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Radiolabelled polymeric materials for imaging and treatment of cancer: Quo Vadis?

Kritee Pant, [a] Ondřej Sedláček, [b] Robin A. Nadar, [a], [c] Martin Hrubý, *[b] and Holger Stephan*[a]

Dedicated to Professor Helmut Ringsdorf

[a] Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiopharmaceutical Cancer Research
   Bautzner Landstraße 400, 01328 Dresden (Germany)
[b] Institute of Macromolecular Chemistry, The Academy of Sciences of the Czech Republic
   Heyrovského náměstí 2, 16206 Prague 6 (Czech Republic)
[c] Current address: Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Department of Biomaterials, Philips van Leydenlaan 25, 6525 EX Nijmegen (The Netherlands)

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Radiolabelled polymeric materials for cancer imaging and therapy: Quo Vadis? This review highlights the historical progress as well as recent advances of radiolabelled natural and synthetic polymers applied in cancer imaging and treatment, including drug delivery
systems. Advantages and disadvantages of different radiolabelling as well as targeting strategies are discussed.

Keywords: polymers, cancer, radiodiagnosis, radiotherapy, targeting, nuclear medicine, theranostics

Abstract
Owing to their tunable blood circulation time and suitable plasma stability, polymer-based nanomaterials hold a great potential for designing and utilising multifunctional nanocarriers for efficient cancer imaging and effective treatment of cancer. When tagged with appropriate radionuclides, they may allow for specific detection (diagnosis) as well as the destruction of tumours (therapy) or even customization of materials, aiming to both diagnosis and therapy (theranostic approach). This review provides an overview of recent developments of radiolabelled polymeric nanomaterials (natural and synthetic polymers) for molecular imaging of cancer, specifically, applying nuclear techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT). Different approaches to radiolabel polymers are evaluated from the methodical radiochemical point of view. This includes new bifunctional chelating agents (BFCAs) for radiometals as well as novel labelling methods. Special emphasis is given to eligible strategies employed to evade the mononuclear phagocytic system (MPS) in view of efficient targeting. The discussion encompasses promising strategies currently employed as well as emerging possibilities in radionuclide-based cancer therapy. Key issues involved in the clinical translation of radiolabelled polymers and future scopes of this intriguing research field are also discussed.
1. Introduction

In the 1920s, the age of macromolecular chemistry was inaugurated by the works of Hermann Staudinger.\textsuperscript{[1-4]} He was awarded the Nobel Prize in chemistry in 1953 for his fundamental contributions in the field of macromolecular chemistry. From the very beginning, the chemistry of the giant molecules was located somewhere between the classical disciplines of chemistry and physics, and it was very application-oriented. For all his life, Hermann Staudinger devoted his visions and hopes to the natural macromolecules, such as cellulose and natural rubber, and their opportunities of application.\textsuperscript{[5]} Early on, he was active at the interface between chemistry and biology. Today, we can encounter the amalgamation of biology and macromolecular chemistry in numerous facets, such as biomacromolecular chemistry, biopolymers, polymeric biomaterials and polymeric therapeutics.

The most attractive domains of research that have high innovation potential include, without any doubt, the areas of drug delivery and drug targeting. In these particular areas, polymeric materials are of enormous importance as a basis for new specific diagnostics and therapeutics, as synthetic vaccines of the future as well as pharmaceutical carrier systems. Meanwhile, the art of synthesising them has advanced so far that both natural and synthetic polymers can be tailored to medical uses. Moreover, complex structures can be created – for example, by defined polymeric building blocks organising themselves –, a way to provide nanocontainers for targeted active substance release. Liposomes, polymersomes and dendrisomes may be mentioned in that context. Altogether, polymer science has greatly influenced the rapid growth of new disciplines such as nanotechnology, nanoscience and nanomedicine that seek to develop new materials by precisely engineering atoms and molecules to yield new molecular assemblies on a scale ranging from few nanometers to about 500 nm. Size and shape of many polymeric materials (natural and synthetic oligomers,
micelles) resemble biological objects such as enzymes and viruses. Vesicles, liposomes and synthetic polymers bridge the gap between smaller nano-objects and bacteria, prokaryotic and eukaryotic cells. However, there is a difference of some orders of magnitude between the size of polymeric materials and that of, for instance, cancer cells (Figure 1). Derived from that, polymeric materials are ideally suited as transport vehicles in vivo.\textsuperscript{[6-17]}

Wide space is taken by the development of polymeric systems for detecting and treating cancer.\textsuperscript{[18-26]} The latter area, in particular, was most sustainably influenced by the works of Ringsdorf on active substance systems based on synthetic polymers.\textsuperscript{[27-28]} Working groups around Duncan, Maeda, Satchi-Fainaro and Folkman have especially advanced the clinical application of polymer therapeutics in cancer treatment.\textsuperscript{[29-37]}

This is where defined, highly selective materials must be developed, which are non-immunogenic and non-toxic, permitting unequivocal detection and targeted destruction of tumours and metastases at the same time. In that respect, information on the biodistribution and on the pharmacokinetic and pharmacodynamic properties of polymers is of great importance, and nuclear techniques, such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT), play a fundamental role.\textsuperscript{[38-39]} These non-invasive methods permit to reliably evaluate properties, such as absorption, distribution, metabolism, and excretion (ADME) of pharmaceutical compounds in living systems. For that purpose, polymeric materials need to be labelled by suitable radionuclides and then investigated by means of special cameras.

This review focuses on recent developments of radiolabelled polymeric materials for application in cancer diagnosis and therapy. We provide a brief description of molecular nuclear imaging modalities using the nuclear techniques positron emission tomography (PET) and single-photon emission computed tomography (SPECT). Destruction of tumours with appropriate radionuclides is considered as well as customisation of diagnosis and therapy
with appropriate materials (theranostic approach). Targeting scenarios and strategies to evade mononuclear phagocyte system (MPS) trapping will be discussed. Emphasis is taken to critical evaluation of the approaches employed, evaluating both advantages and disadvantages of each approach – both from the methodical radiochemical point of view (how to radiolabel the carrier, which radionuclide is most suitable for the particular use) and biological efficacy point of view, which is largely dependent on the targeting efficacy of the carrier.

**Figure 1.** Examples of biological objects and polymeric materials.
2. Cancer imaging and treatment using radionuclides

Early diagnoses, precise monitoring of therapeutic treatment, quantitative imaging and efficient treatment of cancer require highly specific compounds with appropriate pharmacokinetic and pharmacodynamic properties as well as extremely sensitive detection methodologies. Concerning the latter, radiolabelling of cancer-seeking molecules with gamma- and positron-emitting radionuclides permit both high sensitivity and reliable information about \textit{in vivo} distribution using nuclear medicinal imaging techniques such as SPECT and PET (Figure 2). Today, about 80\% of all radio-diagnostic scans are performed by clinical SPECT imaging and about 20\% by PET imaging. In addition to the high sensitivity (nanomolar and even picomolar level) achieved with nuclear imaging techniques, these modalities are far superior with respect to the penetration depth of gamma photons compared to light photons used in fluorescence optical imaging.\textsuperscript{[40]}
Figure 2. Depiction of SPECT imaging (left) and PET imaging (right).

Elements with medically useful radionuclides are summarised in Figure 3. SPECT is still the most widely used imaging technique in the clinic. Dedicated SPECT cameras available in many hospitals allow the detection of gamma rays in an energy window between 100 and 360 keV. Due to appropriate nuclear decay properties, conventional availability and reasonable cost, $^{123}$I ($t_{1/2} = 13.3$ h), $^{111}$In ($t_{1/2} = 67.2$ h) and $^{99m}$Tc ($t_{1/2} = 6.03$ h) are the most applied gamma-emitting radionuclides in medicine. $^{99m}$Tc can be obtained from a generator containing $^{99}$Mo ($t_{1/2} = 66$ h), simply by eluting with saline solution, thus providing a constant supply of $^{99m}$Tc. The steady availability in combination with its ideal nuclear characteristics of 6 h half-life and gamma-ray emission energy of 141 keV has made it the most prevalently used radionuclide in nuclear medicine.\footnote{The other imaging-related radionuclides are}
positron emitters. That is, a positron is ejected from the atomic nucleus and is rapidly slowed down in the surrounding tissue (typical mean range in water < 1 mm) followed by annihilation with an electron, which is accompanied by the collinear emission of two 511 keV gamma photons. These two coincident photons are then detected by scintillation detectors arranged in a circular array, allowing for quantification of radiotracer distribution \textit{in vivo} as well as dosimetry estimations. The era of PET applications began with the use of short half-life isotopes $^{18}$F ($t_{1/2} = 109.7$ min), $^{15}$O ($t_{1/2} = 2.04$ min), $^{13}$N ($t_{1/2} = 9.96$ min) and $^{11}$C ($t_{1/2} = 20.4$ min) readily available in on-site cyclotrons.\cite{42} Today, $^{18}$F is the most prevalent radionuclide used for PET. However, the short half-life of 110 min precludes the investigation of biochemical processes in the range of days and weeks. This is especially important for polymers and nanoparticles, accumulation of which in solid tumours usually takes hours to days (see below). In this perspective, metallic positron-emitting nuclides are being more and more explored. This meets in particular $^{64}$Cu ($t_{1/2} = 12.7$ h) and $^{89}$Zr ($t_{1/2} = 78.4$ h) but also $^{44}$Sc ($t_{1/2} = 3.9$ h) and $^{72}$As ($t_{1/2} = 26$ h). Due to the availability of a $^{68}$Ge/$^{68}$Ga generator being able to steadily supply $^{68}$Ga, this short-lived positron-emitting nuclide ($t_{1/2} = 67.7$ min) has been also considered for PET imaging. A further precedence of metallic radionuclides compared to conventional positron-emitters discussed \textit{vide supra} is that many of these nuclides have radioisotopes, emitting energetic particles such as beta-particles ($\beta^-$), alpha-particles (\textalpha) and Auger electrons. Particle radiation emitted by radionuclides can be used to destroy cancerous cells and tissues. So-called matched pairs of radionuclides that allow for imaging as well as therapy are gaining in importance, preferably originating from the same element.\cite{43} Matched pairs utilising PET imaging and $\beta^-$ radiation are for instance $^{44}$Sc/$^{47}$Sc, $^{64}$Cu/$^{67}$Cu, $^{72}$As/$^{77}$As and $^{86}$Y/$^{90}$Y. $^{99m}$Tc/$^{186/188}$Re makes use of SPECT imaging and $\beta^-$ radiation. Iodine isotopes permit SPECT ($^{123}$I), PET ($^{124}$I) and therapy ($^{131}$I).
Figure 3. Periodic table to show elements useful for internal radiodiagnosis and radiotherapy.

In particular for therapeutic applications, the choice of the radionuclide is of outstanding importance. So, a therapeutically effective dose has to be transported almost solely to tumours and metastases, minimising radiation exposure to the normal tissue. For this purpose, especially Auger electron emitting nuclides such as $^{125}$I, $^{111}$In and $^{99m}$Tc seem to be ideal because they have a short range in biological tissue of some nm; that is much lower than 1 cell diameter.$^{[44-46]}$ However, the critical issue is that Auger emitters have to selectively reach the cell nucleus of the diseased tissue to be effective. Because of their extremely low range, they are highly biologically effective when internalised into the cell nucleus, where they cause unrepairable double-strand breaks of DNA.
Alpha emitters are also highly efficient to destroy cancerous tissue and metastases.\cite{47} They combine a short range in biological tissue up to 80 µm (2 - 3 cell diameters) with a high linear energy transfer (LET) between 50 and 230 keV/µm, \textit{e.g.} generating about $10^5$ ion pairs for alpha particles with an initial energy of 3.5 MeV.\cite{48} This kind of radionuclides finally causes many double-strand breaks of DNA and kills all cells affected. Meanwhile, $^{211}$At ($t_{1/2} = 7.2$ h), $^{213}$Bi ($t_{1/2} = 45.6$ min), $^{223}$Ra ($t_{1/2} = 11.4$ d) and $^{225}$Ac ($t_{1/2} = 10$ d) have also been used for α-particle therapy of cancer.\cite{49}

