although only 0.14% to 0.16% of the microspheres were delivered to the vessel wall, an average of 92% of these microspheres was still present 7 days later. In addition, much higher amounts of the microspheres were found in the periadventitia (the vessel's "outside") and the overlying musculature and are believed to be caused by the increase of vaso vasora present in atherosclerotic patients. Although the targeted amounts of microspheres are small, they can lead to drug concentrations a few hundred times higher than the serum concentrations, allowing for effective restenosis therapy with microspheres containing cytostatic or antiproliferative agents, especially from the periadventitia side.

Therapeutic uses of radioactive microspheres

Many radiolabeled particles, microspheres and liposomes are appropriate for therapy once the encapsulated diagnostic radioisotope has been exchanged for a therapeutic one from the α- or β-emitter group. Typical uses in the last 20 to 40 years include local applications for the treatment of rheumatoid arthritis, liver tumors and cystic brain tumors. However, their use remains experimental because of smaller than expected target uptake, unwanted toxicity and insufficient treatment effects that have resulted from radiochemical instability and suboptimal biodistribution of the radiopharmaceutical. In addition, there exists a general negative attitude towards the use of radioactive substances in spite of proven superior results of many radiation therapies (102-104). What follows is a review of a few α-emitter applications as well as the more established β-emitter therapies.

Therapy with alpha-emitting microspheres

Different α-emitters have been tested in ovarian cancer mouse models. Microsphere-bound ^{211}At, for example, was applied in mice with ovarian cancer metastases and was found to be more effective than the β-emitting ^{32}P- and ^{90}Y-microspheres (63). It was, however, also shown that the amount of radioactivity had to be tailored carefully. More than 1 MBq of ^{211}At per animal led to shorter survival times of the treated mice. This effect is very likely due to the instability of ^{211}At which is highly toxic to the lymphatic tissue and thyroid gland when leakage occurs. First clinical trials with the same α-emitter bound to albumin microspheres have been reported by Wunderlich et al. (105). The authors injected the microspheres into the arteries leading to tongue and larynx tumors. After 4 hours, 80% of the radioactivity was bound to the tongue and 12% to the lungs. The rest was found in the abdomen. The tongue tumor was completely ablated, and no side effects or recurrences were observed at 2 year follow-up. Another α-emitter, ^{212}Pb, in the form of radioactive colloids (106) was also investigated in an ovarian cancer mouse model. Tumor necrosis and decrease in ascites was observed in a dose-related manner, with acute gastro-intestinal toxicity developing at the highest doses. The therapeutically effective radioisotope in these experiments was ^{212}Pb=s daughter nuclide ^{212}Bi (**Table 2**).

To increase the limited range of α-emitters (see general properties of α-emitters), the radiopharmaceutical should be delivered close to the tumor from where it releases the radioisotope, allowing it to diffuse into the surrounding area. Ideally, the released radioactivity binds to the tumor's cell surface and not to the surrounding normal tissue, something that could be accomplished, for example, by pre-targeting the cancer cells with an antibody metallothionein bioconjugate. This approach has been tested *in vitro* with the biodegradable polymer mixture of PHEA (= α,β-poly(hydroxyethyl)-D,L-aspartamide) and Pluronic enclosing ^{212}Bi. Within 1 hour, the polymer began to resemble Swiss cheese, with its many small holes of about 1 Φm in diameter (**Figure 7**). The size of the holes further increased, and after 2 days, more than 75% of the radioactivity had been set free (107). The polymer tested was in the form of a paste, but microspheric radiopharmaceutical delivery forms are also possible. This approach is limited by the diffusion distances of the radioisotope (108). In a chopped meat model, ^{212}Bi has been shown to diffuse a maximum distance of 10 mm. It thus may only be useful for the treatment of very small tumors, metastases or leftover tumor cells from incompletely resected tumors.

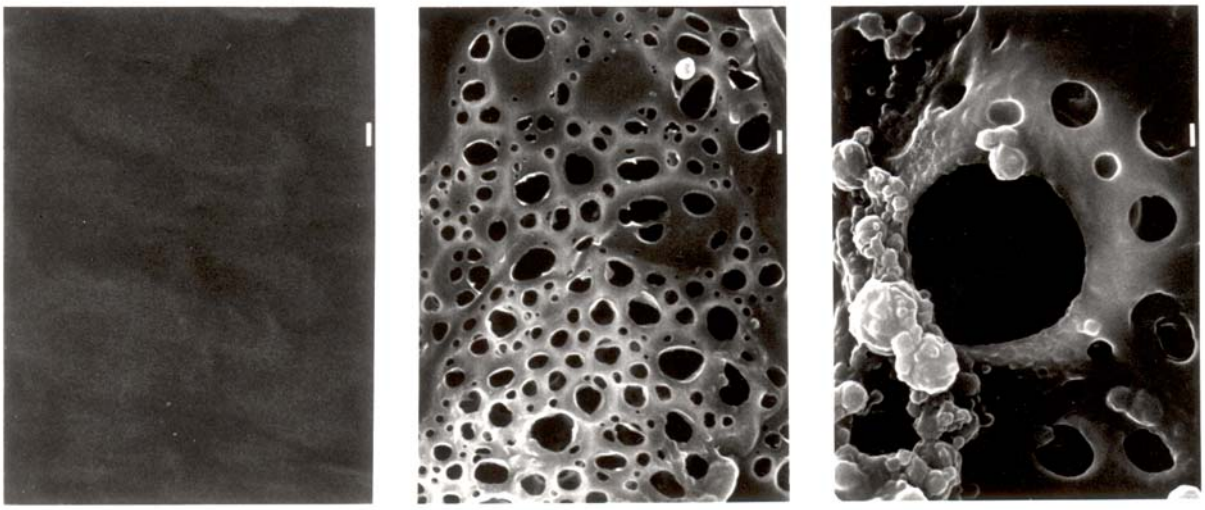

Figure 7. Scanning electron microscopy picture of the biodegradable polymer PHEA/P immediately after preparation (left), after 1 hour (middle) and after 24 hours (right) at 37 EC in PBS pH 7.4. The bar to the right represents 1 Φm.

Therapy with beta-emitting microspheres

One of the first applications of β-emitting microspheres was the treatment of inaccessible tumors (109). In this approach termed **radioembolization therapy**, 20 to 50 Φm microspheres are injected into the artery leading to the tumor. Since the microspheres are larger than the newly formed capillaries in the tumor, they are trapped and become lodged in the tumor from where they irradiate the surrounding cancerous tissue with radiation doses 20 to 30 times higher than what is achievable with external radiation therapy. This approach, pioneered with ^{65}Zn- and ^{198}Au-microspheres by Müller and Rossier in Switzerland (26), was further investigated with ^{198}Au- and ^{90}Y-microspheres in many types of tumors by Ariel (110-112) and with ^{32}P-resin microspheres by Caldarola and Dogliotti (113). Turner et al. investigated ^{166}Ho-labeled cation exchange resins (114) and Hafeli et al. ^{186}Re/^{188}Re-labeled glass microspheres for the same application (65,115). Currently, radioembolization therapy is primarily used for the treatment of liver tumors, both hepatomas and liver metastases (116). Since liver tumors get most of their blood supply from their hepatic artery (117), the radioactive microspheres injected into this artery are preferentially flushed into the tumor. Radiochemically highly stable glass ^{90}Y microspheres sized 25 to 35 Φm (**Figure 4**) are commercially available for the treatment of liver tumors in Canada and since June 1999 also in the United States (Theraspheresϑ; Nordion, Kanata, Ontario, Canada).

