General introduction:
Advances in nuclear oncology, microspheres for internal radionuclide therapy of liver tumours
invited paper (Current Medicinal Chemistry)

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Abstract: Liver metastases cause the majority of deaths from colorectal cancer, and response to chemotherapy and external radiotherapy is poor. An alternative is internal radionuclide therapy using $^{90}$Y labeled microspheres. These microspheres are very stable and have a proven efficacy in the field of treatment of primary or metastatic hepatic cancer. Whilst these spheres showed encouraging results in patients, their high density is a serious drawback. Currently, other materials with lower densities and other radioisotopes are being investigated in order to optimize this promising new therapy. Three major radiolabeled microsphere materials, viz. glass, resin-based and polymer-based, are now available for therapy or are being tested in animals. In this review the preparation, stability and degradation of these spheres are highlighted.
1.1 Introduction
In western countries malignant tumours originating from colorectal carcinoma are frequently seen in the liver. Surgical resection is presently the treatment of choice in patients with liver metastases. After this procedure the 5-year survival is around 35% [1]. However, most tumours are inoperable by the time of diagnosis. Other treatment options for these tumours include conventional chemotherapy and external radiotherapy [2,3]. Unfortunately, neither of the latter regimens have shown an obvious improvement in patient survival. The regional administration of therapeutic agents [4] via the hepatic artery is one strategy that has been developed to improve tumour response, since both primary and metastatic liver tumours are well vascularized and receive the bulk of their blood supply from the hepatic artery [5]. These therapies must satisfy two requirements in order to be successful: (a) the relevant agent must be effective in the in-vivo orthotopic microenvironment of tumours, and (b) this agent must reach the target cells in-vivo in optimal quantities [6]. All conventional and novel therapeutic agents for regional administration may be divided into three categories: molecules, particles and cells. In this review we focus on the injection of particles into the hepatic artery in order to obtain selective delivery of radioisotopes to the tumour, thus maximizing the irradiation effect while sparing toxicity to the surrounding healthy liver [2]. Among the more promising of these radiotherapeuticals are beta-emitting microspheres. These microspheres can be based on polymers, polymeric resins, albumin or inorganic materials e.g. glass. Radioisotopes used for labeling are yttrium-90 (\(^{90}\text{Y}\)), rhenium-186/rhenium-188 (\(^{186}\text{Re}/^{188}\text{Re}\)) and holmium-166 (\(^{166}\text{Ho}\)).

This chapter reviews the current literature on radioactive microspheres used for the treatment of liver malignancies and concludes with the aims and outline of the thesis.

1.2 Microspheres: materials and preparation
The ideal properties for radiolabeled microspheres or particles for intra-arterial therapy are summarised in Table 1. Müller and Rossier [8] first described the use of particles radiolabeled with gold-198 (\(^{198}\text{Au}\)), which were used in the treatment of lung cancer. Early studies used angular fragments that were made by crushing the bulk material and sieving the particles to grade in the desired diameter [9]. The first plastic microspheres were labeled with \(^{90}\text{Y}\) and showed an unpredictable and catastrophic leaching of yttrium, which brought them into question [10]. This problem was solved later by the use of glass beads. As well as these glass beads, resin-based and other polymeric microspheres are currently being used. An overview is given in Table 2.
Table 1. Ideal properties of radiolabeled microspheres for intra-arterial therapy [7]

1. High mechanical stability to resist breakdown and passage through the capillary network
2. High chemical stability to resist elution of radioactive label, macrophage removal, or radiolysis
3. Uniform size
4. Unit density to prevent settling or streaming
5. Relative ease of label
6. Radionuclide label with high-energy beta particle, low photofraction, and intermediate (days) half-life

Table 2. Radiolabeled microspheres currently in use

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Microsphere</th>
<th>Labeling</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{166}$Ho</td>
<td>PLLA</td>
<td>N</td>
<td>[13,29]</td>
</tr>
<tr>
<td></td>
<td>Resin (Aminex A-5)</td>
<td>Lr</td>
<td>[14]</td>
</tr>
<tr>
<td>$^{90}$Y</td>
<td>Glass (TheraSpheres®)</td>
<td>N</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td>Resin (Bio-Rex-70)</td>
<td>Lr</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>Resin</td>
<td>Lr</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>PLLA</td>
<td>Lr/Lp</td>
<td>[33]</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>Lr</td>
<td>[27]</td>
</tr>
<tr>
<td>$^{186}$Re/$^{188}$Re</td>
<td>Glass</td>
<td>N</td>
<td>[16]</td>
</tr>
<tr>
<td></td>
<td>Resin (Aminex A-27)</td>
<td>Lr</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>PLLA</td>
<td>Lr/Lp</td>
<td>[2]</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>Lr</td>
<td>[25]</td>
</tr>
<tr>
<td>$^{32}$P</td>
<td>Glass</td>
<td>N</td>
<td>[18]</td>
</tr>
</tbody>
</table>