To date, however, mainly the β-emitting radionuclides $^{131}$I ($t_{1/2} = 8.02$ d) and $^{90}$Y ($t_{1/2} = 64$ h) are applied for radioimmunotherapy of human tumours.\cite{50} β-particles have much lower LET values (0.2 keV/µm) than α-emitters. They mainly generate single-strand breaks of DNA that are easier to repair comparing to double-strand breaks caused by alpha emitters. Due to the millimeter-range of high-energy β-particles of $^{90}$Y ($E_{\text{max}} = 2.28$ MeV) or $^{188}$Re ($E_{\text{max}} = 2.1$ MeV) the so-called “cross-fire effect” (in internal radiotherapy) allows cells devoid of radioactively labelled carriers to be irradiated and killed. This is particularly relevant to cancer cells which lack antigens, or which cannot be reached due to poor vascularization and intratumoural pressure in a bulky tumour. On the other hand, using a high-LET β-emitter to treat small tumours may cause damage to neighbouring healthy cells. This is a potential disadvantage. To protect healthy tissues from damage, radiometals of lower β-energy such as $^{177}$Lu ($E_{\text{max}} = 0.497$ MeV, $t_{1/2} = 6.7$ days) may be more suitable.\cite{43} Furthermore, besides its β-emission, $^{177}$Lu features a low abundance of photons of almost ideal energy (113 keV, 6.5%; 208 keV, 11%) for SPECT imaging and post-therapeutic dosimetry (Figure 4).
Figure 4. Patterns of cellular damage caused by radiation sorted by LET. a. Radionuclides undergoing beta-decay emit low LET radiation that produces sparse ionizations and excitations within DNA along a contorted track, resulting in individual DNA lesions that are easily repairable. b. Cascades of Auger electrons (with intermediate LET). Auger electrons that produce densely localized ionizations and excitations within DNA, inducing poorly repairable damage. In the context of radioimmunotherapy, ionizations localize mainly at the cell membrane if the antibody binds to cell-surface antigen. However, ionizations are found mainly in the cytoplasm if antibodies are taken up by the cell. c. Alpha-particles with high LET produce densely localized ionizations and excitations along a linear track, resulting in locally, multiple damaged sites that are poorly repairable. Abbreviation: LET, linear energy transfer. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Clinical Oncology, reference 48. Copyright by Nature Publishing Group 2011.
3. Radiolabelling procedures applied for polymers

Radiolabelled polymeric materials used for imaging and therapeutic purposes require optimal carrier systems that form highly stable compounds/complexes \textit{in vivo} with appropriate radionuclides as well as permit a rapid and unsophisticated labelling of biologically active vector molecules for a pharmaceutical targeting.

Radiolabelled polymers are frequently prepared to either follow the polymer properties \textit{in vivo} or to deliver the therapeutic doses of radioactivity to the targeted tissue by the polymer carrier.\textsuperscript{[51-52]} Methods of polymer radioiodination were mostly adapted from the strategies developed for radiolabelling of proteins. These must be rapid, mild and offer high radiochemical yields. Contrary to the labelling of proteins/antibodies, where the direct labelling of tyrosine residues is preferentially exploited, the labelling of polymers requires their functionalisation with ligands that are capable of radionuclide binding (indirect approach). Generally, there are two different labelling approaches that can be distinguished (Figure 5): (A) pre-labelling of bifunctional chelating agents (BFCAs) with radionuclides followed by their attachment to the polymer; (B) post-radiolabelling approach, where the polymers are decorated with radionuclide binding ligands, (\textit{e.g.}, by conjugation reactions or by copolymerisation of suitable monomers), followed by their radiolabelling.\textsuperscript{[53]}
Polymers labelled with radiometals (e.g., $^{99m}$Tc, $^{111}$In, $^{64}$Cu, $^{68}$Ga) are mostly used in biomedicine to track their biodistribution by SPECT or PET techniques. Alternatively, polymers can be used as carrier systems for therapeutic radionuclides (e.g., $^{90}$Y, $^{177}$Lu) to improve the biodistribution and pharmacokinetic properties. In contrary to the radiolabelled low-molecular-weight substances, where the bulkier chelator-metal complex frequently alters the biological properties of the parent molecule, radiolabelling of polymers usually do not significantly alter their biodistribution behaviour, because the attached complex does not represent a significant weight fraction of the polymer conjugate. Labelling of polymers with radiometals employs the use of efficient BFCAs that are linked to the polymers by appropriate spacer elements. There are numerous ‘tailor-made’ bifunctional chelator systems available for different radiometals and may also be utilised to modulate the biodistribution and pharmacokinetic properties of the radiolabelled compounds. To label polymers with radiometals, BFCAs are required to be capable of both, to bind the radionuclide efficiently
and to possess appropriate functional groups for the conjugation to the polymer (Figure 6 (A)). The most frequently used BFCAs rely on derivatives of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and diethylenetriaminepentaacetic acid (DTPA). Even though originally designed to bind lanthanides, DOTA demonstrates sufficient stability for other metals, e.g., $^{68}$Ga, $^{64}$Cu and $^{111}$In. However, heating is usually required to reach high radiolabelling efficiency particularly for radiolanthanide ions, which may limit its use when, e.g., thermosensitive polymers are involved. On the other hand, the formation of DTPA complexes with $^{111}$In or $^{99m}$Tc proceeds smoothly under mild conditions, which predetermined the use of DTPA as a preferred ligand for diagnostic purposes, despite the lower stability of DTPA complexes compared to those with macrocyclic chelators. For the labelling of polymers with $^{64}$Cu, more suitable macrocyclic chelators have been used such as 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA) and 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA). By now, more appropriate copper chelators are available based on hexamine cage ligands, bis(2-pyridylmethyl)-1,4,7-triazacyclononane (DMPTACN) and 3,7-diazabicyclo[3.3.1]nonane (bispidine). Due to the longer half-life compared to other PET nuclides used, $^{89}$Zr ($t_{1/2} = 78.4$ h) is attracting increasing interest particularly to study the pharmacokinetic behaviour of medium and high molecular mass polymers. Recently, desferrioxamine-based BFCAs for $^{89}$Zr have been reported with improved stability that permits reliable in vivo evaluation of polymeric materials. To be attached to the polymer, BFCAs contain a suitable functional group that can react with free amines (active esters, isothiocyanates), sulfhydryl- (maleimides, iodoacetamides), carboxylate- (amines, alcohols) or alkynyl- (azide) groups of the polymer to form stable polymer-chelator conjugates. Particularly for the latter approach, employing click-chemistry has attracted enormous attention in recent times. In this way, alkyne- and azide-decorated polymers can be efficiently functionalised with BFCAs using copper-
catalysed cycloaddition, strain-promoted cycloaddition and Diels-Alder reactions, respectively.\textsuperscript{[67]} Enzyme-mediated site-specific conjugation strategies, predominantly employed for the modification of antibodies, may pave the way to yield highly defined polymers equipped with appropriate metal chelators.\textsuperscript{[68-71]}

(A) Common bifunctional chelators

| DOTA-NHS | p-SCN-Bn-NOTA | TETA-NHS | p-NH$_2$-CHX-A"'-DTPA |

(B) Radioiodination of polymers

\[ \text{Bolton-Hunter's reagent} \]

\[ \text{Radioiodination via nucleophilic or electrophilic aromatic substitution is mostly implemented.} \textsuperscript{[72]} \]

\[ \text{The nucleophilic aromatic substitution reactions are facilitated by the activation of the aromatic ring with electron-withdrawing substituents, e.g., carbonyl or cyano groups.} \textsuperscript{[73]} \]

Figure 6: Methods of polymer radiolabelling. (A) Examples of common bifunctional chelators (B) Common procedures for radioiodination of polymers.

To radiolabel polymers with iodine, classical organic iodination procedures can be adapted. Radioiodination via nucleophilic or electrophilic aromatic substitution is mostly implemented.\textsuperscript{[72]} The nucleophilic aromatic substitution reactions are facilitated by the activation of the aromatic ring with electron-withdrawing substituents, e.g., carbonyl or cyano groups.\textsuperscript{[73]} The simplest nucleophilic radioiodination is the isotopic exchange, where
the non-radioactive iodine atom is replaced by radioiodine. However, relatively harsh reaction conditions (temperature, pressure) are required to obtain at least moderate radiolabelling yields.\textsuperscript{[74]} However, the isotopic exchange is not suitable for the synthesis of compounds with high specific activity. Apart from iodine, more suitable leaving groups for the nucleophilic radioiodination are bromide, and sulfonates, \textit{e.g.}, tosylate, triflate, mesylate and nosylate. In this case, the radioiodinated compounds can be separated from the precursor compounds by chromatographic techniques (HPLC, silica cartridges).\textsuperscript{[75]} Using non-activated aromatic compounds, the nucleophilic radioiodination must be catalysed by transition metals, \textit{e.g.}, \text{Cu}^I or \text{Pd}^{II}.\textsuperscript{[76]} Contrary to the nucleophilic substitution, the high electron density of the aromatic ring enhances the rate of the electrophilic substitution. This can be achieved by activation of the aromatic ring by electron-donating groups, \textit{e.g.}, hydroxyl and phenolic moieties. The electrophilic radioiodine species can be generated from sodium radioiodide by numerous oxidising agents. The most frequently used oxidisers are peracetic acid, chloramine-T, Iodogen\textsuperscript{®} and \textit{N}-halosuccinimides.\textsuperscript{[75]} The use of aromatic precursors such as trialkylstannyl, trialkylsilyl or boronic acid derivatives also provides radioiodinated polymers in high yields.

The most applied labelling method for radioiodination is the direct electrophilic iodination of tyrosine residues (\textbf{Figure 6(B)}). This approach was originally developed for radioiodination of proteins since most proteins contain at least one tyrosine residue. For the labelling of polymers, appropriate amounts of tyrosine-containing monomers can be added to the polymerisation mixture.\textsuperscript{[77]} For example, \textit{N}-\text{methacroyl}methacryloyl-L-tyrosine amide monomers are often used to synthesise methacrylamide-type polymers eligible for radioiodination.\textsuperscript{[78]} Alternatively, polymers can be decorated with tyrosine moieties after the polymerisation. However, this direct method is not applicable, when functional groups of the polymer interfere with the radiolabelling agent. In this case, a two-step indirect
radioconjugation protocol can be used, involving the reaction of the polymer with iodinated precursors. These agents contain functional groups, which permit the rapid, unsophisticated conjugation to the polymers.[77] The most commonly used radioiodinated conjugation agent is the Bolton-Hunter reagent, \textit{i.e.}, radioiodo-labelleld \textit{N}-succinimidyl-3-(4-hydroxyphenyl)propionate.[79]

Radiofluorination of polymers with \textsuperscript{18}F mostly relies on the use of \textsuperscript{18}F-labelled prosthetic groups for coupling to amine, hydroxyl, carboxylate and maleimide groups.[42, 80-81] Recently, clickable reagents have been developed that may facilitate the preparation of \textsuperscript{18}F-labelled polymers.[82]

4. Tumour targeting with polymers

It is fairly challenging to exclusively achieve a pharmaceutical/biological targeting of tumour cells. Until now, several targeting vector molecules have been used, comprising of both relatively small molecules such as specific peptides, oligosaccharides, oligonucleotides, peptide mimetics, and larger constructs such as antibodies and antibody fragments.[83-84]

Due to size, shape and functionalities, especially larger polymers can be passively accumulated in the tumour tissue through the enhanced permeability and retention (EPR) effect first described by Maeda et al.[32] This seminal work inaugurated the era of polymeric therapeutics, including liposomes and polymer-drug conjugates, for cancer treatment.
Figure 7. The enhanced permeability and retention (EPR) effect. In normal tissue (yellow-orange cells), endothelium of the vessels (pink cells) is almost impermeable for polymers. Leaky fenestrated endothelium in the tumour (grey cells) allows extravasation of the polymer from the blood vessels.

To supply the fast growing tumour with blood, the tumour vascularisation proceeds rapidly. This causes an irregular structure of the tumour blood vessels. Their leaky architecture allows permeation of polymers to the cancerous tissue from the bloodstream (not possible in the healthy blood vessels). Furthermore, lymphatic drainage of the tumour is usually disordered or missing, preventing the rapid efflux of polymers from the tumour environment. As a consequence, the concentration of polymers in tumour vascularisation may increase up to one to two orders of magnitude compared to healthy tissues (Figure 7).\textsuperscript{85} The passive targeting is quite general for numerous solid tumours because it relies on characteristic properties of the neoplasia which is very similar for many tumours. The extent of accumulation usually rises gradually with the size of nanoparticles and the cut-off size of
Pores in tumour vessels is 200 nm - 1.2 µm.[86] The maximum therapeutic effect was reported for the particles of 50 - 200 nm in diameter.[87] The accumulation of sub-100 nm polymeric micelles in poorly permeable tumours has been studied in detail, unveiling that the size of polymers is of fundamental importance.[88] In addition to the size, surface charge and shape of nanoscale materials remarkably influence the pharmacokinetic and tumour accumulation characteristics in vivo.[89-93] Furthermore, specific manipulation of either local tumour or systemic conditions can significantly increase nano-sized drug delivery.[94-95] Very recently, Kobayashi et al. could show that after initial near-infrared photoimmunotherapy of tumours the EPR effect is drastically enhanced and termed this phenomenon super EPR (SUPR) effect.[96-97]

All in all, tumour vasculature targeting through the EPR effect is the most exploited principle for polymeric materials, taking as a basis of numerous drug delivery and controlled release systems.[33-34,98-102] However, it has to be mentioned that the EPR effect is a common phenomenon particularly in rodent models using subcutaneous and orthotopic xenografts. In contrast, its occurrence in human tumours may be less widespread and less pronounced.