Prior to radioembolization, a diagnostic step is generally performed in order to prevent arterial shunting in the liver. Arterial shunts can divert large amounts of the highly cytotoxic microspheres to the lungs and thus lead to pneumonitis (118). The diagnostic step consists of determining the "shunt index" by injecting ^{99m}Tc-labeled macro-aggregated albumin microspheres sized between 1 to 10 Φm and imaging their biodistribution. If less than 5% of the radioactivity shunts to the lungs, then β-emitting microspheres such as ^{90}Y-glass microspheres (64) or ^{90}Y-resin microspheres (27) are injected into the hepatic artery of the patient. The first results in a disease which carries a grave prognosis with a survival rate of less than 50% after 1 year are very encouraging. After intra-hepatic injection, the microspheres are preferentially taken up by the tumor at an average ratio of about 4 to 1 (tumor to normal liver ratio) (119,120). Very high radiation doses without side effects can thus be given. Treating 7 patients with doses of 50 to 100 Gy, Houle showed that the larger doses are necessary for successful

treatment results (121), and doses between 80 and 150 Gy are now recommended. Further improvements in treatment outcome are possible by injecting the vasoconstricting agent angiotensin II immediately after the microsphere injection. Normal hepatic vessels are able to react by constriction, but the developing tumor capillaries are not. As a result, larger amounts of the microspheres are diverted to the tumor bed (113). The clinical results regarding radioembolization therapy have been described in detail by Harbert (109).

Table 8: Radioactive microspheres for therapeutic applications

Application	Type of radioactive microspheres used	Particle size
Radioembolization of liver and spleen tumors	^{90}Y-glass microspheres (Therasphereseϑ)	25-35 Φm
	^{186}Re/^{188}Re-glass microspheres	25-35 Φm
	^{188}Re-Aminex A27 microspheres	20-50 Φm
	^{166}Ho-Aminex A-5 microspheres	13 Φm
Radiosynovectomy of arthritic joints	^{35}S-colloid	0.05-0.6 Φm
	^{90}Y-resin microspheres	20-50 Φm
	^{90}Y-silicate, ^{90}Y-citrate	0.01-1 Φm
	^{165}Dy-ferric hydroxide macroaggregates	2-5 Φm
	^{169}Er-citrate	0.1-1 Φm
	^{186}Re-sulfur-colloid	30-50 nm
	^{188}Re-macro-aggregated albumin	10-20 Φm
Local radiotherapy	^{90}Y-labeled poly(lactic acid) microspheres	1-5 or 10-50 Φm
	^{165}Dy-acetylacetone poly(lactic acid) microspheres	1-5 or 10-50 Φm 1-5 or 10-50 Φm 1-5 or 10-50 Φm 1.8 Φm, 3-10 Φm
	^{166}Ho-acetylacetone poly(lactic acid) microspheres	
	^{186}Re/^{188}Re-labeled poly(lactic acid) microspheres	
	^{211}At-microspheres	<1 Φm
	^{212}Pb-sulfur colloid	<1 Φm
	^{212}Pb-ferrous(ferric) hydroxide	
Intracavitary treatment (peritoneal ovarian tumor metastases, cystic brain tumors)	chromic ^{32}P-phosphate	1-2 Φm
	^{90}Y-silicate, ^{90}Y-citrate	0.01-1 Φm
	^{198}Au suspensions	5-25 nm

Another therapeutic application of β-emitting colloids and microspheres is the radioactive ablation of inflamed synovia in arthritic joints, which has been termed **radiosynovectomy** or sometimes radiosynoviorthesis. The first use of ^{198}Au gold colloid for the treatment of rheumatoid arthritis in knees was reported in 1952 by Fellinger and Schmid (122). Their results were not very encouraging probably due to underdosing, but they did not give up and later confirmed the value of this treatment (123). Therapy with ^{198}Au has the drawback of a 411 keV γ-emission. To overcome this drawback other β-emitters such as ^{186}Re (124), ^{90}Y (125), ^{165}Dy (46) and ^{32}P-colloids (126) have been investigated. Today, the choice of the radioisotope is entirely based on the size of the joint and the radioisotopes= treatment range (for example, ^{90}Y and ^{188}Re for knee and shoulder, and ^{186}Re and ^{169}Er for finger or elbow) (**Table 8**). Traditionally used radioactive colloids are not ideal because their small particle size and large size distribution lead to radiation leakage from the joint (126,127). Higher than desired leakage has also been measured in liposomes filled with ^{99m}Tc (128) and in liposomes that contain the chelating DTTA-group covalently bound to cholesterol (129). In the second case, the chelator was incorporated into the liposomes' phospholipid-wall during preparation and was then able to bind different radioisotopes such as the β-emitter ^{177}Lu (**Table 3**) and the γ-emitter ^{67}Ga (**Table 4**).

More radiochemically stable and better-defined microspheres of about 5 Φm seem to be optimal for retention in joints. Many of the recently developed microspheres such as biodegradable glass

microspheres containing ^{153}Sm, ^{166}Ho, ^{90}Y, ^{165}Dy, ^{186}Re or ^{188}Re (130), ^{188}Re-labeled albumin microspheres (78), ^{166}Ho- or ^{165}Dy-enclosing biodegradable poly(lactic acid) microspheres (67,131) and ^{90}Y- or ^{186}Re/^{188}Re-enclosing biodegradable poly(lactic acid) microspheres (79,132) can be produced in the appropriate size, will biodegrade after complete decay and can easily be made radioactive. More information about radiosynovectomy is available in an extensive review written by Harbert (133). It covers the medical applications and procedures in detail. The radiation dosimetry of radiosynovectomy is covered by Johnson et al. (30).

Another important area for β-emitting microspheres is their use in the local treatment of tumors. The delivery of these radioactive microspheres has been attempted in several ways. In one of them, radioactive microspheres are directly injected into the tumor. Wang et al., for example, radiolabeled ion exchange resin microspheres with ^{188}Re and injected them directly into rat hepatomas (134). Twelve out of 15 rats survived longer than 60 days in the treatment group, as compared to 5 out of 15 rats in the control group. In another novel method for the treatment of solid tumors, Order et al. combined embolization therapy and local radiotherapy, injecting first non-radioactive macro-aggregated albumin microspheres followed by colloidal ^{32}P-chromic phosphate (135). The blockage of the capillaries induced before the ^{32}P-injection resulted in a 3-fold increase of colloid uptake, an effect that lasted for at least 48 hours. This technique has been tested in a first clinical phase I trial for the treatment of non-resectable pancreatic cancer (136). Four patients had a complete response with a duration ranging from 2-57 weeks and 5 patients had a partial response with a duration ranging from 4-21 weeks, corresponding to an objective response of 53% (9 of 17 patients). Six of these patients were alive 33-57 weeks after treatment.

At the current time, there is only one approved application for radioactive microspheres in the United States (137). It is the use of ^{32}P-chromic phosphate colloid for the treatment of cystic brain tumors such as craniopharyngiomas and astrocytomas. The radiocolloid is typically instilled using stereotaxic equipment, either with or without surgical resection or drainage of the cyst. There exists persuasive evidence that this therapeutic approach is as or more efficacious than conventional methods not only for patients with recurrent malignancies, but also for patients receiving primary radiocolloid therapy (138). Radioactive glass- and poly(lactic acid)microspheres containing a mixture of ^{186}Re and ^{188}Re have recently been incorporated into a bioadhesive gel of either carboxymethyl cellulose or fibrin glue and applied to the surface of growing rat 9L-glioblastomas (139). The control group=s survival was 18 days, whereas 4 out 6 of the treated animals were still alive on day 35, which represented the end of the experiment (**Figure 8**). The amount of radioactive ^{186}Re and ^{188}Re injected was less than 50 ΦCi combined. The surviving animals showed no signs of toxicity and had not lost any weight. Such microspheres are now planned for a clinical phase I trial of the treatment of recurrent brain tumor metastases intraoperatively after debulking. This therapeutic approach looks especially promising because the likelihood of local recurrence in these patients is very high (140), and the local radiation with β-emitters could be done in addition to chemotherapy or whole brain irradiation without risking undue toxicity.