N = activated by neutron bombardment; Lr = labeled with radioactive compound, retrospectively; Lp = labeled with radioactive compound, preceding production of microspheres.
1.2.1.1 Glass

Glass is relatively resistant to radiation-damage, highly insoluble, and non-toxic. Glass can be easily spheridized in uniform sizes and has minimal radionuclidic impurities. The manufacturing process is described comprehensively by Ehrhardt and Day [10]. The yield of microspheres with the desired diameter, 20-30 µm (see below), is around 15%. Advances in this technology have led to the production of glass microspheres with practically no leaching [11]. Although the glass spheres have several advantages, their high density (3.29 g/ml [12]) and their non-biodegradability are major drawbacks [13,14]. The relatively high density increases the chance of intravascular settling [15]. These glass microspheres produced under the name TheraSpheres® are the first registered microsphere product for internal radionuclide therapy, and are used in patients with primary or metastatic tumours. Because of the lack of $\gamma$-emission of $^{90}$Y, radioactive rhenium ($^{186}$Re/$^{188}$Re) microspheres were also produced. The general method of manufacture of these spheres was the same as for the $^{90}$Y spheres [2,16].

Brown et al. [17] prepared $^{166}$Ho-loaded glass particles (2-5 µm) for direct injection into tumours of mice, which resulted in an effective modality for deposition of intense $\gamma$-radiation for use in localised internal radionuclide therapy. However, no further studies were done.

Kawashita et al. [18] suggested the use of phosphorus-rich $\text{Y}_2\text{O}_3$-$\text{Al}_2\text{O}_3$-$\text{SiO}_2$-glass microspheres containing phosphorus ions, which were produced by thermoelectron bombardment of red phosphorus vapour and implanted into glass, thus resulting in a high phosphorus content and high chemical durability. After activation by neutron bombardment the glass contains phosphorus-32 ($^{32}$P).

1.2.1.2 Resins

Resin-based microspheres are much favoured for radio-embolization. Chloride salts of holmium and yttrium were added to cation exchange resins. Different resins were investigated by Schubiger et al. [19], amongst which were Bio-Rex 70, Cellex-P, Chelex 100, Sephadex SP and AG 50W-X8. The resins with $^{90}$Y bound to the carboxylic acid exchange groups of the acrylic polymer were sterilised and used for renal embolization of pigs. Only the pre-treated Bio-Rex 70 resulted in applicable particles, with a retention of beta activity in the target organ of >95% of injected dose, and no histologically detectable particles in lung tissue samples [20].

As well as Bio-Rex 70, Aminex resins (Bio-Rad Inc. Hercules CA, USA) loaded with $^{166}$Ho or $^{188}$Re, also resulted in applicable preparations. Turner et al. [14] prepared microspheres by addition of $^{166}$Ho-chloride to the cation exchange resin Aminex A-5, which has sulphonic acid functional groups attached to styrene divinylbenzene copolymer lattices. Reproducible, non-uniform distributions of the
Ho-microspheres throughout the liver were observed on scintigraphic images, following intrahepatic arterial administration in pigs. This predictable distribution allowed these investigators to determine the radiation absorbed dose from a tracer activity of Ho-microspheres, and to define the administered activity required to provide a therapeutic dose.

Aminex A-27 was labelled with \( ^{188}\text{Re} \) by adding \( ^{188}\text{Re}\)-perrhenate and SnCl\(_2\) to vacuum-dried resin particles [21]. The mixture was boiled and centrifuged and microspheres were separated and resuspended in saline. Spheres were tested by direct intratumoural injection into rats with hepatoma. Survival over 60 days was significantly better in the treated vs. the control group (80% vs. 27%).

Investigators from Australia and Hong Kong have used unspecified resin-based particles labeled with \( ^{90}\text{Y} \) for treatment of patients with primary or secondary liver cancer [22,23]. The spheres had a diameter of 29-35 \( \mu \text{m} \), a density of 1.6 g/ml and a specific activity of approximately 30-50 Bq per sphere. Treatment was well tolerated with no bone-marrow or pulmonary toxicity. The median survival was 9.4 months (range 1.8-46.4) in 71 patients, and the objective response rate in terms of drop in tumour marker levels was higher than that based on reduction in tumour volume shown by computed tomography [15,24].