Improved targeting efficiency and in particular prolonged residence time of polymeric materials in tumours can be achieved by the specific interaction of tailor-made polymers with biomarkers on the surface of tumour endothelial cells. In this regard, the most relevant targeted moieties are the vascular endothelial growth factor (VEGF), vascular cell adhesion molecule 1 (VCAM-1) and αvβ3-integrin cell adhesion receptors.[103-109] Specific antibodies and their fragments, as well as small peptidic units, are frequently used as recognition units. For example, radiolabelled single-chain VEGF-based probes can be utilised for molecular imaging of angiogenic vasculature.[110] Nanomaterials equipped with cyclic peptides, containing the Arg-Gly-Asp (RDG) amino acid sequence, are efficient targeting elements and tissue penetration devices for αvβ3-integrin cell adhesion receptors.[111]
Specific receptors that are overexpressed on the surface of cancer cells represent prominent clinical targets. In this context, somatostatin receptors play an important role that can be targeted by stabilised somatostatin peptides termed octreotide and octreotate.\textsuperscript{[112-113]} Further prominent peptides for specific targeting of receptors located on the surface of cancer cells are bombesin, neurotensin, vasoactive intestinal peptide (VIP), α-melanocyte stimulating hormone (α-MSH), substance P and neuropeptide Y.\textsuperscript{[114-115]} Such small peptides are capable of achieving cancer cells \textit{in vivo}. However, there are numerous studies with polymeric materials decorated with specific peptides that show that this targeting strategy works only \textit{in vitro} but not \textit{in vivo}. The reason for this finding is that in particular, larger polymers have to overcome biological barriers such as the mononuclear phagocyte system, dense collagen matrix, high interstitial fluid pressure, etc.\textsuperscript{[116]}

The development of polymeric systems featured to target the surface of tumour cells is an emerging and rapidly growing field, but frankly very challenging. One strategy is the use of very small polymers, preferably oligomers and multimers such as hydrophilic dendritic moieties. However, it has to be ensured to keep the compounds small enough even after the functionalisation with targeting vector molecules.\textsuperscript{[117]} On the other, larger constructs trapped in the tumour vasculature can be degraded into small vehicles capable of migrating to the tumour cells. This can be achieved by using of biodegradable polymers like polysaccharides,\textsuperscript{[118]} synthetic block copolymers interconnected with biodegradable bonds\textsuperscript{[119]}, enzyme-mediated and stimuli-driven self-immolating systems\textsuperscript{[120-125]} as well as shell cleavable dendritic polyglycerol derivatives.\textsuperscript{[126]}

5. Strategies to evade MPS trapping

One of the major limitations of an efficient tumour targeting with non-biocompatible polymeric systems, particularly larger than 100 nm, is their rapid body clearance by the
mononuclear phagocyte system (MPS), previously known as reticuloendothelial system (RES). The MPS is a part of the immune system composed of phagocytic cells (e.g., monocytes, macrophages), resulting in unwanted uptake of polymeric drug delivery systems (DDs) in the spleen, liver and the lymphatic system. The process begins with the opsonisation of DDs (Figure 8), involving absorption of blood proteins (e.g., immunoglobulins, serum albumin) on the surface of these nano-objects. As a next step, the polymer-protein complex formed is recognised by phagocytic cells as xenobiotic and is rapidly internalised in the phagosomes. After fusing with lysosomes, protein complexes of exogenous nanomaterials are subjected to decomposition by lysosomal digestive enzymes.[127-129]

To decrease the uptake of nanomaterials by the MPS, different strategies have been proposed. The mostly utilised strategy is the grafting of biocompatible hydrophilic polymers on the surface of the exogenous materials, whereby a hydration layer is formed that prevents/minimises opsonisation and thus, prolongs the plasma circulation lifetime of DDs.[130] In this regard, the most prevalently used polymer is poly(ethylene oxide) (PEO; PEG is also commonly used), providing good stealth and solubility properties. However, there are also some serious drawbacks of PEOylation, e.g., induced hypersensitivity and degradation under stress.[131] It was also shown that PEOylated liposomes are cleared from the body by so-called accelerated body clearance (ABC). The mechanism of this phenomenon is not yet fully understood, but involves the production of anti-PEO IgGs caused by the first administered dose of PEOylated liposomes.[132] The production of anti-PEO antibodies became widespread in the last 20 years among the western population mainly due to excessive overuse of PEO-based non-ionic detergents in everyday life. Therefore, alternatives to PEO have also been widely studied in the recent years.[133] This includes systems’ based on poly(vinylpyrrolidone) (PVP),[134] poly[N-(2-hydroxypropyl)methacrylamide] (PHPMA), poly[N-(2-hydroxypropyl)methacrylamide]
(PHPMA), poly(2-oxaline)s (POXs)\textsuperscript{135} and biopolymers such as polysaccharides.\textsuperscript{136-137} From these, PVP is reported to be inferior to PEO in terms of circulation time and immunogenicity, whereas PHPMA and POXs sustain their stealth properties, and additionally offer broad variability relating to the synthesis of multifunctional (co)polymers on the surface. As an example, modification of liposomes with PHPMA resulted in their prolonged circulation and reduced MPS uptake. Opsonisation and sequestration by MPS is significantly influenced by the surface charge of nanomaterials.\textsuperscript{138} To evade MPS trapping, neutral and slightly negatively charged vehicles are superior to positively charged ones particularly with regard to protein corona formation, resulting in prolonged blood circulation times.\textsuperscript{139-140} Recently, zwitterionic polymers gained attention to avoid non-specific protein adsorption.\textsuperscript{89, 141-142} These electrically neutral polymers are composed of both negatively and positively charged moieties, either within the same monomer or on different monomers (polyampholytes). The surface of zwitterionic polymers favours the formation of a highly stable hydration shell, preventing protein adsorption and thus yielding in stealth properties. Noteworthy, even slight excess of cationic or anionic groups may significantly influence both biodistribution and cellular uptake behaviour of polyzwitterions. Several studies confirmed high protein repellence of zwitterionic polymers. As an example of an organic-inorganic hybrid, gold nanoparticles equipped with poly(2-methacryloyloxyethyl phosphorylcholine) showed remarkably decreased adsorption of serum albumin compared to those coated with PEO.\textsuperscript{143} A similar behaviour was observed in the case of coating gold with poly(carboxybetaine methacrylate).\textsuperscript{144} Smart micellar nanoparticles composed of amphiphilic block polymers for the controlled release of doxorubicin have been reported by Yuan et al.\textsuperscript{93} These zwitterionic polymeric nanoparticles showed reduced serum protein adsorption and prolonged blood circulation, resulting in enhanced tumour accumulation. At a slightly acidic pH of 6.8 that is typical of tumour environment, the neutral surface turned
positive and thus, promoted the cellular internalisation \textit{in vivo}. All in all, coating of nanoparticles with zwitterionic polymers is a very promising strategy to produce stealth vehicles with a low degree of ‘off-target’ binding. Furthermore, it is worth mentioning that innovative approaches based on nanomaterials coating with „self“ peptides\textsuperscript{[145]} as well as membrane compartments of red blood cells\textsuperscript{[146]} and autologous leukocytes\textsuperscript{[147]} are under development, aiming to inhibit phagocytic clearance and to enhance tumour specific drug delivery.

\textbf{Figure 8}: Mechanisms of the mononuclear phagocyte system clearance of non-biocompatible polymeric nanoparticles. Upon exposure to blood, non-biocompatible polymers absorb blood proteins such as IgG antibodies, and such complexes are then recognised and scavenged by mononuclear phagocyte system cells.
6. Radiolabelled natural polymers for cancer imaging and therapy

Natural polymers are macromolecules derived from nature and can be categorised into polysaccharides, polynucleotides and proteins. Their inclusion in theranostic applications has various advantages such as high biocompatibility, biodegradability (biological environment-friendly degradation), availability of many conjugation sites; scope for surface modification to a certain degree, etc.

Biopolymer based nanocarriers normally induce less immunogenic effects and may also overcome biological barriers in the body to reduce retention of nanocarriers by the MPS in vivo.\textsuperscript{148} With the help of genetic engineering, they can be customised and mass produced without losing their inherent biological functions.\textsuperscript{149-150} Thus, biopolymers with required functional groups or targeting moieties could be easily achieved based on application intended requirements. Here, we report the most common radiolabelled biopolymers namely, albumin, alginate, chitosan, dextran, gelatin and heparin, (Figure 9) which are described below.
**Figure 9.** A general overview of radiolabelled biopolymers and their hybrids used in tumour imaging and therapy.

6.1 *Natural polymers for imaging*

**Albumin**

Owing to its abundance in blood, Albumin is a well-known macromolecule exhibiting intriguing properties of long half-life and non-immunogenicity. Due to these remarkable properties, especially human serum albumin (HSA) and bovine serum albumin (BSA) has driven extensive research in (radio)pharmaceutical applications.[151-159] 99mTc-labelled macroaggregated (MAA) albumins are commercially available as SPECT imaging agents such as 99mTc-Pulmolite® and 99mTc-HSA microspheres B20® for lung perfusion imaging; 99mTc-Albures® useful for liver and spleen imaging and 99mTc-Nanocoll® as well as 99mTc-Nanotop® for bone marrow and sentinel lymph node (SLN) imaging.[160]

Different strategies can be employed for efficient radiolabelling of albumins to achieve the desired stability and suitable pharmacokinetic properties either by the introduction of both macrocyclic and acyclic chelators (DOTA, DTPA) as well as via direct labelling approaches.[161-164] For instance, Choi et al. developed a highly efficient method for 125I-labelling using the inverse-electron-demand Diels-Alder reaction between trans-cyclooctene-functionalised HSA and a 125I-containing tetrazine component.[165] This technique overcomes the disadvantage of traditional radioiodine labelling methods for proteins such as accumulation of non-specific bound radioiodine in the thyroid. With regard to radiometal labelling, Zeng et al. introduced an automated microfluidic radiolabelling system for BSA with PET nuclides such as 64Cu and 68Ga using DOTA and NOTA as BFCAs.[166]
Irrespective of the choice of appropriate BFCAs as well as suitable labelling methods, a major focus has also been set on final product application such as engineering HSA microspheres surface for slow proteolytic degradation and appropriate passive tumour accumulation. HSA nanoparticles can also be decorated with active targeting moieties such as single-chain antibody fragments (scFv), nanobodies, affibodies, folic acid, luteinizing hormone releasing hormone (LHRH), utilising overexpressed receptor-mediated targeting. In addition, drugs can be loaded to augment their tumour localisation further. Jain et al. developed $^{99m}$Tc-labelled methotrexate-loaded HSA. To render them long-circulating, folic acid as a targeting moiety was conjugated through a hydrophilic PEO spacer. The *in vivo* results clearly demonstrated a tumour-specific localisation upon folic acid conjugation and PEOylation. These targeted NPs also inhibited the tumour growth more efficiently, confirming the therapeutic potential of such multifunctional systems.

Recently, Farkas et al. synthesised albumin nanoparticles with folic acid as targeting vector and conjugated them with NODAGA(1,4,7-triazacyclononane-1-glutaric acid-4,7-acetic acid) as a chelator. The folate-based radioconjugates ($^{64}$Cu, $^{68}$Ga) exhibited high accumulation in KB tumours of mice with 14.5% ($^{64}$Cu) and 11.9% ($^{68}$Ga) of injected activity per gram. Interestingly, this labelling strategy was found to yield enhanced *in vivo* stability compared to $^{64}$Cu-DOTA-conjugates, which showed a high liver uptake most likely due to the release of $^{64}$Cu. As an alternative system to overcome the issue of transchelation/demetalation *in vivo* of radiometal-chelator complexes, Gao et al. developed ultra-small ‘chelator-free’$^{64}$Cu-labelled BSA nanoclusters with the tumour targeting peptide LHRH for fast and sensitive diagnosis of lung cancers (Figure 10). These renally excretable nanoclusters showed high biocompatibility and four times higher tumour deposition as compared to the non-targeted control. Similarly, multi-functional HSA-based formulations are also currently pursued combining dual modalities such as PET/OI (optical
imaging) or SPECT/OI systems using radionuclides along with optical dyes incorporated via non-covalent interactions or covalent conjugation techniques. These strategies can also be employed to investigate the metabolic fate of such systems in vivo.