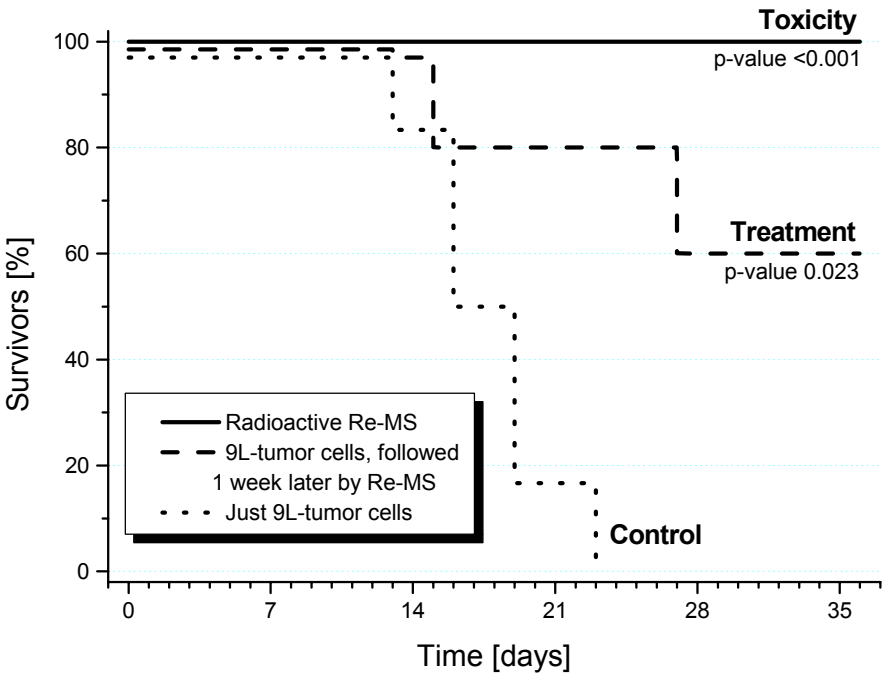

Figure 8. Treatment of 9L-glioblastoma brain tumors in Sprague Dawley rats. The treatment and toxicity group received 50 ΦCi ^{186}Re and ^{188}Re in 0.5 mg glass microspheres contained in 30 Φl of fibrin glue.

Radioactive microspheres filled with magnetite and radiolabeled with the β-emitter ^{90}Y can also be used for targeted cancer therapy. This has been shown with 30% magnetite-containing poly(lactic acid) microspheres sized 20 to 30 Φm that were injected intraperitoneally into C57BL6/N mice and targeted to a subcutaneously growing EL-4 murine lymphoma of about 0.5 g (141). The injection of microspheres took place inside the peritoneal cavity as far from the tumor as possible. After injection, a round, 2 mm thick rare earth magnet with a diameter of 10 mm was taped directly above the tumor. The magnetic field on top of the magnet was 0.12-0.16 Tesla. A dose dependent decrease in tumor size was observed after the 7 day treatment period (**Figure 9**). Close examination revealed that 3 out of 4 tumors in the 80 Gy group and 2 out of 4 tumors in the 120 Gy group were completely eradicated, but that the remaining 1 or 2 tumors, respectively, had grown. It was precisely these tumors that had initially been found to be oblong or flattened out, thus causing the magnetic microspheres to be concentrated farther than 5 mm away from the edges of the tumor. Considering that 90% of the dose of ^{90}Y is deposited within 2.8 mm (30), it follows that the tumor cells farther away were undertreated with the applied amount of radioactivity. The tumors which were not eradicated were therefore local treatment failures.

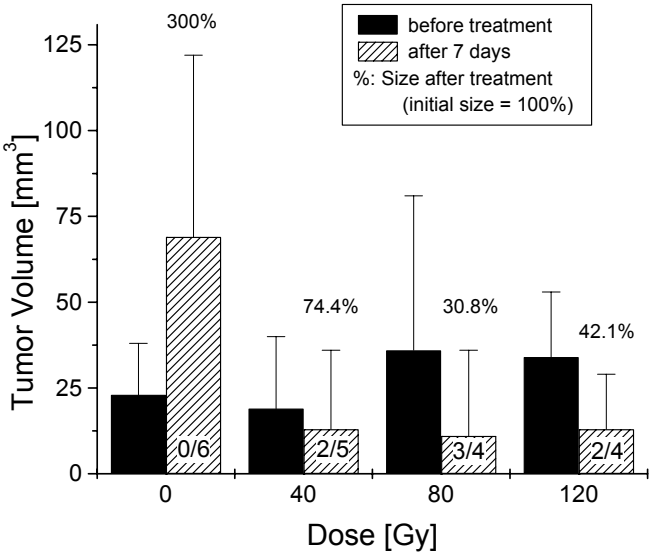

Figure 9. Treatment results of subcutaneous EL-4 lymphomas in mice after magnetic targeting of ^{90}Y-PLA microspheres (n = 6). The numbers inside the bars represent the ratio of completely eradicated tumors to the total number of tumors.

Considerations for the use of radioactive microspheres

The recent surge in the evaluation and clinical testing of radiopharmaceuticals is closely related to the recent development of user-friendly kits which allow the user to prepare radioactive microspheres or other radiolabeled agents in a hospital=s radiopharmacy. These kits have served to reduce concerns about the safety, cost and handling of radioactive pharmaceuticals. Current manipulations needed in most kit preparations typically include the addition of a radioisotope, incubation for a predetermined length of time between 5 and 60 minutes, verification of the activity of the radiopharmaceutical by a simple measurement in a dose calibrator and, sometimes, a thin layer chromatogram for quality control. Ideally, the kit preparation leads to highly stable radioactive microspheres with no purification needed. Additional information is given by Saha in an excellent up-to-date introduction into the currently used radionuclides, radionuclide generators and radiopharmaceuticals in a nuclear pharmacy (142). Saha covers not only all technical aspects of a nuclear pharmacy, but also the radiation regulations and radiation protection aspects.

Increased interest in radiopharmaceuticals is also explained by easier access to generator-produced α- and β-emitting radioisotopes. The β-emitter ^{188}Re, for example (**Table 3**), can now be inexpensively obtained by any hospital radiopharmacy, in the form of a ^{188}W/^{188}Re generator from Oak Ridge National Laboratories. This generator contains the parent nuclide ^{188}W with a half-life of 69.4 days permanently bound to an alumina column. Because of ongoing decay into the daughter-nuclide ^{188}Re, hundreds of mCi of a sterile ^{188}Re-solution can be eluted from the column every day over the course of about 3 months (143). Rhenium-188 is currently being tested in clinical trials for the radioactive treatment of restenosis (144), for local cancer therapy (134,145,146), for radioembolization therapy (147) and for radioimmunotherapy (148).

Two of the most important parameters for the in vivo use of radiopharmaceuticals are their stability and target specificity. The stability can be improved by using more specific chelators and by attaching the chelators to the microspheres through a linker that does not interfere with their metal-binding properties. Many of these stability issues have already been optimized for radiolabeled antibodies (149) and can thus be directly applied to radioactive microspheres and their kit preparation. The second

parameter, target specificity, can be addressed by using additional surface chemistry to modify and functionalize the microspheres= surface. This allows for circulation time optimization and foreign body response minimization. In addition, more specific targeting of microspheres to areas other than the reticuloendothelial system (mainly liver and spleen) is possible, as well as the modification of the microspheres' adsorption behavior to blood proteins (150).

Also important for the application of therapeutic radioactive microspheres is that the radioisotope be chosen for radiobiologic and dosimetric reasons. The target size should, for example, be matched with the radiation range of the radioisotope, thus maximizing the therapeutic effect and minimizing the toxicity (151). Also, dose rates should be taken into account and radioisotopes chosen so that dose rate and total dose deposited are optimal for the target lesion (152). These parameters are not yet well established, but are nevertheless important and should be investigated further.