1.2.1.3 Albumin

Technetium-99m-microspheres (\( ^{99m}\text{Tc}\)-microspheres) of human serum albumin (HSA) have been widely used for clinical nuclear medicine, particularly for lung scanning, since 1969 (25,26). \( ^{188}\text{Re} \) labeled HSA microspheres used by Wunderlich et al. [25] are uniform in size, with a mean diameter of 25 \( \mu \text{m} \), and are biocompatible and biodegradable. However, the labeling process is time-consuming and depends on SnCl\(_2\).2H\(_2\)O and gentisic acid concentration. On the surface of the microspheres a shell of about 1 \( \mu \text{m} \) thickness was seen, probably consisting of precipitated tin hydroxide. The particle labeling (coating) may be achieved by a combination of the reduction reaction of Re(VII) with Sn(II) and a particle surface-related coprecipitation effect of tin hydroxide colloid with high adsorption capacity and reduced, hydrolysed rhenium. The labeling yield under optimal reaction conditions is more than 70%. Biodistribution experiments in rats, using the lungs as a model for a well-perfused tumour, resulted in excellent in vivo stability.

As well as rhenium, yttrium was bound to HSA for internal radiotherapy [27]. \( ^{90}\text{Y} \)-acetate and macroaggregates of HSA (MAA) (Macrokit\(^\text{\textregistered}\), Dainabot, Tokyo, Japan) were suspended in sodium acetate buffer and incubated at room temperature. Experiments in mice were carried out in order to investigate the possibility of using \( ^{90}\text{Y-MAA} \) as an internal radiotherapeutic agent for whole-lung irradiation. Yttrium-activity in the lung was cleared within 72h post injection and activity was
redistributed in other organs, especially in the bone, but this could be prevented by the combined use of CaNa$_3$DTPA. Based on its rapid clearance $^{90}$Y-MAA was suggested as being useful for fractionated internal radiotherapy of the lung.

1.2.1.4 Polymers
Polymer-based microspheres have many advantages over other materials, in particular their near-plasma density, biodegradability and biocompatibility. However, their major disadvantage is their inability to withstand high thermal neutron fluxes [16]. Additives [13,28] and adjustment of irradiation-parameters [29] can overcome this problem.

Polymer-based microspheres used for internal radionuclide therapy are mainly prepared by a solvent evaporation technique. In the solvent evaporation process, the polymer is dissolved in a suitable water immiscible volatile solvent, and the medicament is dispersed or dissolved in this polymeric solution. The resulting solution or dispersion is then emulsified by stirring in an aqueous continuous phase, thereby forming discrete droplets. In order that the microspheres should form, the organic solvent must first diffuse into the aqueous phase and then evaporate at the water/air interface. As solvent evaporation occurs the microspheres harden, and free flowing microspheres can be obtained after suitable filtration and drying [30]. The solvent evaporation method has been used for preparation of poly(L-lactic acid) (PLLA) microspheres containing $^{166}$Ho, $^{90}$Y and $^{186}$Re/$^{188}$Re.

Mumper et al. [13,28,31] and also our group [29] prepared PLLA microspheres with holmium-165-acetylacetonate (HoAcAc) [32]. HoAcAc complex and PLLA were dissolved in chloroform and the solution was added to a polyvinyl alcohol (PVA) solution and stirred until the solvent had evaporated. Microspheres were graded and collected according to size, on stainless steel sieves of 20-50 µm. These microspheres can be dispensed in patient-ready doses, that only need to be activated by neutron bombardment to a therapeutic amount of radioactivity in a nuclear reactor [29]. These holmium loaded microspheres are currently being tested by intrahepatic arterial administration to rat liver tumours (Fig. 1a). A seven-fold increase of the $^{166}$Ho-microspheres in and around the tumour compared with normal liver is found (Fig. 1b), based on distribution of radioactivity.
Fig. 1. Scanning electron micrograph of holmium loaded PLLA microspheres (a). Radioactive holmium loaded microspheres in an artery of tumour-tissue in rat liver (white arrow). Artificial black line shows the border between tumour tissue (left) and liver tissue (right) (b).