**Chitosan**

Chitosan is a linear polymer composed of β-(1→4)-linked D-glucosamine units with residual N-acetylation, determining its water solubility with latter increasing its solubility. This positively charged polysaccharide possesses favourable properties including biodegradability, low toxicity, easy functionalisation etc., which may be efficiently utilised for cancer diagnosis as well as therapy. Unlike albumin, radiolabelling strategies in case of chitosan need to be carefully considered to avoid the formation of large aggregates that causes increasing MPS uptake in vivo. Various formulations based on radiolabelled chitosan have been reported, which make use of actively targeting moieties such as antigen subunits, VEGF and specific analogous peptides. These chitosan-based nanoplatforms, incorporating anticancer drugs such as methotrexate have been reported as effective tumour therapeutics, and can simultaneously be used as guiding agents (99mTc/131I-labelled) to tailor therapeutic dosing.

Exploring its biodegradability, targeted imaging approaches have also been exploited with distinct water-soluble chitosan derivatives as SPECT/PET imaging agents. In this regard, 99mTc-labelled chitosan derivatives have been used for imaging of liver tumours and metastases. Hawary et al. reported specific tumour accumulation, using 99mTc-labelled lactosaminated N-succinyl-chitosan (LNSC) as water-soluble chitosan derivatives. Biodistribution data showed a long-term retention in liver followed by its degradation via kidney over time. Similarly, water-soluble carboxymethyl-chitosan (CMC) and micellar N-lauryl-carboxymethyl-chitosan (LCMC) exhibited partial excretion via kidneys and accumulation in the liver. Such chitosan derivatives are superior to proteins/peptides that
degrade in liver rapidly and thus may be utilised as liver-targeted agents for (radio)therapeutic applications with low accumulation in non-targeted tissues.\textsuperscript{[192-193]} Akhlaghi et al. optimised the biodistribution profile of \textsuperscript{66}Ga-labelled DTPA-chitosan derivatives (\textsuperscript{66}Ga: $t_{1/2} = 9.5$ h is better suited to investigate longer periods as compared to the short-lived \textsuperscript{68}Ga) as a function of the number of chelators, specific activity and concentration of carriers by intratumour administration in fibrosarcoma bearing mice. DTPA-modified chitosan (DTPA degree: 10.3\%) showed the highest efficiency to prevent \textsuperscript{66}Ga-leakage. 97\% of the injected dose remained in the tumour, even after 54 h with low uptake in lungs, liver, spleen and kidneys.\textsuperscript{[194]} Recently, Lee at al. reported a facile method to label PEOylated-chitosan nanoparticles with \textsuperscript{64}Cu, applying copper-free click chemistry (SPAAC = strain promoted alkyne-azide cycloaddition) using azide-functionalised chitosan and DOTA derivatives with appending dibenzyl cyclooctyne moieties. This formulation allowed for fast labelling (within 30 mins) with high radiolabelling yield (98\%) and showed a tumour uptake plateau (6.2\% ID/g) at 24 h p.i. in A549 tumour-bearing mice mainly attributed to the EPR effect. However, a high uptake was also found in the kidneys, liver and the spleen. Using the same approach, the dibenzyl cyclooctyne-containing cyanine dye Cy5 attached to an activatable matrix metalloproteinase (MMP)-sensitive peptide was clicked to the azide-functionalised chitosan particles to yield a dual-labelled probe capable of combining PET with optical imaging. Such dual probes provide reliable data of biodistribution and pharmacokinetic properties as well as additional biological information on tumour microenvironment.\textsuperscript{[195-196]}
Figure 10. *In vivo* imaging and biodistribution. Representative PET images of coronal single slices on orthotopic A549 lung tumor bearing mice after intravenous injection of 6.7 MBq of $[^{64}\text{Cu}]\text{Cu NC@BSA}$ (a) and $[^{64}\text{Cu}]\text{CuNC@BSA-LHRH}$ (b). Images were acquired at 0.5, 1, 2, and 4 h. White arrows indicate the lung tumor. Corresponding whole body three-dimensional (3D) PET reconstruction images are shown in supplementary videos 1-4 (after intravenous injection of $[^{64}\text{Cu}]\text{CuNC@BSA}$) and videos 5-8 (after intravenous injection of $[^{64}\text{Cu}]\text{CuNC@BSA-LHRH}$). (c) Corresponding organ biodistribution of $[^{64}\text{Cu}]\text{CuNC@BSA}$ and $[^{64}\text{Cu}]\text{CuNC@BSA-LHRH}$ at 4 h after iv injection in mice bearing orthotopic A549 lung tumor, calculated by $\gamma$-counter (n=3). L= Lung: left lung; R-lung: right Lung. (Asterisk (*) denotes statistical significance, *, $p < 0.05$). Reprinted by permission from ACS Nano, reference 176, copyright American Chemical Society 2015.

**Dextran**

Derivatives of dextran are non-toxic and biocompatible, which is favourable for pharmaceutical applications. Furthermore, the terminal hydroxyl groups render them water-soluble and neutral as well as provide several attachment sites for biomolecules, linkers, etc.
Since the past decade, radiolabelled dextran derivatives have been exploited as blood pool agents as well as tumour imaging agents. These applications were further extended on the modification of monoclonal antibodies, whereby dextran acts as a linker to a radiolabelled moiety and the biomolecule, conferring a prolonged half-life while preserving its enzymatic activity. An anti-VEGF receptor 2 antibody DC10 conjugated to $^{99m}$Tc-DTPA-dextran provides better images compared to the non-conjugated antibody.

Dextran derivatives have been applied as lymphatic mapping agents, for example, to predict breast cancer staging, which was found to be 100% sensitive and surpassed blue dye staining in human patients. Vera et al. reported benzoyl-mercaptoacetylglucylglycyl-glycine-mannosyl-dextran (MAG$_3$-mannosyl dextran) for sentinel node detection via targeting the DC-SIGN receptor on macrophages and dendritic cells, whereby the MAG$_3$ chelation system was used to ensure high $^{99m}$Tc-labelling stability. Furthermore, it was demonstrated that mannosyl-dextran (termed Lymphoseek/Tilmanocept) could be employed as SLN imaging agent for mannose receptor targeting. This formulation exhibited favourable SLN imaging properties such as high signal to background ratio, high receptor affinity and fast clearance from the injection site as compared to Nanocoll. It was proved to be effective in clinical studies (phase 1 and 2), and a clinical phase 3 study with oral cavity squamous cell carcinoma patients confirmed the detection and accurate prediction of the pathologic nodal status with 0% false negative results. Lymphoseek/Tilmanocept formulations were also found useful to guide invasive surgery for colon cancer, which was then, also confirmed for stomach, gastric, colon and prostate cancers in pigs. Based on the Tilmanocept approach, pyrazolyl-diamine (pz), cysteine and dicysteine derivatives were prepared in order to improve the stability of the $^{99m}$Tc-labelled dextran. Recently, dual probes of 10 kDa-dextran-based polymers equipped with DOTA ($^{68}$Ga-labelling) and pyrazol-diamine ($[^{99m}$Tc(CO)$_3$]$^+$-labelling) as well as an NIR-fluorophore were reported for
SLN imaging (Figure 11, compound 5a: Dx-Man-$^{99m}$Tc-IR775; compound 6a: Dx-Man-$^{68}$Ga-IR775). \[^{[227]}\] Besides SLN mapping, $^{99m}$Tc-labelled dextran derivatives are also known as efficient carrier systems for hydrophobic drugs. \[^{[228-230]}\] Altogether, dextran derivatives play an important role as radionuclide/drug/fluorophore carriers for cancer imaging/treatment as well as modifiers to improve the pharmacokinetic and pharmacodynamics properties of other NPs platforms.

Other radiolabelled natural polymers based on gelatin, heparin, collagen, etc. have also proven to increase the half-life of the conjugates or act as passive carriers to increase the tumour targeting. \[^{[231-234]}\]

**Figure 11.** A. Planar gamma-image of a Wistar rat injected with 5a at 60 min p.i. (a). SPECT/CT image of the same animal at 90 min p.i. (b) B. NIR optical images of Wistar rat leg injected with 5a (left,180 min p.i.) or 6a (right, 90 min p.i.) respectively. The yellow arrows indicate the localization of the bimodal probes in the popliteal lymph node. Reprinted by permission from Bioconjugate Chemistry, reference 227, copyright American Chemical Society 2014.
6.2 Natural polymers for therapy

Natural polymers can be used in cancer therapy to deliver therapeutic radionuclides and to transport anticancer drugs. In regard to the former, albumin has been radiolabelled with $^{188}$Re, $^{90}$Y and $^{177}$Lu for therapeutic applications. Du et al. reported about $^{188}$Re-labelling of cysteine-containing dextran using $^{188}$Re-gluconate as a precursor. However, only a modest stability was obtained, which could be improved in the presence of antioxidants as ascorbic acid.[235] $^{188}$Re-labelled HSA microspheres B20 (about 25 µm) were found to have sufficient in vitro stability in human plasma, blood and saline (> 88% of $^{188}$Re was bound after 30 h on the microspheres). For an easy handling, a kit preparation procedure was developed.[236] A similar approach was carried out with DOTA-functionalised HSA microspheres using $^{90}$Y and $^{177}$Lu as therapeutic radionuclides. However, the radiolabelled microspheres were found to be less stable and undergo a radiation-induced cleavage.[237]

Chitosan has also been used as a carrier for particle-emitting radiometals. Suzuki et al. synthesised a $^{166}$Ho-chitosan complex for local radiotherapeutic application by intrahepatic, intratumoural and intravenous administration in B16 melanoma mice. The biodistribution data showed high localisation of the radioactivity at the injected site in either liver or tumour followed by a low uptake in lungs, spleen and bone.[238] This finding is most likely due to the spontaneous demetalation of the weak $^{166}$Ho complex formed with chitosan. Similarly, $^{153}$Sm/$^{166}$Ho-amino acid-chitosan complexes were used in internal radiation therapy. Marques et al. reported on the fabrication of chitosan derivatives with appending amino acids (valine and aspartic acid). These water-soluble chitosan-amino acid conjugates form stable complexes with $^{153}$Sm and $^{166}$Ho, and are discussed as potential candidates for liver-targeted radionuclide therapy.[239] $^{166}$Ho-labelleld alginate microspheres were also employed for localised radiotherapy.[240]
Recently, Lee at al. described the preparation of \(^{131}\)I-labelled chitosan microhydrogels loaded with doxorubicin. These microgels showed significant synergistic therapeutic effects of radiation \((^{131}\)I) and chemotherapy (doxorubicin) on mouse breast cancer models.\(^{[241]}\) 99mTc-labelled chitosan conjugated to a glycopeptide was used to image the tumour uptake in breast tumour-bearing rats. Such chitosan-glycopeptide conjugates show interesting pharmacokinetic properties and may be useful as carrier systems for anticancer drug delivery.\(^{[242]}\)

For targeted radionuclide therapy, specific localisation of radioactivity in diseased tissue is crucial in order to achieve a high therapeutic effect as well as to avoid systemic side effects. In this regard, different combinations of biopolymers, as well as thermosensitive synthetic-natural co-polymeric systems, are under development.\(^{[243-247]}\)

7. Radiolabelled synthetic polymers for cancer imaging and therapy

Advances in polymer science have resulted in the development of synthetic polymers which can be ‘tailored for the need’ with advanced properties that can be engaged in their development as drug delivery devices or theranostic applications. Synthetic polymers such as poly(ethylene oxide) (PEO), poly(glutamic acid) (PGA) and poly(lactic-co-glycolic acid) (PLGA) have already been approved for clinical use in macroformulations. Other examples include \(N\)-(2-hydroxypropyl) methacrylamide (HPMA) and styrene-maleic acid/anhydride (SMA) copolymers. These structures can be easily modified to permit their extensive use in drug delivery, imaging and/or targeting. More complex structures such as polymeric assemblies like liposomes, micelles, polymersomes or polymer-protein conjugates have also been reported as drug delivery devices (DDD) and/or tumour targeting systems (Figure 12).\(^{[17, 248]}\) They can be passively accumulated in the tumour tissue due to the EPR effect up to 100 times higher than in the surrounding tissue.\(^{[85, 249]}\) Moreover, by bundling imaging
agents along with drugs, analysis of drug distribution as well as its release at the target site can also be achieved in real time. Polymeric nanoparticles, therefore, play a vital role in the architecture of many passively as well as specifically-accumulated drug delivery and diagnostic systems.

![Polymeric nanoparticles](image)

**Figure 12.** Architectures of polymeric systems for tumour imaging and therapy.

### 7.1 Synthetic polymers for imaging

Radiolabelled linear hydrophilic biocompatible polymers represent the simplest synthetic polymer-based cancer imaging systems, whereby the tumour accumulation can be boosted with increasing chain length. However, a narrow distribution of the polymer molecular weights is crucial to assure a reproducible biodistribution profile.