Another area for the optimization of radioactive microspheres is research into the most appropriate size of microspheres for *in vivo* application. It has long been known that differently sized microspheres of identical composition show different biodistribution profiles (153,154). In addition, for both diffusion and erosion release mechanisms, the release rate of the encapsulated drugs is theoretically dependent on the available surface area. Smaller microspheres with a much larger surface area should thus release their contents at a much faster rate with all the other parameters being equal. Unfortunately, no homogenous, mono-sized microspheres made from biodegradable materials are currently available.

The use of radioactive microspheres is the basis of a large variety of well-established and original concepts for future biomedical, diagnostic and therapeutic applications. Optimally, radioactive microspheres should be combined with biologically active molecules such as proteins, peptides, hormones, lectins, and antibodies. This will allow for the diagnosis and treatment of many different diseases with microsurgical precision and will lead to better treatment concepts with fewer side effects.

References

(1) Wilder RB, DeNardo GL, and DeNardo SJ. Radioimmunotherapy: Recent results and future directions. *J. Clin. Oncol.* **14:** 1383-1400 (1996).

(2) Papatheofanis FJ and Munson L. Peptide radiopharmaceutical imaging. *Appl. Radiol.* **June:** 11-17 (1994).

(3) Cleland JL and Jones AJS. Stable formulations of recombinant human growth hormone and interferon-ô for microencapsulation in biodegradable microspheres. *Pharmaceut. Res.* **13:** 1464-1475 (1996).

(4) Mehta RC, Jeyanthi R, Calis S, Thanoo BC, Burton KW, and DeLuca PP. Biodegradable microspheres as depot system for parenteral delivery of peptide drugs. *J. Contr. Rel.* **29:** 375-384 (1994).

(5) Mathiowitz E, Jacob JS, Jong YS, Carino GP, Chickering DE, Chaturvedi P, Santos CA, Vijayaraghavan K, Montgomery S, Bassett M, and Morrell C. Biologically erodable microspheres as potential oral drug delivery systems. *Nature* **386:** 410-414 (1997).

(6) Langer R. Drug delivery and targeting. *Nature* **392:** 5-10 (1998).

(7) Chen H and Langer R. Oral particulate delivery: status and future trends. *Adv. Drug Del. Rev.* **34:** 339-350 (1998).

(8) Muir W, Husband AJ, Gipps EM, and Bradley MP. Induction of specific IgA responses in rats after oral vaccination with biodegradable microspheres containing a recombinant protein. *Immunol. Lett.* **42:** 203-207 (1994).

(9) Hanes J, Chiba M, and Langer R. Polymer microspheres for vaccine delivery. *Pharm. Biotech.* **6:** 389-412 (1995).

(10) Smith OP, Hann IM, Cox H, and Novelli V. Visceral leishmaniasis: rapid response to AmBisome treatment. *Arch. Dis. Childhood* **73:** 157-159 (1995).

(11) Codde JP, Lumsden AJ, Napoli S, Burton MA, and Gray BN. A comparative study of the anticancer efficacy of doxorubicin carrying microspheres and liposomes using a rat liver tumor model. *Anticancer Research* **13:** 539-544 (1993).

(12) Treleaven JG. Bone marrow purging: An appraisal of immunological and non-immunological methods. *Adv. Drug Del. Rev.* **2/3:** 253-269 (1988).

(13) Arshady R. Polymer supports, reagents and catalysts. *In* Arshady R (Ed.). Microspheres, microcapsules and liposomes. Citus Books, London, 1999, pp. 197-235.

(14) Mikhalovsky SV. Microparticles for hemoperfusion and extracorporeal therapy. *In* Arshady R (Ed.). Microspheres, microcapsules and liposomes. Citus Books, London, 1999, pp. 133-169.

(15) Bangs LB. Microspheres for medical diagnostics: Specific tests and assays. *In* Arshady R (Ed.). Microspheres, microcapsules and liposomes. Citus Books, London, 1999, pp. 71-96.

(16) Flandroy PMJ, Grandfils C, and Jerome RJ. Clinical applications of microspheres in embolization and chemoembolization: A comprehensive review and perspectives. *In* Rolland A (Ed.). Pharmaceutical particulate carriers: Therapeutic applications. Marcel Dekker Inc., New York, 1993, pp. 321-366.

(17) Boschetti E and Schwarz A. Polymer microbeads: Biological applications. *In* Arshady R (Ed.). Microspheres, microcapsules and liposomes. Citus Books, London, 1999, pp. 191-224.