Magnetic PLLA microspheres loaded with yttrium were made by Häfeli et al. [33,34], in order to direct them to the tumour. Their method resulted in stably loaded spheres, with the possibility of pre- or afterloading. To produce preloaded microspheres PLLA was dissolved with L-α-phosphatidylcholine in methylene chloride. Commercially available $^{90}$YCl$_3$ and magnetite Fe$_3$O$_4$ were added to the solution, vortexed, and sonicated. The suspension was injected into PBS with PVA, and microspheres were prepared following a solvent evaporation technique. Afterloaded spheres were prepared by suspending dried microspheres in a solution of PBS, after which $^{90}$YCl$_3$ in HCl was added. Spheres were subsequently vortexed, incubated, and washed, resulting in labeled microspheres. Leaching of $^{90}$Y was around 4% after 1 day in PBS at 37°C. Specific activity was 1.85 MBq/mg in both methods. $^{90}$Y was bound to the carboxylic endgroups of the PLLA. Experiments in mice showed a 12-fold increase in activity in the tumour with a directional magnet fixed above it [34]. Rhenium loaded PLLA microspheres were also developed, but these microspheres were unable to withstand the high neutron fluxes in a nuclear reactor which are necessary to achieve the high specific activity required in the treatment of liver tumours [2].

1.2.2.1 Size of microspheres for treatment of hepatic malignancies
The size of the microsphere is an important factor for the distribution in the liver and tumour. The production parameters of microspheres can be modified to result in high yields of spheres with the desired diameter. For example, lowering the stirring rate during production and a higher viscosity of the aqueous phase, result in increasing diameters of the polymeric microspheres. Although the size of the microspheres can
be regulated by production methods, a sifting step is necessary after production of cold or radioactive spheres.

A range of sizes has been reported in the literature with microsphere diameters varying from 13 to 75 µm [14,22,23,35,36]. The widely used ⁹⁰Y glass microspheres have a mean diameter of 22 µm with a range of 15 to 30 µm [10,12,37].

Larger microspheres, 40-50 µm, are distributed more homogeneously in the liver and with fewer spills to other organs such as lungs and spleen than smaller particles. However, with larger particles the tumour/liver ratio decreases. Microspheres of around 30 µm are the optimum size for hepatic radionuclide therapy, as they are most evenly distributed within the normal liver tissue, yet still provide a concentrated dose of radiation to tumour tissue [8,38,39]. Distribution of intra-arterial microspheres with a diameter of 32 µm was extensively investigated in the microvasculature of tumour in human liver by Campbell et al. [40]. Microspheres were found to deposit preferentially in the richly vascularized periphery of the tumour.

1.2.2.2 Choice of radionuclide

The ultimate radionuclide suitable for internal radionuclide therapy of primary and metastatic malignancies requires the following properties: 1) The radioisotope must have an appropriate radiation spectrum for treating small to large multiple tumours. Large tumours with a vascular periphery but a necrotic centre take up less microspheres per volume, therefore a high energy β-emitter with a subsequently high tissue range is needed to reach the interior of the tumour. 2) A high dose rate is advantageous for the radiobiological effect [41,42]. Consequently, a short half-life is preferable. 3) For external imaging of the biodistribution of the radioisotope with a gamma camera, a γ-emitter is necessary. However, the energy should be low to prevent unnecessary radiation burden to the patient and environment [13]. 4) The labeling of particles has to be simple without any leakage of the isotope. 5) A large thermal neutron cross section is needed to enable high specific activities to be achieved within short neutron activation times [16].

Only a few radioisotopes have characteristics, which make them potentially suitable for the treatment of tumours (see Table 3). Taking into account the aforementioned properties three radioisotopes are the most likely candidates, yttrium-90, rhenium-188 and holmium-166. Yttrium-90 has two major disadvantages as a radioisotope for therapy. First, long neutron activation times (>2 weeks) are needed to achieve therapeutic activities of yttrium because ⁹⁰Y’s precursor has a small thermal neutron cross section of 1.28 barn. Secondly, the biodistribution of microspheres loaded with ⁹⁰Y cannot be directly determined in clinical trials, since ⁹⁰Y is a pure β-emitter and does not produce imageable γ-rays.
Table 3. Characteristics of radionuclides suitable for therapeutical application [43-46]