**PHPMA** Poly[N-(2-hydroxypropyl)methacrylamide]

In this regard, PHPMA is a polymer with great potential for both cancer imaging and therapy.\(^{(250)}\) Its molecular weight can be precisely adjusted by changing the polymerisation conditions, whereas diverse functional/targeting groups can be incorporated either by copolymerisation of suitable (co)monomers or by derivatisation of the chain end groups. Linear HPMA polymers have been radiolabelled with radionuclides such as \(^{131}\)I and \(^{18}\)F for
prostate cancer tumour imaging or ADME studies.\textsuperscript{251-252} Lammers et al. explored the potential of $^{131}$I-labelled HPMA loaded with the antitumour drugs doxorubicin and gemcitabine (putative radiosensitiser) to study the theranostics capabilities of these long circulating systems (\textbf{Figure 13}).\textsuperscript{253} Comparing two different molecular weights (31 kDa and 65 kDa) of $^{131}$I-labelled HPMA polymers, the 65 kDa polymer showed an almost three-fold increase in tumour accumulation attributed to its long blood circulation followed by an increased uptake in the spleen (\textbf{Figure 14}).\textsuperscript{253-254} Further, the functionalisation of these polymers with cationic or anionic groups resulted in their decreased tumour accumulation.\textsuperscript{255} This effect was more profound in the case of the positively charged polymer.
Figure 13. Drug targeting improves doxorubicin (Dox)-based radiochemotherapy. (A) Growth inhibition of Dunning At1 tumours induced by three intravenous (i.v.) injections (days 1, 8, and 15; see vertical arrows) of saline, of free doxorubicin and HPMA copolymer-bound doxorubicin. PK1: pHMPA- GFLG-Dox (28 kDa). IgG-PKI: human IgG- modified pHMPA-GFLG-Dox (900 kDa). Values represent average ± s.e.m. (n= 6-12). *Indicates P<0.05 vs control (Mann-Whitney U-test; Bonferroni-Holm post hoc analysis). (B) Tumour growth inhibition induced by three i.v. injections of the abovementioned chemotherapeutic agents in combination with a clinically relevant regimen of fractionated radiotherapy (20 X 2 Gy; see vertical lines). Values represent average ± s.e.m. (n= 8-10). *Indicates P<0.05 vs control, # indicates P<0.05 vs free Doc, and + indicates P<0.005 vs free Dox (Mann-Whitney U-test; Bonferroni-Holm post hoc analysis). (C) Representative images (day 50) of tumours treated with the indicated combination regimens. (D) Weight loss induced by doxorubicin-based combined modality therapy. Values represent average ± s.e.m. (n= 4-5). Reprinted by permission from Macmillan Publishers Ltd on behalf of Cancer Research UK: British Journal of Cancer, reference 253, copyright Nature Publishing group 2008.
Figure 14. HPMA copolymers localise to tumours both effectively and selectively. (A) Scintigraphic analysis of the biodistribution of two differently sized iodine-131-I labelled HPMA copolymers in Copenhagen rats bearing subcutaneously transplanted Dunning AT1 tumours, demonstrating prolonged circulation and effective tumour accumulation (H: heart (blood), B: bladder, S: spleen, L:Liver, T: tumour. (B) Analysis of the blood concentrations of the two radiolabelled copolymers. Values represent average ± s.d. (n=6). (C) Quantification of the tumour and organ concentrations of the two radiolabelled copolymers at 24 and 168h post intravenous injection. Values represent average ± s.d. (n=6). Except for lung and spleen, concentrations in tumours were always significantly higher than those in healthy organs (P<0.05; two-tailed t-test). (D) Quantification of the tumour-to-organ ratios of the copolymers analysed in (C), pointing out (in green) that they accumulate more selectively in tumours than in seven out of nine healthy tissues. Reprinted by permission from Macmillan
Tumour accumulation can be influenced by fine-tuning various physicochemical characteristics such as charge, hydrophobicity (mainly by attachment of drug) and by increasing the size for passive targeting, which is often followed by an unwanted MPS accumulation.[256-257] Thus, in order to evade the MPS uptake, Zhang et al. developed degradable polymers based on PHPMA to achieve a longer blood circulation without impairing their biocompatibility, and thereby, increasing their therapeutic/targeting efficiency. These second generation PHPMA-epirubicin (2P-EPI) polymers were decorated with cleavable sequences at both the polymeric backbone and the side chains.[258] Dual-labelling of the polymer and the drug with $^{125}\text{I}$ and $^{111}\text{In}$ ($^{111}\text{In} \cdot \text{2P-EPI} \cdot ^{125}\text{I}$ or $^{125}\text{I} \cdot \text{2P-EPI} \cdot ^{111}\text{In}$) did not affect the tumour targeting, indicating that the labelling strategy does not significantly alter the pharmacokinetics of HPMA copolymers.

Active targeting of polymeric systems can also contribute to an enhanced tumour accumulation, leading to improved tumour imaging. As an example, Schieferstein et al. reported the increased tumour uptake of folate-targeted $^{18}$F-PHPMA copolymers in Walker-256 mammary carcinoma-bearing mice compared to the non-targeted ones.[259] In one of the few clinical trials with polymer-drug conjugates in human patients with liver cancer, the organ distribution of $^{123}\text{I}$-labelled HPMA polymers, bearing doxorubicin and galactosamine as a targeting unit was determined using SPECT imaging.[260] 24 hours after administration, 16.9 ± 3.9 % of the administered dose of doxorubicin targeted to the liver and 3.3 ± 5.6 % of dose was accumulated in the tumour. Interestingly, doxorubicin-polymer conjugate without galactosamine showed no tumour targeting. In another study, $^{64}$Cu-DOTA-labelled PHPMA targeted with c(RGDyK) oligopeptide was used as a theranostic scaffold for cancer imaging.
by PET.\textsuperscript{[261]} Again, the tumour uptake of actively targeted polymer was significantly higher than that of non-targeted one, as the latter was accumulated mainly in the liver.

**Poly(2-alkyl-2-oxazoline)s**

Lower molecular weight (about 5 kDa) polymers based on poly(2-methyl-2-oxazoline) and poly(2-ethyl-2-oxazoline) radiolabelled with $^{111}$In-DOTA showed a rapid excretion via kidneys with only minimal uptake by MPS.\textsuperscript{[262]} To increase the plasma circulation time and to achieve information about their renal clearance, Wyffels et al. synthesised desferrioxamine-containing poly(2-ethyl-2-oxazoline) polymers of different molecular weight. Their pharmacokinetic behaviour was studied with $^{89}$Zr-labelled compounds using microPET.\textsuperscript{[62]} As a result, the renal threshold for poly(2-ethyl-2-oxazoline) polymers was determined to be around 40 kDa.

**PLGA Poly(lactic-co-glycolic acid)**

Poly(lactic-co-glycolic acid) (PLGA) belongs to the most advanced biodegradable polymers used in the synthesis of degradable nanoparticles.\textsuperscript{[263]} To prevent the opsonisation by plasma proteins, the surface of PLGA nanoparticles are frequently coated with biocompatible hydrophilic polymers (e.g., PEO). Such particles, labelled with $^{99m}$Tc, were used for imaging of lungs\textsuperscript{[264]} and sentinel lymph nodes.\textsuperscript{[265]} When coated with Pluronic\textsuperscript{®} surfactant [poly(ethylene oxide)-$b$-poly(propylene oxide)-$b$-poly(ethylene oxide)], the nanoparticles can be used as theranostics for simultaneously prolonged blood circulation of drug etoposide (chemotherapy) and imaging via the $^{99m}$Tc-label (here, etoposide was used both as chemotherapeutic and as $^{99m}$Tc chelator).\textsuperscript{[266]} Further, $^{111}$In-labelled galactosylated PLGA nanoparticles were developed as trackable carriers for the liver-specific delivery of drugs.\textsuperscript{[266]}
Self-assembled polymeric amphiphilic block copolymer supramolecular structures (nanoparticles, micelles, liposomes, polymericosomes)

Tumour accumulation and thus the tumour imaging quality may be positively affected by an increasing size of polymeric systems. Besides the polymer chain elongation, optimal size can be achieved by preparation of various self-assembly polymer architectures like micelles, liposomes, nanovesicles and other nanoscale systems. In general, amphiphilic copolymer micellar systems comprise of a hydrophobic block which forms the core of the micelle, and the hydrophilic blocks, forming the micellar shell in aqueous conditions. The size of self-assembled micelles is usually large enough to evade the renal elimination, which may improve its tumour accumulation by prolonged blood circulation time of the system. Moreover, the micelle-unimer equilibrium assures slow body excretion of the system, providing the size of the unimeric block copolymers below the renal threshold. Different polymers have been used for the construction of both hydrophilic and hydrophobic blocks. However, some of the most commonly studied systems are based on the diblock copolymers of PEO and poly(ε-caprolactone) (PEO-b-PCL). After intravenous injection of radiolabelled $^{111}$In-DTPA PEO$_{5kDa}$-b-PCL$_{5kDa}$ nanoparticles (hydrodynamic diameter 58 nm) to an MDA-MB-231 tumour bearing mice, a clear visualisation of the tumour, liver and spleen could be observed after 48 hours via μSPECT/CT imaging. Comparing the different size of block copolymer micelles (25 nm and 60 nm), the micelles with a diameter of 60 nm lead to an almost two-fold tumour accumulation (EPR effect), which could be further improved by active targeting with the human epidermal growth factor (EGF). In another report, Park et al. used the $^{99m}$Tc-DTPA-labelled PEO-PCL copolymer as a targetable bone imaging system. This system showed an increased bone accumulation, while the liver and spleen uptake was suppressed. Other widely studied diblock copolymers comprise of PEO and polylactide blocks (PEO-b-PLA). As an example, this polymer was labelled with $^{125}$I via
tyrosine moieties to track its biodistribution in mice.\textsuperscript{[140]} Interestingly, the study revealed that the introduction of negative charge to the micellar surface (by functionalisation with glutamic acid) lowered the uptake of the system in liver and spleen compared to the neutral micelles.

ABA triblock copolymers poly[2-methyl-2-oxazoline – \textit{block} – (2-isopropyl-2-oxazoline – \textit{co} – 2-butyl-2-oxazoline) – \textit{block} – 2-methyl-2-oxazoline] with two hydrophilic A blocks and one central thermo-responsive B block with different monomer ratios have also been synthesised.\textsuperscript{[271]} These polymers are well-soluble in aqueous millieu, being non-assembled below the cloud point temperature (CPT) of the thermo-responsive block and as micelles at a higher temperature. Micelles are formed within a narrow temperature range. The CPT of the thermoresponsive block was adjusted by the 2-butyl-2-oxazoline (hydrophobic monomer lowering the CPT) to 2-isopropyl-2-oxazoline (main monomer giving thermosresponsive properties to its copolymers) ratio, and size of the micelles is influenced also by the A to B block weight ratio. Phenolic groups were introduced into the above-stated polymer to allow radionuclide labelling with radioiodine for both diagnosis and therapy of solid tumours. Such polymer was then radiolabelled with \textsuperscript{125}I in good yields with sufficient \textit{in vitro} stability under model conditions.

Radiolabelled liposomes stabilised with hydrophilic polymers can be utilised as excellent tools for tumour imaging, as well. Liposomes prepared from hydrogenated soybean phosphatidylcholine, cholesterol and \textit{N}-(carbamoyl-PEO)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine labelled with \textsuperscript{111}In-DTPA were injected into KB squamous cell tumour-bearing mice, and the biodistribution was assessed by a gamma scintillation.\textsuperscript{[272]} The system showed substantial tumour accumulation, but also considerable liver and spleen uptake. Further, the system was administered into 17 human patients with different advanced cancers (breast, head, bronchus, glioma and cervix).\textsuperscript{[273]} After 72 hours, the levels of tumour
liposome uptake were estimated from gamma camera images. The highest levels of tumour uptake were seen in the patients with head and neck cancers, while the breast tumours showed relatively low uptake. Also, significant localisation of radiolabelled liposomes was observed in the MPS.