(18) Papisov MI. Modeling in vivo transfer of long-circulating polymers (two classes of long circulating polymers and factors affecting their transfer in vivo). *Adv. Drug Del. Rev.* **16:** 127-139 (1995).
(19) Torchilin VP and Trubetskoy VS. Which polymers can make nanoparticulate drug carriers long-circulating? *Adv. Drug Del. Rev.* **16:** 141-155 (1995).
(20) Ackerman NB. The blood supply of experimental liver metastases. IV. Changes in vascularity with increasing tumor growth. *Surgery* **75:** 589-596 (1974).
(21) Gupta PK. Review article: Drug targeting in cancer chemotherapy: A clinical perspective. *J. Pharm. Sci.* **79:** 949-962 (1990).
(22) Roser M, Fischer D, and Kissel T. Suface-modified biodegradable albumin nano- and microspheres. Part II: Effect of surface charges on in vitro phagocytosis and biodistribution in rats. *Eur. J. Pharm. Biopharm.* **46:** 255-263 (1998).
(23) Macklis RM, Kinsey BM, Kassis AI, Ferrara JLM, Atcher RW, Hines JJ, Coleman CN, Adelstein SJ, and Burakoff SJ. Radioimmunotherapy with alpha-particle-emitting immunoconjugates. *Science* **240:** 1024-1026 (1988).
(24) Humm JL, Macklis RM, Bump K, Cobb LM, and Chin LM. Internal dosimetry using data derived from autoradiographs. *J. Nucl. Med.* **34:** 1811-1817 (1993).
(25) McDevitt MR, Sgouros G, Finn RD, Humm JL, Jurcic JG, Larson SM, and Scheinberg DA. Radioimmunotherapy with alpha-emitting nuclides. *Eur. J. Nucl. Med.* **25:** 1341-1351 (1998).
(26) Muller JH and Rossier PH. A new method for the treatment of cancer of the lungs by means of artificial radioactivity. *Acta Radiologica* **35:** 449-468 (1951).
(27) Rösler H, Triller J, Baer HU, Geiger L, Beer HF, Becker C, and Blumgart LH. Superselective radioembolization of hepatocellular carcinoma: 5-year results of a prospective study. *Nucl. Med.* **33:** 206-214 (1994).
(28) Hall EJ and Brenner DJ. The dose-rate effect in interstitial brachytherapy: a controversy resolved. *Brit. J. Radiology.* **65:** 242-247 (1992).
(29) Kinsey RR. National Nuclear Data Center: Nuclear Data from NuDat at Brookhaven National Laboratory [http://www.nndc.bnl.gov/nndc/nudat/]. **:** (1998).
(30) Johnson LS, Yanch JC, Shortkroff S, Barnes CL, Spitzer AI, and Sledge CB. Beta-particle dosimetry in radiation synovectomy. *Eur. J. Nucl. Med.* **22:** 977-988 (1995).
(31) Loevinger R, Budinger TF, and Watson EE. MIRD primer for absorbed dose calculations. Society of Nuclear Medicine, New York, 1991.
(32) Russell JL, Carden JL, and Herron L. Dosimetry calculations for Yttrium-90 used in the treatment of liver cancer. *Endocurietherapy/Hyperthermia Oncology* **4:** 171-186 (1988).
(33) Stabin MG. MIRDOSE: Personal computer software for internal dose assessment in nuclear medicine. *J. Nucl. Med.* **37:** 538-546 (1996).
(34) Harbert JC, Eckelman WC, and Neumann RD. Nuclear medicine: Diagnosis and therapy. Thieme Medical Publishers, New York, 1996.
(35) Bardies M, Lame J, Myers MJ, and Simoen JP. A simplified approach to beta dosimetry for small spheres labelled on the surface. *Phys. Med. Biol.* **35:** 1039-1050 (1990).
(36) Akabani G, Poston JW, and Bolch WE. Estimates of beta absorbed fractions in small tissue volumes for selected radionuclides. *J. Nucl. Med.* **32:** 835-839 (1991).
(37) Siegel JA and Stabin MG. Absorbed fractions for electrons and beta particles in spheres of various sizes. *J. Nucl. Med.* **35:** 152-156 (1994).
(38) Duncan R, Kopeckova-Rejmanova P, Strohalm J, Hume I, Cable HC, Pohl J, Lloyd JB, and Kopecek J. Anticancer agents coupled to N-(2-hydroxypropyl)methacrylamide copolymers. I. Evaluation of daunomycin and puromycin conjugates in vitro. *Br. J. Cancer* **55:** 165-174 (1987).
(39) Nelp WB. Evaluation of colloids for RES function studies. *In* Subramanian G, Rhodes B, Cooper JF, and Sodd VJ (Eds.). . Society of Nuclear Medicine, New York, NY, 1975, pp. 349-356.
(40) Goodwin DA, Stern HS, and Wagner HN. Ferric hydroxide particles labeled with indium In-113m for lung scanning. *JAMA* **206:** 339-343 (1968).
(41) Lin MS and Winchell HS. A "kit" method for the preparation of technetium-tin(II) colloid and a study of its properties. *J. Nucl. Med.* **13:** 58-65 (1972).
(42) Zolle I. Method for incorporating substances into protein microspheres. US Patent No. 3937668, 1976.
(43) Amersham. Guide to radioiodination techniques: Iodine-125. Amersham International, Little Chalfont, England, 1993.
(44) Yang DJ, Kuang LR, Li C, Kan Z, and Wallace S. Computed tomographic liver enhancement with poly(d,l-lactide)-microencapsulated contrast media. *Invest. Radiol.* **29 Suppl. 2:** S267-S270 (1994).
(45) Häfeli U, Tiefenauer LX, Schubiger PA, and Weder HG. A lipophilic complex with ^{186}Re/^{188}Re incorporated in liposomes suitable for radiotherapy. *Nucl. Med. Biol. Int. J. Rad. Appl. Instr. Part B* **18:** 449-454 (1991).
(46) Sledge CB, Noble J, Hnatowich DJ, Kramer RT, and Shortkroff S. Experimental radiation synovectomy by 165Dy ferric hydroxide macroaggregate. *Arthritis Rheum.* **20:** 1334-1342 (1977).
(47) Howson MP, Shepard NL, and Mitchell NS. Colloidal chromic phosphate P-32 synovectomy in antigen-induced arthritis in the rabbit. *Clin. Orthopaed. Rel. Res.* **229:** 283-293 (1988).
(48) Gürkan H, Yalabik-Kas HS, Hincal AA, and Ercan MT. Streptomycin sulphate microspheres. Formulation and in vivo distribution. *J. Microencapsulation* **3:** 101-108 (1986).
(49) Reza MS and Whateley TL. Iodo-2'-deoxyuridine (IUdR) and I-125-IUdR loaded biodegradable microspheres for controlled delivery to the brain. *J. Microencapsulation* **15:** 789-801 (1998).
(50) Senyei AE and Widder KJ. Drug Targeting: Magnetically responsive albumin microspheres - a review of the system to date. *Gynecol. Oncol.* **12:** 1-13 (1981).
(51) Teder H, Johansson CJ, d'Argy R, Lundin N, and Gunnarsson PO. The effect of different dose levels of degradable starch microspheres (Spherex) on the distribution of a cytotoxic drug after regional administration to tumor-bearing rats. *Europ. J. Cancer* **31A:** 1701-1705 (1995).
(52) Burch WM, Sullivan PJ, and McLaren CJ. Technegas - a new ventilation agent for lung scanning. *Nuclear Medicine Communications* **7:** 865-871 (1986).
(53) Schubiger PA, Beer HF, Geiger L, Rösler H, Zimmermann A, Triller J, Mettler D, and Schilt W. ^{90}Y-resin particles - Animal experiments on pigs with regard to the introduction of superselective embolization therapy. *Nucl. Med. Biol. Int. J. Rad. Appl. Instr. Part B* **18:** 305-311 (1991).
(54) Quinlan MF, Salman SD, Swift DL, Wagner HN, and Proctor DF. Measurement of mucociliary function in man. *Am. Rev. Respir. Dis.* **99:** 13-23 (1969).
(55) Stivland T, Camilleri M, Vassallo M, Proano M, Rath D, Brown M, Thomforde G, Pemberton J, and Phillips S. Scintigraphic measurement of regional gut transit in idiopathic constipation. *Gastroenterology* **101:** 107-115 (1991).
(56) Simon H, Drettner B, and Jung B. Messung des Schleimhauttransportes in der menschlichen Nase mit Cr-51 markierten Harzkügelchen. *Acta Otolaryngol.* **83:** 378-390 (1977).
(57) Zimmermann A, Schubiger PA, Mettler D, Geiger L, Triller J, and Rösler H. Renal pathology after arterial Y-90 microsphere administration in pigs: A model for superselective radioembolization therapy. *Invest. Radiol.* **30:** 716-723 (1995).