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Production</th>
<th>Half-life (h)</th>
<th>( \beta_{\text{max}} ) (MeV)</th>
<th>( \gamma ) (MeV)</th>
<th>Max. tissue range (mm)</th>
<th>Cross section (barn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{32})P</td>
<td>(^{31})P(n, ( \gamma ))&lt;br&gt;(^{34})S(d, ( \alpha ))&lt;br&gt;(^{32})S(n, p)</td>
<td>343.2</td>
<td>1.71</td>
<td>7.9</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>(^{90})Y</td>
<td>(^{89})Y(n, ( \gamma ))&lt;br&gt;(^{90})Sr/(^{90})Y</td>
<td>64.1</td>
<td>2.27</td>
<td></td>
<td>11</td>
<td>1.3</td>
</tr>
<tr>
<td>(^{109})Pd</td>
<td>(^{108})Pd(n, ( \gamma ))&lt;br&gt;(^{112})In</td>
<td>13.4</td>
<td>1.03</td>
<td>0.088</td>
<td>4.2</td>
<td>8.8</td>
</tr>
<tr>
<td>(^{140})La</td>
<td>(^{139})La(n, ( \gamma ))&lt;br&gt;(^{140})Ba/(^{140})La</td>
<td>40</td>
<td>1.31 (79%)&lt;br&gt;2.18 (6%)</td>
<td>0.487&lt;br&gt;0.329</td>
<td>10&lt;br&gt;8.9</td>
<td></td>
</tr>
<tr>
<td>(^{153})Sm</td>
<td>(^{152})Sm(n, ( \gamma ))&lt;br&gt;(^{150})Nd (( \alpha ), n)</td>
<td>46.8</td>
<td>0.80</td>
<td>0.070&lt;br&gt;0.103</td>
<td>3.0&lt;br&gt;220</td>
<td></td>
</tr>
<tr>
<td>(^{165})Dy</td>
<td>(^{164})Dy(n, ( \gamma ))&lt;br&gt;(^{166})Hf</td>
<td>2.4</td>
<td>1.29</td>
<td>0.095</td>
<td>5.7</td>
<td>800</td>
</tr>
<tr>
<td>(^{166})Ho</td>
<td>(^{165})Ho(n, ( \gamma ))&lt;br&gt;(^{166})Hf</td>
<td>26.8</td>
<td>1.84 (51%)&lt;br&gt;1.78 (48%)</td>
<td>0.081&lt;br&gt;0.9</td>
<td>8.6&lt;br&gt;64</td>
<td></td>
</tr>
<tr>
<td>(^{169})Er</td>
<td>(^{168})Er(n, ( \gamma ))&lt;br&gt;(^{168})Er(n, ( \gamma ))</td>
<td>230.4</td>
<td>0.34</td>
<td>0.008</td>
<td>0.9</td>
<td>2</td>
</tr>
<tr>
<td>(^{186})Re</td>
<td>(^{185})Re(n, ( \gamma ))&lt;br&gt;(^{186})W/(^{184})W</td>
<td>90.6</td>
<td>1.07</td>
<td>0.137</td>
<td>4.5</td>
<td>110</td>
</tr>
<tr>
<td>(^{188})Re</td>
<td>(^{187})Re(n, ( \gamma ))&lt;br&gt;(^{188})Re/(^{188})Re</td>
<td>17</td>
<td>2.11</td>
<td>0.155</td>
<td>10</td>
<td>70</td>
</tr>
<tr>
<td>(^{198})Au</td>
<td>(^{197})Au(n, ( \gamma ))&lt;br&gt;(^{198})Pt(p, n)</td>
<td>64.8</td>
<td>0.96</td>
<td>0.412</td>
<td>3.9</td>
<td>99</td>
</tr>
</tbody>
</table>
Natural rhenium is composed of two isotopes (\(^{185}\text{Re}\) and \(^{187}\text{Re}\)) that form β-emitting \(^{186}\text{Re}\) and \(^{188}\text{Re}\) radioisotopes respectively, upon neutron activation. The nuclear and dosimetric properties of the rhenium radioisotopes are comparable to those of \(^{90}\text{Y}\), but they have imageable γ-photons and \(^{188}\text{Re}\) is easily available from a \(^{188}\text{W}/^{188}\text{Re}\)-generator system.

Like the rhenium radioisotopes, \(^{166}\text{Ho}\) emits β-particles and photons. It has a physical half-life of 26.8h resulting in a high dose rate. Its cross section is comparable with rhenium, but \(^{165}\text{Ho}\) has a natural abundance of 100% and thus only one radioisotope, \(^{166}\text{Ho}\), is formed by neutron bombardment. Taking these characteristics into consideration, \(^{166}\text{Ho}\) is therefore an attractive candidate for use in future treatments.