7.2 Synthetic polymers for therapy

Although not as common as for the imaging, synthetic polymeric systems can also be used to transport therapeutic radionuclides into solid tumours. In this case, the systems resemble “classical” drug delivery devices, where the chemotherapeutic payload is replaced with the radionuclide. In regard to polymeric radiotherapeutics equipped with $\beta$ emitting particles, the radionuclide retains its therapeutic effectivity without being released from the polymer due to the higher range of radiation. The radiation burden of the tissues occurs in a rather unspecific way in the surroundings of the polymer. As an example, Mitra et al. synthesised $^{90}$Y-DTPA labelled HPMA polymer decorated with $\alpha_\text{V}\beta_3$ integrin-targeting peptide RGD4C. The system showed an enhanced tumour accumulation and substantial suppression of the tumour growth in the human prostate (DU145) tumour-bearing mice when treated with 3.7, respectively 9.25 MBq of this $^{90}$Y-labelled polymer. This effect may be further enhanced by the combination of radionuclide therapy and local hyperthermia caused by PEOylated gold nanorods. Wang et al. synthesised dextran grafted poly (N-methacryloylglycylglycine) copolymer–tyrosine conjugates, which were successfully iodinated with $^{125}$I and injected into healthy mice (see also section of natural polymers). Aside from the kidney clearance, the copolymer showed an increased accumulation in liver and spleen, which may limit their use as radiotherapeutics. The biodistribution profile can be improved by conjugation of HPMA units to the methacrylate chains of this copolymer. Supramolecular systems exploiting micelles, liposomes or nanovesicles can also be used for the radionuclide delivery. As an example, Hara et al. synthesised a diblock micellar system...
composed of hydrophobic poly(lactic acid) and hydrophilic poly(sarcosine) blocks, which were labelled with $^{131}$I and then administered in the 4T1 mammary carcinoma mice. This resulted in the tumour growth suppression, which was further improved by simultaneous injection of ethanol percutaneously to the tumour region.\textsuperscript{[279]} Sofou et al. proposed liposomes prepared from PEOylated lipids, 1,2-dinonadecanoyl-glycero-3-phosphocholine and cholesterol, for tumour treatment with the $\alpha$-emitter $^{225}$Ac-DOTA.\textsuperscript{[280-281]} The nuclide $^{225}$Ac is an efficient \textit{in situ} radiation nanogenerator due to its fast decay cascade. When attached to a mouse anti-human PSMA J591 antibody, radiolabelled-liposomes showed an enhanced cellular uptake and cytotoxicity towards prostate-specific membrane antigen expressing human cell lines LNCaP and HUVEC.\textsuperscript{[282]} Werner et al. described folate-targeted $^{90}$Y-DTPA-bearing nanoparticles comprised of poly(lactic-co-glycolic acid) (PLGA) hydrophobic core and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[[polyethylene oxide]] outer shell.\textsuperscript{[283]} Paclitaxel (anticancer drug) was then encapsulated in the hydrophobic core. \textit{In vivo} studies showed an increased survival time of SKOV-3 murine ovarian cancer-bearing mice after therapy with folate-targeted nanoparticles (1.85 MBq of $^{90}$Y per mouse) compared to the non-targeted ones.\textsuperscript{[283]} These efforts strongly suggest that synthetic polymers hold a significant potential for improving cancer theranostics. However, a comprehensive understanding is critical between the pharmacokinetics and targeting of the systems for an improved efficacy.

8. Dendritic polymers

Dendrimers are highly branched spherical macromolecules with a controlled size resulting in a near-perfect three-dimensional architecture.\textsuperscript{[284-286]} These monodisperse, perfectly branched scaffolds can mainly be synthesised through divergent and convergent approaches which, however, often involve tedious multi-step reactions. Owing to their facile one-pot synthesis and their compatibility with a range of conventional polymerisation
techniques, a significant subclass of dendrimers, hyperbranched polymers have also garnered substantial interest to be considered as favourable alternatives.\textsuperscript{[287-289]} Numerous dendritic polymers have been developed over the years for various imaging, and therapeutic systems which can be categorized at least into (a) dendrimers; (b) hyperbranched polymers; (c) star polymers; (d) dendrigrafts; or hybrid systems such as (e) polymersomes; (f) amphiphilic core-shell dendrimers forming, unimolecular micelles; (g) Dendrimer-DNA conjugates, called as dendriplexes, and (f) self-assembled Janus dendrimers forming uniform ‘dendrosomes’ (Figure 15).

**Figure 15.** Different multifunctional dendritic and similar polymers for theranostic applications.

Such structures are recognised as versatile nanoscale devices regarding their structural control as well as their composition which can be utilised to fine-tune their tumour specificity as well as their biocompatibility. Their physical characteristics such as the size, degree of
branching (DB), and surface functionalities can be efficiently adjusted and tailor-made for numerous applications. The multiple peripheral groups allow a high payload of drugs, imaging agents or an amalgamation of various moieties through chemical modification, encapsulation or in a combination of both. Furthermore, dendrimers allow multivalent interactions by increasing the number of effectors within the same controlled backbone which can exhibit amplified substrate avidity (polyvalent effect).\textsuperscript{290-291} Combining imaging technologies such as PET, CT and SPECT with therapeutic agents on a single core, not only aids in evaluating a diseased state \textit{via} imaging prior to therapy but also increases the sensitivity and resolution of the diagnosis. Due to these intriguing characteristics, dendritic polymers are promising candidates in the theranostics and biomedical fields.

Over the years, some excellent reviews have been published discussing the applications of dendrimers in theranostics, bioimaging, protein engineering, as well as MRI contrast agents, \textit{etc.}\textsuperscript{292-299} In the present chapter, we will focus on the ‘state of the art’ of various radiolabelled dendritic polymers as potential candidates for diagnostic, imaging as well as therapeutic applications.

8.1 Dendritic polymers for imaging

Dendritic polymer-based SPECT imaging has proven to be a useful technique using radionuclides like $^{99m}$Tc and $^{111}$In, \textit{etc}. Incorporation of these radionuclides can be done at the terminal groups of the structures after functionalisation with chelating agents such as diethylenetriamine (DTPA) or 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) derivatives. These studies are typically the basis of the rationale to design the optimal structure to satisfy their requirements for their diagnostic or therapeutic applications.\textsuperscript{300-301} The use of chelating units does not seem to alter the biodistribution of these macromolecules. To design dendrimers for nuclear medicine imaging applications,
pioneering work has been done by Mukhtar et al. using generation 1 and 2 dendritic porphyrins capable of glioma tumour imaging.\textsuperscript{302} Kobayashi et al. evaluated \textsuperscript{111}In and \textsuperscript{88}Y-labelled G2 PAMAM dendrimer-monomclonal antibody conjugates for tumour imaging.\textsuperscript{303-304} These studies showed a significant accumulation on the tumour. Unfavourably, a high accumulation was also observed in other organs such as liver, spleen, kidneys, etc. Interestingly, they showed that MPS accumulation is significantly decreased when the chelates were saturated with non-radioactive indium or yttrium ions. A high uptake by the MPS, however, indicates a detrimental effect of PAMAM dendrimers from an imaging point of view. In this case, dendrimers with differing structure and size may be used to improve the organ distribution. Parott et al. prepared different generations (G5-G7) of polyester dendrimers.\textsuperscript{305} To minimise the impact of chelator functionalisation on the dendrimer periphery to its biological environment, they used an alternate approach by introducing the bis(2-pyridyl)amine chelating unit for \textsuperscript{99m}Tc into the core of the dendrimers. MicroSPECT studies showed that regardless of the generation, all the dendrimers were rapidly eliminated from the bloodstream via the renal pathway into the bladder within 15 mins post-injection most likely due to the small size of the dendrimers significantly below the renal exclusion limit. Despite decreasing the blood residence time, renally clearable systems are still preferred for excretion of any non-biodegradable drug delivery or imaging systems.

Conversely, passive targeting, utilising the leaky vasculature of tumours requires a long circulating system (more than 6 h) to have sufficient time for accumulation on the target. Moreover, for an efficient targeting, the macromolecules must also overcome several biological barriers such as opsonisation and should possess appropriate hemocompatibility, \textit{etc.}\textsuperscript{116} PEOylation is a known strategy for surface modulation to increase the size of the targeting systems for such purposes. It not only provides stealth-like properties to the structure but also enables to potentially evade MPS uptake and phagocytosis by macrophages.
This can enable the conjugates to bind selectively to the tumour cells. Such PEOylated dendrimers have been used to visualise tumour tissue, sentinel lymph nodes as well as melanoma with high resolution and to gain information about the state of angiogenesis.[306-309]

The tree-like structure of dendritic polymers also permits multi-functionalisation of different functionalities such as chelating, targeting and solubilising moieties as well as fluorescent tags on the surface. In principle, radionuclide imaging is majorly dependent on the half-life of the radionuclide in use, ranging from a few hours to a few days. To overcome this challenge, attaching a fluorescent label simultaneously onto a probe can thus, enable longer period evaluation resulting in multimodal probes. For example, Kobayashi et al. developed an $^{111}$In-labelled generation 6 PAMAM-based nanoprobe with multimodal and multicolour potential to permit lymphatic imaging.[310] About 120 DTPA BFCAs on the surface of the dendrimer permit non-sophisticated radiolabelling and $^{111}$In scintigraphic imaging, which allowed increased depth penetration and whole body quantification, whereas five NIR dyes offered a real-time spatial resolution for each of the five lymphatic basins (FIGURE 16). This demonstrates the potential of these multimeric systems in particular for cancer diagnosis, further reinforcing the importance of multifunctional systems.
Figure 16. *In vivo* dual-modal, five-color lymphatic drainage imaging with the ability to visualize five distinct lymphatic drainages. (a) *In vivo* multiexcitation spectral fluorescence (right) and *post mortem in situ* radionuclide (left) images of a mouse injected with five distinct G6-[Bz-DTPA]_{119}-(NIR)_{4}-(Bz-DTPA-^{111}In)_{1} nanoprobes intracutaneously into the middle digits of the bilateral upper extremities, the bilateral ears, and at the median chin, as shown in the schema (mouse 7 in Table 1). Five primary draining lymph nodes were simultaneously visualized with different colors through the skin in the *in vivo* spectral fluorescence image and are more quantitatively seen in the radionuclide image. (b) *Ex vivo* spectral fluorescence and radionuclide images of resected eight draining lymph nodes correlate well to the *in vivo* imaging. (c,d) Images of a different mouse (mouse 8 in Table 1), given shuffled injections. The imaging results were consistent for all mice examined. Reprinted by permission from ACS Nano, reference 310, copyright American Chemical Society 2007.

Exploring the multivalent effects, dendrimer-based PET imaging was reported by Tanaka et al. using various generations’ of ^{68}Ga-DOTA labelled glycoclusters, exhibiting a size dependent renal filtration.\[^{311}\] Multimeric ‘dendritic’ probes have also been developed to target and image angiogenesis, using α,β₃ integrin targeting in order to overcome the otherwise poor *in vivo* radiostability and lack of selectivity of small-molecule probes such as RGD-based peptides using ^{64}Cu or ^{68}Ga with DOTA as the BFCA. Increasing the number of RGD peptides on the scaffold was shown to enhance the integrin-binding affinity and hence, the tumour uptake.\[^{312-316}\] An impressive example was illustrated by Fréchet et al., who developed a multivalent nanoprobe with a core-shell architecture consisting of a biodegradable heterobifunctional core, chemoselectively functionalised with heterobifunctional polyethylene oxide (PEO) chains, to impart biological stealth.\[^{317}\] The
radionuclide $^{76}$Br was incorporated in the tyrosine moieties to the core to avoid dehalogenation, and the cRGD peptides were functionalised on the PEO surface to enhance accessibility to $\alpha_v\beta_3$ integrin receptors. The nanoprobe of a hydrodynamic size of 12 nm exhibited 50-fold enhancement of the binding avidity with respect to the monomeric cRGD peptides alone. In vivo studies in a murine hindlimb ischemia model revealed excellent bioavailability and a highly specific tumour accumulation of $^{76}$Br-labelled nanoprobe allowing selective imaging of angiogenesis.