(58) Willmott N, Murray T, Carlton R, Chen Y, Logan H, McCurrach G, Bessent RG, Goldberg JA, Anderson J, McKillop JH, and McArdle CS. Development of radiolabelled albumin microspheres: A comparison of gamma-emitting radioisotopes of Iodine (^{131}I) and Indium (^{111}In/^{113m}In). *Nucl. Med. Biol. Int. J. Rad. Appl. Instr. Part B* **18:** 687-694 (1991).
(59) Wagner SJ and Welch MJ. Gallium-68 labeling of albumin and albumin microspheres. *J. Nucl. Med.* **20:** 428-433 (1979).
(60) Ercan MT, Tuncel SA, Caner BE, and Piskin E. Tc-99m-labeled monodisperse latex particles with amine or carboxylic functional groups for colon transit studies. *J. Microencapsulation* **10:** 67-76 (1993).
(61) Day DE, Ehrhardt GJ, and Zinn KR. Radiolabeled protein composition and method for radiation synovectomy. U.S.A. Patent No. 5403573, 1995.
(62) Yan C, Li X, Chen X, Wang D, Zhong D, Tan T, and Kitano H. Anticancer gelatin microspheres with multiple functions. *Biomaterials* **12:** 640-644 (1991).
(63) Vergote I, Larsen RH, de Vos L, Nesland JM, Bruland O, Bjorgum J, Alstad J, Trope C, and Nustad K. Therapeutic efficacy of the á-emitter ^{211}At bound on microspheres compared with ^{90}Y and ^{32}P colloids in a murine intraperitoneal tumor model. *Gynecol. Oncol.* **47:** 366-372 (1992).
(64) Ehrhardt GJ and Day DE. Therapeutic use of 90Y microspheres. *Nucl. Med. Biol. Int. J. Rad. Appl. Instr. Part B* **14:** 233-242 (1987).
(65) Conzone SD, Häfeli UO, Day DE, and Ehrhardt GJ. Preparation and properties of radioactive rhenium glass microspheres intended for in-vivo radioembolization therapy. *J. Biomed. Mat. Res.* **42:** 617-625 (1998).
(66) Brown RF, Lindesmith LC, and Day DE. ^{166}Holmium-containing glass for internal radiotherapy of tumors. *Nucl. Med. Biol. Int. J. Rad. Appl. Instr. Part B* **18:** 783-790 (1991).
(67) Mumper RJ and Jay M. Poly(L-lactic acid) microspheres containing neutron-activatable Holmium-165: A study of the physical characteristics of microspheres before and after irradiation in a nuclear reactor. *Pharmaceut. Res.* **9:** 149-154 (1992).
(68) Nijsen JFW, Zonnenberg BA, Woittiez JRW, Rook DW, Swildens-van Woudenberg IA, van Rijk PP, and van het Schip AD. Holmium-166 poly lactic acid microspheres applicable for intra-arterial radionuclide therapy of hepatic malignancies: effects of preparation and neutron activation techniques. *Eur. J. Nucl. Med.* **26:** 699-704 (1999).
(69) Häfeli UO, Roberts WK, Pauer GJ, Kraeft SK, and Macklis RM. Preparation and stability of biodegradable radioactive rhenium microspheres (Re-186 and Re-188) for use in radiotherapy. *J. Pharm. Sci.* **submitted:** (1999).
(70) Ercan MT. Rapid determination of hydrolyzed-reduced Technetium-99m in particulate radiopharmaceuticals. *Appl. Radiat. Isot. - Int. J. Radiat. Appl. Instrum. Part A* **43:** 1175-1177 (1992).
(71) Chinol M, Vallabhajosula S, Goldsmith SJ, Klein MJ, Deutsch KF, Chinen LK, Brodack JW, Deutsch EA, Watson BA, and Tofe AJ. Chemistry and biological behavior of Samarium-153 and Rhenium-186-labeled hydroxyapatite particles: Potential radiopharmaceuticals for radiation synovectomy. *J. Nucl. Med.* **34:** 1536-1542 (1993).
(72) Junghans RP, Dobbs D, Brechbiel MW, Mirzadeh S, Raubitschek AA, Gansow OA, and Waldmann TA. Pharmacokinetics and bioactivity of 1,4,7,10-tetra-azacyclododecane N,N',N",N"'-tetraacetic acid (DOTA)-bismuth-conjugated anti-Tac antibody for á-emitter 212Bi therapy. *Cancer Res.* **53:** 5683-5689 (1993).
(73) Camera L, Kinuya S, Garmestani K, Wu C, Brechbiel MW, Pai LH, McMurry TJ, Gansow OA, Pastan I, Paik CH, and Carrasquillo JA. Evaluation of the serum stability and in vivo biodistribution of CHX-DTPA and other ligands for Yttrium labeling of monoclonal antibodies. *J. Nucl. Med.* **35:** 882-889 (1994).
(74) Fritzberg AR. Radioimmunotherapy with Rhenium-186 and Rhenium-188. *In* Bryskin BD (Ed.). Rhenium and Rhenium Alloys. TMS (Minerals, Metals and Materials Society), Warrendale, PA, 1997, pp. 479-487.
(75) Fritzberg AR, Abrams PG, Beaumier PL, Kasina S, Morgan AC, Rao TN, Reno JM, Sanderson JA, Srinivasan A, Wilbur DS, and Vanderheyden JL. Specific and stable labeling of antibodies with Tc-99m with a diamide dithiolate chelating agent. *Proc. Natl. Acad. Sci. USA* **85:** 4025-4029 (1988).
(76) Eckelman WC, Steigman J, and Paik CH. Radiopharmaceutical chemistry. *In* Harbert JC, Eckelman WC, and Neumann RD (Eds.). Nuclear medicine: Diagnosis and therapy. Thieme Medical Publishers, New York, 1996, pp. 213-266.
(77) Griffiths GL, Goldenberg DM, Diril H, and Hansen HJ. Technetium-99m, Rhenium-186, and Rhenium-188 direct labeled antibodies. *Cancer* **73:** 761-768 (1994).
(78) Wunderlich G, Pinkert J, and Franke WG. Studies on the processing and in vivo stability of Re-188 labeled microspheres. *In* Nicolini M and Mazzi U (Eds.). Technetium, rhenium and other metals in chemistry and nuclear medicine. SGE Ditoriali, Padova, Italy, 1999, pp. 709-712.
(79) Häfeli UO, Sweeney SM, Beresford BS, Sim EH, and Macklis RM. Biodegradable magnetically directed ^{90}Y-microspheres: Novel agents for targeted intracavitary radiotherapy. *J. Biomed. Mat. Res.* **28:** 901-908 (1994).
(80) Day DE and Day TE. Radiotherapy Glasses. *In* Hench LL and Wilson J (Eds.). An Introduction to Bioceramics. World Scientific, New Jersey, 1993, pp. 305-317.
(81) Conzone SD. Glass microspheres for medical applications. *Ph.D. thesis*, University of Missouri, Rolla, 1999.
(82) Locher GL. Biological effects and therapeutic possibilites of neutrons. *AJR* **36:** 1-13 (1936).
(83) Mehta SC and Lu DR. Targeted drug delivery for boron neutron capture therapy. *Pharmaceut. Res.* **13:** 344-351 (1996).
(84) Akine Y, Tokita N, Tokuuye K, Satoh M, Fukumori Y, Tokumitsu H, Kanamori R, Kobayashi T, and Kanda K. Neutron capture therapy of murine ascites tumor with gadolinium-containing microcapsules. *J. Cancer Res. Clin. Oncol.* **119:** 71-73 (1992).
(85) Tokumitsu H, Ichikawa H, Fukumori Y, and Block LH. Preparation of gadoptentetic acid-loaded chitosan microparticles for gadolinium neutron capture therapy of cancer by a novel emulsion-droplet coalescence technique. *Chem. Pharm. Bull.* **47:** 838-842 (1999).
(86) Yanagie H, Tomita T, Kobayashi H, Fujii Y, Nonaka Y, Saegusa Y, Hasumi K, Eriguchi M, Kobayashi T, and Ono K. Inhibition of human pancreatic cancer growth in nude mice by boron neutron capture therapy. *Br. J. Cancer* **75:** 660-665 (1997).
(87) Rawls RL. Bringing boron to bear on cancer. *C&EN* **March 22:** 26-29 (1999).
(88) Hawthorne MF and Shelly K. Liposomes as drug delivery vehicles for boron agents. *J. Neuro-Oncol.* **33:** 53-58 (1997).
(89) Heymann MA, Payne BD, Hoffman JI, and Rudolph AM. Blood flow measurements with radionuclide-labeled particles. *Progress in Cardiovascular Diseases* **20:** 55-79 (1977).
(90) Peters AM, Danpure HJ, Osman S, Hawker RJ, Henderson BL, Hodgson HJ, Kelly JD, Neirinckx RD, and Lavender JP. Clinical experience with Tc-99m-hexamethyl propylene amine oxime for labeling leukocytes and imaging inflammation. *The Lancet* **2:** 946-949 (1986).
(91) Knight L. Thrombus-localizing radiopharmaceuticals. *In* Fritzberg AR (Ed.). Radiopharmaceuticals: Progress and clinical perspectives. CRC Press, Boca Raton, Florida, 1986, pp. 23-40.
(92) Marcus ML, Heistad DD, Ehrhardt JC, and Abboud FM. Total and regional cerebral blood flow measurement with 7- 10-, 15-, 25-, and 50-ìm microspheres. *J. Appl. Physiol.* **40(4):** 501-507 (1976).
(93) Triller J, Rösler H, Geiger L, and Baer HU. Methodik der superselektiven Radioembolisation von Lebertumoren mit Yttrium-90-Resin-Partikeln. *Fortschr. Röntgenstr.* **160:** 425-431 (1994).
(94) Lin M. Radiation pneumonitis caused by Yttrium-90 microspheres: Radiologic findings. *AJR* **162:** 1300-1302 (1994).
(95) Papisov MI and Brady TJ. System of drug delivery to the lymphatic tissues. U.S.A. Patent No. 5582172, 1996.