1.3 Characteristics in-vitro and in-vivo

1.3.1 Irradiation damage of neutron activated microspheres

Glass is relatively resistant to irradiation damage, as is shown by the long irradiations that are necessary to produce rhenium loaded glass microspheres [16]. The γ-heating, neutrons and other radiation conditions in a nuclear reactor result in considerable irradiation damage to polymers. Literature concerning γ-irradiations for sterilisation of the product can be found to gain an impression of the damage caused by irradiation of polymeric microspheres, which must be irradiated in a nuclear reactor to produce radioactive pharmaceuticals. We investigated the changes in morphology of the surface of holmium loaded microspheres after irradiation [29]. Non-irradiated \(^{165}\text{Ho}\)-PLLA-microspheres show a smooth, spherical appearance, whilst irradiated spheres show minor surface changes of small free PLLA fragments. These fragments represent, under the irradiation conditions used, a negligible part of total particle volume. However, changes in molecular weight of PLLA were substantial [29] and this was also confirmed in other studies using γ-sterilisation [47,48]. Two major mechanisms of degradation (Fig. 2) take place in a polymer as it is subjected to radiation: (1) chain scission occurs as a random rupturing of bonds, resulting in reduction of the molecular weight or, (2) cross-linking which results in the formation of three-dimensional networks. These mechanisms usually occur simultaneously. The cause of the decrease in molecular weight of polymers is mainly radiation chain scission owing to radical formation [49]. In γ-irradiation of PLLA chain scission occurs predominantly in the amorphous phase of the polymer [31,47]. High crystalline polymers are more radiation resistant. The reduction in molecular weight is also dependent on the environment. Oxygen, water, additives and device dimensions are major factors influencing the degradation [29,49,50]. In most polymers such as PLLA, dl-PLA [51], poly(lactic-co-glycolic acid) (PLGA) [52,53,54] or polyglycolic acid (PGA) [55] the mechanisms of degradation are comparable.
1.3.2 Degradation, biodegradability, biocompatibility

Glass and resin particles do not undergo degradation in vivo. Unlike resin or glass microspheres, polymers such as PLLA and PLGA do degrade [10,56]. Polymeric microspheres <300 µm in diameter undergo a homogeneous hydrolytic degradation, the extent of degradation of the core being equivalent to the degradation at the surface [57]. Factors affecting the hydrolytic degradation of biodegradable polyesters are indicated in Table 4. The porosity of microspheres may play a major role in enhancing the rate of biodegradation. Micropores in the spheres permit the release of low molecular weight degradation products, whose carboxylic end groups may facilitate the autocatalytic degradation of the polymer. During the degradation process, the crystallinity of the polymer gradually increases, resulting in a relatively stable product [57].

Fig. 2. Hypothetical degradation mechanisms of PLLA upon γ-irradiation [49].
Table 4. Factors affecting the hydrolytic degradation behaviour of biodegradable polyesters [57]

- Water permeability and solubility (hydrophilicity/hydrophobicity)
- Chemical composition
- Mechanism of hydrolysis (noncatalytic, autocatalytic, enzymatic)
- Additives (acidic, basic, monomers, solvents, drug)
- Morphology (crystalline, amorphous)
- Device dimensions (size, shape, surface to volume ratio)
- Porosity
- Glass transition temperature (glassy, rubbery)
- Molecular weight and molecular weight distribution
- Physico-chemical factors (ion exchange, ionic strength, pH)
- Sterilization
- Site of implantation

Anderson and Shive reported in detail the biocompatibility and tissue/material interactions of biodegradable microspheres [57]. Injection of microspheres, either subcutaneously or intramuscularly, results in the implantation of a high surface area/low volume of material into a given tissue volume. Depending upon the packing and volume of microspheres within an implant site, days to weeks may be required for cellular infiltration from the surface of the microsphere volume, to its centre. The infiltration of inflammatory cells and in particular, monocytes, macrophages and fibroblasts, results in each microsphere having its attendant tissue/material interaction. The volume of microspheres also elicits a response, which is generally seen early as a granulation tissue response leading to a fibrous site. Given biocompatible biodegradable microspheres, the response during the first two weeks is generally similar, regardless of the degradation rate of the biodegradable polymer. A minimal inflammatory reaction is observed. The second phase of the tissue response is initiated by the predominance of monocytes and macrophages. This response occurs after 50-60 days with poly(DL-lactide-co-glycolide) particles, and after more than 350 days with PLLA [58]. The last phase is the breakdown of spheres in particles smaller than 5 µm, small enough to initiate a tissue response which is predominated by macrophages.

1.3.3 Release

Improvements in radiolabelling techniques have resulted in increasingly stable $^{90}$Y microspheres. A new generation of glass (TheraSpheres®, Theragenics, Atlanta, GA, USA) and resin microspheres (supplied by the Australian Nuclear Science and
Technology Organisation) have overcome the problem of leaching. Routine tests on resin and glass microspheres showed that less than 0.1% of the activity leaches from the microspheres. Clinical studies on metastatic liver cancer, using these newer glass [59] and resin microspheres, have produced good results with very little toxicity [23].