Hyperbranched polymers offer facile, easier one-pot syntheses strategies with high-end functionalities which also makes them favourable for the development of multimodal imaging agents.$^{[318]}$ In this regard, polyglycerols represent a unique class of hyperbranched polymers that can be synthesised in a controlled manner via a ring opening multi-branching polymerisation reaction with sizes ranging from 1-10 nm.$^{[319-320]}$ Modification of surface functionalities can also impart inherited targeting to the dendritic scaffolds. In this framework, dendritic polyglycerols (dPG) show excellent biocompatibility in the range of linear polymers like PEO as well as anti-fouling properties, which make them attractive for diagnostic approaches. Their sulphated derivatives (dPGS) show an enhanced anti-inflammation behaviour with respect to heparin which has also been revealed in various in vivo inflammation-targeting models.$^{[321]}$ Recently, Pant et al. showed that $^{64}$Cu- and $^3$H- labelled neutral polyglycerols of a hydrodynamic size of 3.5 nm (i.e., below renal threshold) exhibit fast renal clearance into the urine bladder and moderate blood circulation time.$^{[322-323]}$ On the other hand, dPGS showed a high hepatic and splenic uptake and retention of activity in the kidney cortex, most likely due to the electrostatic repulsion of the negatively charged dPGs with the glomerular basement membrane (GBM) of the kidneys.$^{[323]}$ This finding, unfavourably, impairs its use for diagnostic approaches. The effects of charge on the pharmacokinetic properties of dendritic polymers have also been highlighted by other
In general, the results indicate that with amine-containing dendritic scaffolds, decreasing the isoelectric potential (IEP) between 5 and 6 would be appropriate to reduce the non-specific accumulation and lead to prolonged circulation times. Acetylation and succinylation of the residual primary surface amine groups of dendritic derivatives to neutralise the surface charge without significantly altering the size of the conjugates are other approaches to reduce the non-specific uptake.\textsuperscript{[326]} Moreover, these studies emphasise that - in view of the development of an effective theranostic dendritic probe - it is very crucial to determine the synergy between the optimal size as well as surface charge in order to achieve the desired excretory and circulatory profile for targeting. In this perspective, highly hydrophilic dendritic multimers equipped with TRAP (triazacyclononanephosphinic acids) ligands for ⁶⁸Ga and ⁶⁴Cu binding have also a great potential to be employed in nuclear medicine.\textsuperscript{[327-328]}

8.2 Dendritic polymers in therapy

Dendritic polymers have also been studied for therapeutic applications using radionuclide such as ¹³¹I, ¹⁸⁸Re, ⁹⁰Y, etc.\textsuperscript{[329-330]} An intriguing feature of dendritic polymers is their ability to encapsulate radionuclides and drugs. Concerning the latter, their controlled release is very attractive. Zhao et al. explored the potential of radiolabelled dendrimers for therapy using ¹³¹I-radiolabelled PAMAM dendrimers.\textsuperscript{[331-332]} To enhance the specificity and efficiency, either targeting peptides or drugs such as chlorotoxin were encapsulated in the scaffolds followed by acetylation of the residual amine groups to reduce opsonisation. The results exhibited prolonged tumour accumulation accompanied by markedly improved survival rates of the tumour mice manifested in a decrease of the tumour size (around 8 times). PEO-block dendrons and other amphiphilic dendrimers, which can assemble to form unimolecular micelles, have also been studied to show efficient antitumour activity and
promising tumour imaging properties.\textsuperscript{[333-335]} These nanoplatforms can be equipped with radionuclides such as \textsuperscript{131}I that allow for imaging and therapy, and simultaneously a high payload of drugs can be encapsulated that can be specifically released to tumour cells via stimuli such as a pH trigger, \textit{etc.} Positively charged dendrimers, \textit{e.g.} polyamidoamine (PAMAM) and polypropylene amine (POPAM) form stable complexes with negatively charged DNA, oligonucleotides, \textit{etc.}, resulting in stable 50–200 nm-sized DNA/dendrimer aggregates termed dendriplexes.\textsuperscript{[336]} Such rod- and toroid-shaped dendriplexes are capable of penetrating into cells and reach the cell nucleus, resulting in enhanced transfection efficiency.\textsuperscript{[337]} Recently, Hsu et al. demonstrated a design rationale for developing dendron polymeric micelles by varying their PEO-chain lengths and their surface charge to modulate their cellular interactions and their potential use as drug delivery platforms.\textsuperscript{[338]}

In 2013, Grünwald et al. developed adenovirus vectors coated with PAMAM dendrimers (selective or deficient), carrying the hNIS gene (sodium iodide symporter) to evaluate their potential for systemic radiovirotherapy using \textsuperscript{123}I-scintigraphy.\textsuperscript{[339]} The dendrimer-coated adenovirus showed enhanced transduction efficiency \textit{in vitro}. When injected into liver cancer mice (xenograft mouse model), a significant reduction in hepatic accumulation and toxicity was revealed as compared to the uncoated adenovirus, corroborating with an enhanced oncolytic effect. To further improve the targeting, they expanded their work by modification of the PAMAM-coated adenovirus receptors (hNIS gene) with an EGFR-specific peptide GE11.\textsuperscript{[340]} PET-imaging using \textsuperscript{124}I-labelled adenovirus vectors revealed a high tumour accumulation. As expected, decreased tumour accumulation was obtained after pre-treatment with the anti-EGFR specific antibody cetuximab, confirming the specificity of the peptide conjugates to EGFR-rich tumours. The radiotherapeutic effect could be shown by systemic administering of the \textsuperscript{131}I-labelled dendrimer-coated adenovirus
vectors (Figure 17). These results unveil the potential of radiolabelled dendritic polymers for cancer therapy.

Figure 17. In vivo analysis of EGFR-specificity. $^{124}$I-PET-imaging demonstrated strong hepatic transduction after i.v. injection of the uncoated vector (Ad5-E1/ RSV/NIS) (a) and quantification of radiiodine accumulation revealed only poor tumoral transduction (a,d). In contrast, coating of the adenovirus with PAMAM-G2-PEG-GE11 (dc$_{300}$/GE11 Ad5-E1/AFP-RSV/NIS) before systemic injection resulted in prevention of hepatic radioiodine accumulation and distinct transduction of tumor xenografts (b,d). By pretreatment of mice with the monoclonal anti-EGFR antibody cetuximab before systemic dc$_{300}$/GE11 Ad5-E1/AFP-RSV/NIS administration tumoral radioiodine accumulation was significantly reduced while liver detargeting of NIS expression was still effective (c,d;**P<0.01). AFP, α – fetoprotein; EGFR, epidermal growth factor receptor; NIS, sodium iodide symporter. Reprinted by permission from Macmillan Publishers Ltd: Molecular Therapy– Nucleic Acids, reference 340. Copyright by Nature Publishing Group 2013.
Despite several unique features of radiolabelled dendritic polymers that disclose their suitability to be utilised as theranostic agents, their clinical use in cancer medicine has not been reported yet. The undesired accumulation of in particular larger and/or charged dendritic structures in the MPS is the main reason for this. Although, dendritic polymers are relatively monodisperse, multimodal and multistep modification may lead to a loss of their homogeneity, and thus biodistribution profiles and targeting efficiency is impaired. One way to develop more appropriate tumour theranostics could be the development of smaller, nearly neutral dendritic scaffolds and the use of multi-step pretargeting approaches. Size switchable pH-sensitive scaffolds can also be envisaged for efficient tumour therapy that allows high payload drug delivery as well as the desired biodistribution.\[343\]

**Future Directions**

An alternate approach to commonly used tumour-targeted contrast agents, based on metal complexes as well as inorganic materials, is the development of organic-inorganic hybrids. These exploit the inherent properties of inorganic materials, such as gold and superparamagnetic iron oxide nanoparticles (SPIONs), while simultaneously increasing their biocompatibility by using polymeric materials. Thereby, in many cases, these formulations prolong the blood half-life, which often entails high tumour uptake (EPR effect) that can be utilised for passively-targeted tumour imaging.\[344-348\] Additionally, hybrid systems allow for the combination of two or more modalities integrated on a ‘single nano-platform’ for dual/multimodal imaging, offering synergistic advantages over a single modality alone, such as PET/MRI, PET/OI, and SPECT/MRI, SPECT/OI or PET/CT/OI systems amongst others. These materials can even be coupled with therapeutic approaches such as chemotherapy (controlled release of anticancer drugs), hyperthermia, photodynamic therapy or internal radionuclide therapy.\[349-350\]
As an illustrative example of a theranostic platform, Zolata et al. developed carboxymethyl chitosan (CMC) modified SPIONs conjugated with \(^{64}\)Cu-labelled antibodies as active MRI/PET dual modal system for targeting of HER2 overexpressed on breast adenocarcinoma tumours. Furthermore, doxorubicin was embedded in the CMC-coated SPIONs via a pH sensitive acrylic acid linker for the controlled drug release to the tumour.\(^{[351]}\) However, the limitations of such systems include that, upon in vivo injection, the drug can sometimes prematurely dissociate from the carrier, resulting in insufficient tumour therapy of the drug delivery system. To overcome this, therapeutic radionuclides such as \(^{188}\)Re can be employed so that the therapeutic agent is coupled to the system throughout the treatment.\(^{[352]}\) Recently, \(^{198}\)Au (a \(\beta^+\) emitter) has been considered promising for cancer treatment and imaging. To increase its biocompatibility as well as provide conjugation sites for further functionalisation, \(^{198}\)Au nanoparticles were coated with PAMAM dendrimers or chitosan as stabilising agents.\(^{[353-354]}\) When injected intratumourally to a C57BL/6J melanoma mouse model, PAMAM-coated \(^{198}\)Au(0) nanocomposites showed a 45% reduction of tumour volume after eight days using only 2.74 MBq per mouse.

Despite the great potential, the development of radiolabelled multimodal hybrid systems is fairly challenging. One reason for this is that the increased complexity of the system may lead to reduced radiochemical stability, an increase of size and higher polydispersity of the systems and, thereby, phagocytic arrest will be induced.\(^{[355-357]}\) In this regard, the strong binding ability of \(^{99m}\)Tc-bisphophonates (BP) towards metals was employed by Rosales et al. to develop a dextran-coated SPION (SPECT/MRI) system where the \(^{99m}\)Tc-BP was conjugated directly on the magnetic core (instead of the polymeric coating) to improve stability.\(^{[358]}\) Furthermore, Wong et al. reported a rapid synthesis method for \(^{64}\)Cu-doped, dextran coated SPIONs with a controlled size by employing microwaves.\(^{[359]}\)
In another study, Jarett et al. illustrated the difficulties in radiolabelling DOTA incorporated dextran-coated SPIONs using traditional conjugation methods; presumably, due to the active functional groups present around the hybrids causing steric hindrance.\textsuperscript{[360]} Radiolabelling the chelators prior to conjugation (pre-labelling) could be one approach to avoid these problems. To improve the radiolabelling, new bifunctional chelating systems are also reported to reduce the drawbacks associated with steric hindrance.\textsuperscript{[361]} Nevertheless, inorganic-organic hybrids represent effective carriers for localised radionuclide therapies and have the potential to revolutionise cancer theranostics.

Recently, novel “smart” polymeric radiotherapeutics have also been developed as promising candidates for cancer therapy. Polymer-targeted Auger electron (AE) emitters offer excellent prospects. Aside from the aforementioned systems based solely on the active targeting, a universal passive targeting approach can be utilised as well.\textsuperscript{[362]} Allen et al. proposed \textsuperscript{111}In-containing block copolymer micelles consisting of PEO and poly(\(\varepsilon\)-caprolactone) (PCL), with or without human epidermal growth factor targeting, for therapy of EGFR overexpressing tumours. The micelles (with a hydrodynamic diameter of 15 nm) showed an increased receptor-mediated uptake and cytotoxicity in EGFR-overexpressing MDA-MB-468 breast cancer cells. However, only 1.9\% of the radioactivity was localised in the nucleus.\textsuperscript{[363]} A similar micellar system, bearing the specific antibody trastuzumab, revealed an improved (about 5-fold) cellular nucleus uptake by attaching a cell nucleus-localising sequence peptide. Furthermore, 4.8 wt.-\% of the anticancer drug and radiosensitizing agent methotrexate was incorporated into the hydrophobic micellar core to further increase the cytotoxicity.\textsuperscript{[363]} An efficient way to improve the cell nucleus targeting of AEIs is their attachment to DNA intercalators. Gedda et al. synthesised a \textsuperscript{125}I-labelled daunomycin derivative, which was subsequently entrapped in PEOylated liposomes (called “nuclisome”), bearing the epidermal growth factor as targeting vector. This ensured a
selective uptake in EGFR-containing U-343MGaCl2:6 cells, whereas the uptake into white blood cells was negligible. Autoradiography confirmed the localisation of $^{125}$I in the cell nucleus. This system was five-times more cytotoxic than the same liposomes loaded with doxorubicin. A similar $^{125}$I-labelled nuclisome system was investigated in mice, bearing human ovarian adenocarcinoma SKOV-3. The targeted nuclisome system was proven again to have higher therapeutic activity than the non-targeted one. Sedlacek et al. developed a polymeric delivery system for the $^{125}$I-labelled DNA-intercalator ellipticine (Figure 18). The radiolabelled intercalator was bound to a PHPMA copolymer using an acid-sensitive hydrazone linker; the structure of the linker plays a crucial role in the biological effectivity of the system. In this regard, it was optimized to be stable at pH 7.4 (representing the pH of blood plasma), whereas in acidic pH (typically in endosomes) the radioiodine-labelled intercalator is promptly released from its polymeric carrier. Due to the fact that hydrazone conjugates are incompatible with standard radioiodination conditions, the intercalator was first radiolabelled by an ioddestannylation procedure followed by the polymer conjugation. In vivo experiments in 4T1 murine breast cancer bearing mice resulted in a statistically significant increase in the survival time of mice treated with this polymeric radioconjugate.
Figure 18. Mechanism of the multiple targeting of a polymer-intercalator conjugate labelled with an Auger emitter: (A) Passive accumulation of the system in solid tumour; (B) release of the biologically active radiolabelled intercalator due to the change of pH; (C) intercalation of the radiolabelled intercalator with DNA followed by its decay, DNA breaks and cell apoptosis.