(96) Frier M and Perkins AC. Radiopharmaceuticals and the gastrointestinal tract. *Eur. J. Nucl. Med.* **21:** 1234-1242 (1994).
(97) Caner BE, Ercan MT, Kapucu LO, Tuncel SA, Bekdik CF, Erbengi G, and Piskin E. Functional assessment of human gastrointestinal tract using Tc-99m-latex particles. *Nuclear Medicine Communications* **12:** 539-544 (1991).
(98) Proffitt RT, Williams LE, Presant CA, Tin GW, Uliana JA, Gamble RC, and Baldeschwieler JD. Tumour-imaging potential of liposomes loaded with 111In-NTA: Biodistribution in mice. *J. Nucl. Med.* **24:** 45-51 (1983).
(99) Ogihara-Umeda I, Sasaki T, and Nishigori H. Development of a liposome-encapsulated radionuclide with preferential tumor accumulation - the choice of radionuclide and chelating ligand. *Nucl. Med. Biol. Int. J. Rad. Appl. Instr. Part B* **19:** 753-757 (1992).
(100) Diaz RV, Mallol J, Delgado A, Soriano I, and Evora C. Tc-99m microspheres based on biodegradable synthetic polymers (MSP). A new patented radiopharmaceutical. *Poster* **:** (1997).
(101) Wilensky RL, March KL, Gradus-Pizlo I, Schauwecker D, Michaels MB, Robinson J, Carlson K, and Hathaway DR. Regional and arterial localization of radioactive microparticles after local delivery by unsupported or supported porous balloon catheters. *Am. Heart J.* **129:** 852-859 (1995).
(102) Molho P, Verrier P, Stieltjes N, Schacher JM, Ounnoughene N, Vassilieff D, Menkes CJ, and Sultan Y. A retrospective study on chemical and radioactive synovectomy in severe haemophilia patients with recurrent haemarthrosis. *Haemophilia* **5:** 115-123 (1999).
(103) Keys HM, Bundy BN, Stehman FB, Muderspach LI, Chafe WE, Suggs CL, Walker JL, and Gersell D. Cisplatin, radiation, and adjuvant hysterectomy compared with radiation and adjuvant hysterectomy for bulky stage IB cervical carcinoma. *The New England Journal of Medicine* **340:** 1154-1161 (1999).
(104) Rose PG, Bundy BN, Watkins EB, Thigpen JT, Deppe G, Maiman MA, Clarke-Pearson DL, and Insalaco S. Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. *The New England Journal of Medicine* **340:** 1144-1153 (1999).
(105) Wunderlich G, Franke WG, Doberenz I, and Fischer S. Two ways to establish potential At-211 radiopharmaceuticals. *Anticancer Research* **17:** 1809-1814 (1997).
(106) Rotmensch J, Atcher RW, Schlenker R, Hines J, Grdina D, Block BS, Press MF, Herbst AL, and Weichselbaum RR. The effect of the á-emitting radionuclide Lead-212 on human ovarian carcinoma: a potential new form of therapy. *Gynecol. Oncol.* **32:** 236-239 (1989).
(107) Häfeli U, Atcher RW, Morris CE, Beresford B, Humm JL, and Macklis RM. Polymeric radiopharmaceutical delivery systems. *Radioactivity & Radiochemistry* **3:** 11-14 (1992).
(108) Macklis RM, Atcher R, Morris C, Beresford B, Häfeli U, and Humm J. Controlled release biodegradable radiopolymers for intracavitary radiotherapy using a Pb-212 alpha emitting generator system. **:** (1992).
(109) Harbert JC. Therapy with intra-arterial radioactive particles. *In* Harbert JC, Eckelman WC, and Neumann RD (Eds.). Nuclear medicine: Diagnosis and therapy. Thieme Medical Publishers, New York, 1996, pp. 1141-1155.
(110) Ariel IM. The treatment of metastases to the liver with interstitial radioactive isotopes. *Surgery, Gynecology & Obstetrics* **110:** 739-745 (1960).
(111) Ariel IM. Treatment of inoperable primary pancreatic and liver cancer by the intra-arterial administration of radioactive isotopes (Y-90 radiating microspheres). *Ann. Surg.* **162:** 267-278 (1965).
(112) Ariel IM. Radioactive isotopes for adjuvant cancer therapy. *Arch. Surg.* **89:** 244-249 (1964).
(113) Burton MA, Gray BN, Kelleher DK, Klemp P, and Hardy N. Selective internal radiation therapy: Validation of intra-operative dosimetry. *Radiology* **175:** 253-255 (1990).
(114) Turner JH, Claringbold PG, Klemp PFB, Cameron PJ, Martindale AA, Glancy RJ, Norman PE, Hetherington EL, Najdovski L, and Lambrecht RM. Ho-166-microsphere liver radiotherapy: a preclinical SPECT dosimetry study in the pig. *Nuclear Medicine Communications* **15:** 545-553 (1994).
(115) Häfeli UO, Casillas S, Dietz DW, Pauer GJ, Rybicki LA, Conzone SD, and Day DE. Radioembolization of Novikoff hepatomas using radioactive rhenium (Re-186/Re-188) glass microspheres. *Int. J. Radiat. Oncol. Biol. Phys.* **44:** 189-199 (1999).
(116) Andrews JC, Walker SC, Ackermann RJ, Cotton LA, Ensminger WD, and Shapiro B. Hepatic radioembolization with Yttrium-90 containing glass microspheres: Preliminary results and clinical follow up. *J. Nucl. Med.* **35:** 1637-1644 (1994).
(117) Ackerman NB, Lien WM, Kondi ES, and Silverman NA. The blood supply of experimental liver metastases. I. The distribution of hepatic artery and portal vein blood to "small" and "large" tumors. *Surgery* **66:** 1067-1072 (1969).
(118) Leung TWT, Lau WY, Ho SKW, Ward SC, Chow JHS, Chan MSY, Metreweli C, Johnson PJ, and Li AKC. Radiation pneumonitis after selective internal radiation treatment with intraarterial Y-90-microspheres for inoperable hepatic tumors. *Int. J. Radiat. Oncol. Biol. Phys.* **33:** 919-924 (1995).
(119) Ho S, Lau WY, Leung TWT, Chan M, Chan KW, Lee WY, Johnson PJ, and Li AKC. Tumor-to-normal uptake ratio of Y-90 microspheres in hepatic cancer assessed with Tc-99m macroaggregated albumin. *Brit. J. Radiology.* **70:** 823-828 (1997).
(120) Blanchard RJ, Grotenhuis I, LaFave JW, Frye CW, and Perry JN. Treatment of experimental tumors. *Arch. Surg.* **89:** 406-410 (1964).
(121) Shepherd FA, Rotstein LE, Houle S, Yip TCK, Paul K, and Sniderman KW. A phase I dose escalation trial of Yttrium-90 microspheres in the treatment of primary hepatocellular carcinoma. *Cancer* **70:** 2250-2254 (1992).
(122) Fellinger K and Schmid J. Die lokale Behandlung der rheumatischen Erkrankungen. *Wien Z. Inn. Med.* **33:** 351 (1952).
(123) Delbarre F, Cayla J, Roucayrol JC, et al. Synoviortheses (synoviotherapie par les radioisotopes). Etude de plus de 400 traitements et perspectives d'avenir. *Ann. Med. Interne* **121:** 441 (1970).
(124) Delbarre F, Roucayrol JC, Ingrand J, Sanchez A, Menkes CJ, and Aignan M. Une nouvelle preparation radioactive pour la synoviorthese: le rhenium 186 colloidal: Avantages par rapport au colloide d'or 198. *Nouvelle presse medicale* **2:** 1372 (1973).
(125) Gumpel JM, Beer TC, Crawley JCW, and Farran HEA. Yttrium 90 in persistent synovitis of the knee - a single centre comparison. The retention and extra-articular spread of four 90Y radiocolloids. *Brit. J. Radiology.* **48:** 377-381 (1975).
(126) Winston MA, Bluestone R, Cracchiolo A, and Blahd WH. Radioisotope synovectomy with P-32-chromic phosphate - kinetic studies. *J. Nucl. Med.* **14:** 886-889 (1973).
(127) Noble J, Jones AG, Davies MA, Sledge CB, Kramer RI, and Livni E. Leakage of radioactive particle systems from a synovial joint studied with a gamma camera. *J. Bone Joint Surg.* **65A:** 381-389 (1983).
(128) Zalutsky MR, Noska MA, Gallagher PW, Shortkroff S, and Sledge CB. Use of liposomes as carriers for radiation synovectomy. *Nucl. Med. Biol. Int. J. Rad. Appl. Instr. Part B* **15:** 151-156 (1988).
(129) Knight CG, Bard DR, and Page Thomas DP. Liposomes as carriers of antiarthritic agents. *Ann. N. Y. Acad. Sci.* **:** 415-428 (1988).
(130) Day DE and Ehrhardt GJ. Composition and method for radiation synovectomy of arthritic joints. U.S.A. Patent No. 4889707, 1989.
(131) Mumper RJ, Mills BJ, Yun Ryo U, and Jay M. Polymeric microspheres for radionuclide synovectomy containing neutron-activated Holmium-166. *J. Nucl. Med.* **33:** 398-402 (1992).
(132) Häfeli U, German R, Pauer G, Casillas S, and Dietz D. Production of Rhenium-Powder with a jet mill and its incorporation in radioactive microspheres for the treatment of liver tumors. *In* Bryskin BD (Ed.). Rhenium and Rhenium Alloys. TMS (Minerals, Metals and Materials Society), Warrendale, PA, 1997, pp. 469-477.