Polymeric microspheres loaded with $^{166}$Ho showed a high stability. Mumper et al. and also our group showed that more than 98% of $^{166}$Ho activity was retained in the microspheres after 192h incubation at 37°C, in several physiological media [13,29]. On the one hand, this is a favourable characteristic of these microspheres for the attempted application, but on the other hand this is surprising, since generally low molecular weight compounds are released to a high extent and relatively rapidly from PLLA microspheres [57,60-62]. We found that HoAcAc can be dispersed in the amorphous PLLA phase up to 17% (w/w) indicating the presence of favourable PLLA/HoAcAc interactions in the microspheres. Carbonyl groups of PLLA are likely to interact with the holmium ion in the HoAcAc complex, by which this complex is immobilized in the PLLA matrix. This interaction thus accounts for the high stability (= low holmium release) found in HoAcAc loaded microspheres.

1.3.4 Radiotoxicity
Radiotoxic effects are reported from the old generation of glass and resin microspheres, by leaching of the radioisotope from the particles, which can result in pancytopenia [63]. The new generation of spheres showed no such leaching. These spheres were used for the first time in the well-known dog experiments by Wollner et al. [64], showing that the animals survived a liver dose of up to 350 Gy. However, at such exceedingly high (and clinically unrealistic) doses, sequelae related to cirrhosis would be likely to become a significant problem. Even 800 Gy to the whole liver of rabbits was tolerated [65]. These experiments showed that internal radiotherapy using non-leaking microspheres can be performed safely with high doses. In patients in which doses up to 100 Gy were directed to the liver, the hepatic toxicity of the treatment appears to be low, as is shown by the liver enzyme levels [2]. Gastroduodenal ulceration has been reported as a complication of intra-hepatic arterial glass microsphere therapy, and this may be related to migration of microspheres to organs outside the liver, caused by the high density of the spheres [15,29,66,67]. Radiation pneumonitis was documented in a patient in which the lung dose was greater than 30 Gy, whereas most patients received a lung dose of less than 20 Gy [11,68].
1.4 Application in oncology
Since recent developments, especially the increased skills of interventional radiologists, there is an awakened interest in unique selective radionuclide therapy. Many kinds of radiolabeled particles and radionuclides have been tested for local treatment of a variety of tumours in organs, including liver, lung, tongue, spleen and soft tissue of extremities. The purpose of this treatment is the superselective application of suitable radioactive (high energetic $\beta$-emitters) particles to deliver high doses to the tumour, with as little surrounding tissue damage as possible. These new embolization methods are extremely important particularly for cancers with an extremely poor prognosis and without other adequate therapies, such as primary and metastatic malignancies of the liver.

1.4.1 Liver cancer
Patients with primary or metastatic tumours were treated by radio-embolization via a catheter [59,69] or direct injection of beads into the tumour, with a needle [21,70]. Most studies describe administration of microspheres to patients via a catheter, whereby the tip was placed in the hepatic artery. The spheres eventually lodge in the microvasculature of the liver and tumour, remaining until the complete decay of the radioisotope. Lung shunting and tumour-to-normal liver ratio was determined after infusion of $^{99m}$Tc-labeled macroaggregated albumin, and microspheres were subsequently administered to patients [71,72]. Tumour-to-normal liver ratio was approximately 3-5 [12,22,56,73]. In some studies the blood flow within the liver was temporarily redirected in favour of the tumour by a bolus infusion of a vasoconstrictor, and the spheres were then embolized into the arterial circulation [69]. While external beam radiation causes radiation hepatitis at doses above 30-35 Gy [74] the liver can tolerate up to 80-150 Gy, using internal radionuclide therapy [12,69,72]. Increased longevity, pain relief, tumour response and total clinical improvement are frequently reported [12,24,37,70].

1.4.2 Head-and-neck cancer
Chemo-embolization with ethylcellulose microspheres of 100-450 µm has been used in the treatment of maxillary tumours. The role of intra-arterial radioisotope therapy in the treatment of head-and-neck cancer is just beginning in rabbits, in the work of van Es et al. [75]. The optimal size of microspheres for treatment of unresectable head-and-neck cancer is still to be established. Van Es et al. suggest that large microspheres of 40-65 µm should be used for the embolization of these tumours. Other embolizations in the treatment of head-and-neck cancer have been carried out with particles of 100-450 µm [76].
1.4.3 Ovarian cancer