Brachytherapy (BRT) is another form of internal radiotherapy, where sealed emitters with radiation sources (radioactive fillings of metal containers) are implanted into the target site. This approach has been commonly used especially for treatment of prostate, breast and cervical cancer. However, after the radionuclide decay, the emitter needs to be surgically removed, which (as well as the implantation procedure itself) is inconvenient for the patient. Therefore, injectable depots comprising of radionuclides connected to thermo-responsive polymers are currently widely studied. Water-soluble, thermo-responsive polymers with a low cloud point temperature (CPT) (i.e. those which are soluble at lower (room) temperature in aqueous milieu but insoluble at higher (body) temperature) allow for the construction of advanced self-assembling systems that form the depot at the place of injection, simply by heating to the body temperature. When this polymer is decorated with a therapeutically effective radionuclide the intratumoural depot burdens the tumour tissue (Figure 19). The advantage of these systems is that they do not require surgical intervention for implantation or removal of the radionuclide container, as the equilibrium between the phase-separated (solid) depot polymer and dissolved polymer chains ensure the gradual degradation of the radioactive depot. Currently, most of these radiolabelled thermo-responsive polymers are based on poly(N-isopropylacrylamide) (PNIPAM, CPT ~ 30 °C). In this regard, Hruby et al. developed ¹³¹I-labelled PNIPAM by copolymerisation of N-isopropylacrylamide and trace amounts of N-methacryloyl tyrosinamide. When applied to femoral muscle of Balb/c mice, the radiolabelled polymer depot retained at the application
sit, with 90% of radioactivity on the tumour site 2 hours after injection. This decreased gradually to 60% of retained activity 42 days from application. In vivo results showed major elimination into the urine and faeces with no organ-specific accumulation of the released activity (of note this included the thyroid, for which accumulation is frequently observed for radioiodinated compounds). Furthermore, a single dose of this system lead to a substantial tumour growth inhibition in a murine xenograft model (PC3 human prostate adenocarcinoma) with a dose of 25 MBq/ mouse, causing gradual tumour volume reduction. 2 out of the 6 mice were completely cured.\textsuperscript{[375]} To fine-tune the depot degradation rate, different methacrylamide-type co-monomers (containing hydrophobic alkyl chains attached to the backbone by a hydrolytically labile hydrazone bond) were incorporated into the PHPMA copolymer using the therapeutically relevant radionuclide \textsuperscript{64}Cu.\textsuperscript{[376]} Additionally, anticancer drugs can also be entrapped in such polymeric depots; in this regard, a thermo-responsive system was developed based on a \textsuperscript{125}I-radiolabelled polymer with doxorubicin bound to the polymer via a hydrolytically labile N-glycosylamine bond for a controlled DOX release.\textsuperscript{[377]} Other thermo-responsive polymers based on poly(2-alkyl-2-oxazolins), elastin-like polypeptides and systems based on natural polymers are also known for the development of injectable depots for brachytherapy.\textsuperscript{[378-381]}

\textit{Injectable radiotherapy by thermoresponsive polymers}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{injectable_radiotherapy_diagram.png}
\caption{Diagram illustrating the injectable radiotherapy process using thermoresponsive polymers.}
\end{figure}

\begin{itemize}
\item Polymer gel
\item Polymer solution
\item Formation of radioactive depot (by heating to body temperature)
\end{itemize}
Apart from radiolabelled polymers applied for internal radionuclide therapy, polymers can also be used to transport and accumulate radiosensitizing molecules in tumours, which are then more sensitive to the external beam radiotherapy than the surrounding tissue. This method benefits from easier preparation and storage conditions. As an example, Menon et al. synthesised a nanoparticle system consisting of a PLGA core and a poly(vinyl alcohol) shell with the radiosensitizer 8-dibenzothiophen-4-yl-2-morpholin-4-yl-chromen-4-one (NU7441) encapsulated and the cell penetrating peptide R11 conjugated. The nanoparticles showed high uptake in PC3 prostate cancer cells and strong inhibition of DNA-double strand breaks repair kinetics in vitro after external radiation.\textsuperscript{[382]} In another study, Jin et al. prepared PLGA nanoparticles containing the SR-2508 (etanidazole) radiosensitizer. They showed a significantly higher cellular uptake in vitro as well as radiation sensitivity in the cell lines HeLa (human cervix carcinoma) and MCF-7 (human breast carcinoma) compared to the free drug.\textsuperscript{[383]} Curcumin- and paclitaxel-containing PLGA polymeric materials are further examples which inhibit ovarian cancer cell growth after external radiation exposure\textsuperscript{[384]} as well as increase the radiation sensitivity of hypoxic MCF-7 cells in vitro.\textsuperscript{[385-386]}

A number of key challenges must be addressed, e.g. biocompatibility, easy clearance from the body as well as toxicity issues. In this context, the development of ‘smart probes’ based on self-assembled polymeric materials as well as ‘intelligent’ organic-inorganic hybrids that can exploit minimal changes in the tumour environment is particularly promising. Possible solutions are: stimuli-responsive nanomaterials, using physico-chemical stimuli (magnetic field, pH, temperature, etc.) or even biological stimuli (enzyme- and receptor-mediated).\textsuperscript{[122, 124-125, 387-389]} These ‘smart nanoprobes’ can produce and amplify signals in the tumour tissue when activated by a certain stimuli (e.g. an enzymatic activity that is associated
with the tumour or can be constructed to accumulate in the tumour site and then disintegrate into smaller particles, thus facilitating clearance from the body. In the past decade, significant progress has been made in the development of ‘smart nanoprobes’ with a particular focus on drug delivery. However, the use of smart nanoprobes for radionuclide therapy is still in its infancy and opens up a fascinating field of research especially for cancer theranostics.

**Summary and conclusions**

The interest in the field of developing polymeric materials for imaging and the treatment of cancer is being maintained unabatedly. Thanks to their structural variability, which facilitates setting up the basic structure, modifying the periphery as well as creating complex structures, their properties allow being tailored to – for example – targeted anticancer drug delivery systems.

The development of new techniques of manufacture, such as RAFT (reversible addition-fragmentation chain transfer) polymerisation and ATRP (atom-transfer radical-polymerisation) allows the targeted synthesis of polymers of a well-defined molar mass or of a degree of polymerisation, respectively, of low polydispersity and of well-known final functionality, which is indispensable for medical application. Meanwhile, a multitude of polymeric materials of defined size, structure and charge has become available and also the relevant analytical methods that allow these parameters to be exactly defined. The *in vivo* application requires reliable data regarding biodistribution, pharmacokinetic and pharmacodynamic properties of polymeric materials. Radiolabelled compounds play a prominent role in that aspect, as quantitative data can be gained particularly on the basis of the positron emission tomography, which currently no other method permits. Bifunctional chelating agents for binding suitable radionuclides can be included in polymeric systems in
methods of bioorthogonal chemistry, such as the Staudinger-Bertozzi ligation, the strain-promoted alkyne-azide cycloaddition and the inverse electron demand Diels-Alder reaction, are also being increasingly used. By doing so, both mild reaction conditions can be applied, and elaborate purification operations can be avoided at the same time. Moreover, it is expected that also enzyme-mediated conjugation strategies, which are presently preferred to be applied in the functionalisation of proteins (antibodies and their fragments) are used with a view to polymeric materials. This facilitates the site-specific introduction of BFCAs. By now, a series of chelator systems with adjustable solubility behaviour has been made available, which permit a very stable binding of different radionuclides in polymeric systems. This, for one thing, allows gathering reliable data on the biodistribution over longer periods of time (e.g., $^{64}$Cu several hours; $^{89}$Zr several days). From the point of view of radiochemical stability for the other, the application of radiolabelled polymeric materials with therapeutic radionuclides is also conceivable. However, these compounds must be used only when they reach the specific target site (tumour, metastases) quite fast in order to protect healthy tissue from ionising irradiation to the largest possible extent. Alternatively, more recent approaches, such as the pretargeting approach, can be applied here, too. This is a field that is at its very beginning. If we succeed to deposit the polymeric materials with therapeutic radionuclides almost exclusively in the tumour, especially $\beta^-$ particles will seem to be fit to destroy the tumour tissue. Their long range in tissues (up to 12 mm, cross-fire effect) allows various tumour cells (receptor-positive and receptor-negative ones); including cancer stem cells, to be destroyed. Especially effective is the application of Auger electrons in order to destroy tumour cells. For that purpose, however, the Auger electrons need to be transported into the nucleus of the cell. This is where interesting developments are becoming apparent, which
make use of the shuttle systems or polymer-conjugates, respectively, with DNA-intercalating agents for transporting Auger electrons to the nucleus of tumour cells.

Current developments concentrate on an improvement of the biological/pharmaceutical targeting and also on an effective treatment of cancer using polymeric materials. This is where especially the different properties of healthy and tumour tissue can be exploited to purposefully release anticancer drugs. In this context, the use of response to external stimuli is very promising. Tumour tissue differs from healthy tissues in the pH value (it is more acidic), in temperature (tumour tissue is usually warmer than surrounding tissues due to an intensive metabolism) and in the redox potential (fast growing zones may produce large amounts of reactive oxygen species while central areas of large tumours are hypoxic). With a view to that, the application of radiolabelled stimuli-responsive polymeric materials and drug molecules will provide valuable information on the residence time of the polymers and on the course of releasing the drug molecules over time.

Animal experiments have shown that the EPR effect can be exploited for the passive targeting of polymeric therapeutics. It was possible to show in a recent mouse model that this effect can clearly be increased (super enhanced permeability and retention effect) if the tumour is treated by near-infrared photoimmunotherapy. Investigations in humans, however, regarding the passive and particularly the active targeting of polymeric therapeutics are still in a very early stage. This is where knowledge, especially of the size and density of tumour blood vessels and of the kind and concentration of receptors on the surface of tumour endothelial cells, must be added in order to design polymeric materials for passive and active targeting. However, quite a few more hurdles need to be addressed when it comes to clinical application. This relates especially to issues of toxicity and immunogenicity. Due to severe problems observed for PEO polymers in this regard, other polymers such as poly(2-alkyl-2-oxazolines) and poly[N-(2-hydroxypropyl)methacrylamide] are currently undergoing
intensive investigation as an alternative. For all polymeric medicines to be used parenterally, the major issue is to eliminate the system from the organism after it fulfils its task. The system must be able to be eliminated directly as-is by renal filtration or be completely degraded into fragments that can be eliminated in a reasonable time horizon. There are interesting developments in both strategies. These are, on the one hand, very small (< 5.5 nm) hydrophilic oligomeric (dendritic) materials as well as small poly(2-ethyl-2-oxazoline) polymers that can quickly be excreted renally. Breakable (self-immolative) polymeric systems are being intensively investigated, on the other.

\(^{99}\text{Tc}\)-labelled HSA microspheres and mannosyl dextran derivatives are already being used clinically, especially in imaging liver tumours/metastases and sentinel lymph nodes (SLN). Presently, a development of dual (multimodal)-labelled imaging agents is becoming apparent, which unites the benefits of several different methods. This, on the one hand, relates to the high detection sensitivity and almost infinite depth of penetration in biological tissues, which is characteristic for nuclear techniques of PET and SPECT. In contrast to that, magnetic resonance imaging and optical fluorescence imaging, for example, provide high spatial resolution. Attractive combinations may be seen in the simultaneous tagging of polymeric materials with radiolabels and near-infrared fluorescent dyes or contrast agents (gadolinium-containing compounds, superparamagnetic iron oxides, PARACEST agents). At the same time, rapid development can be observed in the field of new polymeric cancer therapeutics. There are hopes for an enhanced effectiveness of treatment and lower side effects at the same time, by combining several therapeutical approaches, such as radionuclide therapy with other methods, for instance, nanomaterial-enabled radiosensitization, photothermal therapy, photodynamic therapy, near-infrared photoimmunotherapy, chemotherapy and hyperthermia. In that context, hybrids must be mentioned based on organic (polymeric)-inorganic materials.
The results obtained so far in the field of radiolabelled polymeric materials for imaging and treatment of cancer show an enormous potential for clinical use. Further developments of interest can be expected there in the future. However, it will only be the polymeric materials that will succeed in being translated into a clinical routine by being granted regulatory approval and, at the same time, being able to be manufactured under the conditions of good manufacturing practice (GMP). This requires a joint approach and extensive multidisciplinary cooperation of experts with synthetic, bio(analytical), bio(physical), bio(chemical), bio(medical), clinical expertise and stakeholders of regulatory authority. Without any doubt, this will lead to new radiolabelled polymeric materials for both non-invasive imaging and treatment of cancer.

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