(133) Harbert JC. Radionuclide therapy in joint diseases. *In* Harbert JC, Eckelman WC, and Neumann RD (Eds.). Nuclear medicine: Diagnosis and therapy. Thieme Medical Publishers, New York, 1996, pp. 1093-1109.
(134) Wang SJ, Lin WY, Chen MN, Chi CS, Chen JT, Bo WL, Hsieh BT, Shen LH, Tsai ZT, Ting G, Mirzadeh S, and Knapp FF. Intratumoral injection of rhenium-188 microspheres into an animal model of hepatoma. *J. Nucl. Med.* **39:** 1752-1757 (1998).
(135) Order SE, Siegel JA, Lustig RA, Principato R, Zeiger LS, Johnson E, Zhang H, Lang P, Pilchik NB, Metz J, DeNittis A, Boerner P, Beuerlein G, and Wallner PE. A new method for delivering radioactive cytotoxic agents in solid cancers. *Int. J. Radiat. Oncol. Biol. Phys.* **30:** 715-720 (1994).
(136) Westlin JE, Andersson-Forsman C, Garske U, Linne T, Aas M, Glimelius B, Lindgren PG, Order SE, and Nilsson S. Objective responses after fractionated infusional brachytherapy of unresectable pancreatic adenocarcinomas. *Cancer* **80:** 2743-2748 (1997).
(137) Harbert JC. Radiocolloid therapy of cystic brain tumors. *In* Harbert JC, Eckelman WC, and Neumann RD (Eds.). Nuclear medicine: Diagnosis and therapy. Thieme Medical Publishers, New York, 1996, pp. 1083-1091.
(138) Backlund EO. Colloidal radioisotopes as part of a multi-modality treatment of craniopharyngiomas. *J. Neurosurg. Sci.* **33:** 95-97 (1989).
(139) Häfeli UO and Pauer GJ. Brachytherapy of brain tumors using rhenium microspheres in fibrin glue. *J. Natl. Cancer Inst.* in preparation (1999).
(140) Wallner KE, Galicich JH, Krol G, Arbit E, and Malkin MG. Patterns of failure following treatment for glioblastoma multiforme and anaplastic astrocytoma. *Int. J. Radiat. Oncol. Biol. Phys.* **16:** 1405-1409 (1989).
(141) Häfeli UO, Pauer GJ, Roberts WK, Humm JL, and Macklis RM. Magnetically targeted microspheres for intracavitary and intraspinal Y-90 radiotherapy. *In* Häfeli U, Schütt W, Teller J, and Zborowski M (Eds.). Scientific and clinical applications of magnetic carriers. Plenum, New York, 1997, pp. 501-516.
(142) Saha GB. Fundamentals of nuclear pharmacy. Springer, New York, 1998.
(143) Knapp FF, Beets AL, Guhlke S, Zamora PO, Bender H, Palmedo H, and Biersack HJ. Availability of Re-188 from the alumina-based W-188/Re-188 generator for preparation of Re-188-labeled radiopharmaceuticals for cancer treatment. *Anticancer Research* **17:** 1783-1795 (1997).
(144) Waksman R. Clinical trials in radiation therapy for restenosis: Past, present and future. *Vascular radiotherapy monitor* **1:** 10-18 (1998).
(145) Bender H, Zamora PO, Rhodes BA, Guhlke S, and Biersack HJ. Clinical aspects of local and regional tumor therapy with Re-188-RC-160. *Anticancer Research* **17:** 1705-1712 (1997).
(146) Blower PJ, Lam ASK, O'Doherty MJ, Kettle AG, Coakley AJ, and Knapp FF. Pentavalent rhenium-188 dimercaptosuccinic acid for targeted radiotherapy: synthesis and preliminary animal and human studies. *Eur. J. Nucl. Med.* **25:** 613-621 (1998).
(147) Wang SJ, Lin WY, Chen MN, Hsieh BT, Shen LH, Tsai ZT, Ting G, and Knapp FF. Radiolabeling of lipiodol with generator-produced Re-188 for hepatic tumor therapy. *Appl. Radiat. Isot.* **47:** 267-271 (1996).
(148) Chen JQ, Strand SE, Tennvall J, Lindgren L, Hindorf C, and Sjögren HO. Extracorporeal immunoadsorption compared to avidin chase: Enhancement of tumor-to-normal tissue ratio for biotinylated rhenium-188 chimeric BR96. *J. Nucl. Med.* **38:** 1934-1939 (1997).
(149) Schubiger PA, Alberto R, and Smith A. Vehicles, chelators, and radionuclides: Choosing the "building blocks" of an effective therapeutic radioimmunoconjugate. *Bioconj. Chem.* **7:** 165-179 (1996).
(150) Lück M, Pistel KF, Li YX, Blunk T, Müller RH, and Kissel T. Plasma protein adsorption on biodegradable microspheres consisting of PLGA, PLA or ABA triblock copolymers containing poly(oxyethylene). Influence of production method and polymer composition. *J. Contr. Rel.* **55:** 107-120 (1998).
(151) O'Donoghue JA, Bardies M, and Wheldon TE. Relationships between tumor size and curability for uniformly targeted therapy with beta-emitting radionuclides. *J. Nucl. Med.* **36:** 1902-1909 (1995).
(152) Hall EJ and Brenner DJ. The dose-rate effect revisited: Radiobiological considerations of importance in radiotherapy. *Int. J. Radiat. Oncol. Biol. Phys.* **21:** 1403-1414 (1991).
(153) Ogawara KI, Yoshida M, Higaki K, Kimura T, Shiraishi K, Nishikawa M, Takakura Y, and Hashida M. Hepatic uptake of polystyrene microspheres in rats: Effect of particle size on intrahepatic distribution. *J. Contr. Rel.* **59:** 15-22 (1999).
(154) Papisov MI, Savelyev VY, Sergienko VB, and Torchilin VP. Magnetic drug targeting. In vivo kinetics of radiolabeled magnetic drug carriers. *Int. J. Pharm.* **40:** 201-206 (1987).