The overall 5-year survival in patients with epithelial ovarian cancer is as low as 35-38%. Initially, intraperitoneal $^{198}\text{Au}$-colloid was used as an adjunct in the treatment of ovarian cancer, but this resulted in a significant number of major complications and associated deaths [77]. Subsequently $^{32}\text{P}$ became the radioisotope of choice because of a higher $\beta$ energy compared with $^{198}\text{Au}$. Vergote et al. [45] used $^{211}\text{At}$-microspheres for the treatment of mice with ovarian cancer. This was prepared by copolymerization of glycidyl methacrylate and ethylene glycol dimethacrylate with a diameter of 1.8 $\mu\text{m}$. The $\alpha$-emitters such as $^{211}\text{At}$, $^{212}\text{Bi}$ and $^{212}\text{Pb}$ may be a useful addition to future treatment of small clusters of cancer cells.

1.4.4 Other cancers

Intra-arterial administration of $^{90}\text{Y}$-microspheres has been carried out in the spleen [78]. Of nine patients with lymphosarcoma, five manifested no clinical response after splenic irradiation. One patient who complained of weakness, rapid fatigue and anorexia, had relief of all symptoms after splenic irradiation.

1.5 Perspectives

Improvements in radiolabelling techniques have resulted in increasingly stable microspheres with a leakage of less than 0.1% of the activity. Three major radiolabeled microsphere materials, glass, resin-based and polymer-based are now available for therapy or are being tested in animals. The only commercially available glass-type $^{90}\text{Y}$ microspheres are very stable and have a proven reputation in the treatment of primary or metastatic hepatic cancer, but their high density is a serious drawback. Consequently, less dense particles have been developed in the form of resin and polymeric spheres. Resin-based spheres also have a high chemical stability and, with half the density of glass, they provide good prospects for the treatment of liver cancer. Resin particles are currently being investigated in patients, and these particles show results comparable to glass. Polymeric microspheres like poly(L-lactic acid) spheres have a near plasma density and additional advantages such as biocompatibility and biodegradability. A polymeric sphere appears to be the best particle for this kind of therapy, although with the drawback of low resistance to irradiation in a nuclear reactor. Recent studies have demonstrated that polymeric microspheres can be prepared with sufficient amounts of activity for therapeutic application. These microspheres are therefore one of the future materials for use in the battle with liver cancer. Other oncological applications such as the treatment of head-and-neck cancer, bone metastases and ovarian cancer are in the pipeline. Internal radionuclide therapy is likely to play a substantial role in the control of hepatic and other types of cancer in the future.
1.6 Aims and scope of the thesis
In this thesis the feasibility of poly(L-lactic acid) microspheres loaded with \(^{166}\text{Ho}\) for internal radiation therapy of liver malignancies is investigated. Aspects of preparation, characterization, biodistribution and therapeutical effect in animals were addressed in order to answer the following questions:
1. Is it possible to prepare holmium containing microspheres in a reproducible way to enable routine production and facilitate therapeutic application?
2. What are the physical characteristics of these microspheres before and after neutron activation?
3. Can selective targeting of liver tumours be accomplished in vivo?
4. Is there a therapeutic effect of these microspheres on liver tumours?

1.7 Outline of the subsequent chapters
In Chapter 2 the preparation and neutron activation of holmium containing poly(L-lactic acid) microspheres is presented. The microspheres were prepared using a solvent evaporation technique and the effect of the formulation and processing parameters on the holmium loading were evaluated. Further, the effects of neutron irradiation conditions were examined and defined in detail. In Chapter 3 the crystal structures of two holmium acetylacetonate complexes were elucidated using X-ray diffraction. Chapter 4 describes an in depth characterization of PLLA microspheres loaded with HoAcAc. PLLA microspheres, and as a control PLLA films, with and without HoAcAc were investigated using a variety of advanced techniques. Chapter 5 deals with the influence of neutron irradiation on the HoAcAc loaded PLLA microspheres. Possible radiation damage of both the PLLA matrix and the Ho-complex was investigated. In Chapter 6 the biodistribution of PLLA microspheres loaded with \(^{166}\text{Ho}\) is studied in rats with liver tumours. The distribution of the activity, especially the tumour-to-normal liver-tissue ratio, after administration of \(^{166}\text{Ho}\)-PLLA microspheres with sizes between 20 and 50 µm was determined. Also, an extensive histological evaluation was performed. Chapter 7 reports on the therapeutic effect of \(^{166}\text{Ho}\)-PLLA microspheres in a rabbit model. Liver function was examined by histology and analysis of liver enzyme activity. Chapter 8 summarises this thesis and gives suggestions for further research to enable clinical application of Ho-loaded PLLA microspheres for the internal radionuclide therapy of tumours.
References


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