Progress in research and application of PLGA embolic microspheres

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1. ABSTRACT

Transcatheter arterial chemoembolization was an important method in the treatment of solid cancers and the effectiveness of such treatment depends on the nature of embolic agent. Due to the biocompatibility, controllable degradation rate, and both hydrophobicity–hydrophilicity, researches of application on poly(lactic-co-glycolic acid) (PLGA) in medical practice has been ongoing for more than 40 years. We have seen many benefits for patients in recent years. There were five different methods of preparing micrometer-scale microspheres, and three kinds of PLGA microspheres have been subjected to experimental research or used in clinical applications, namely blank microspheres, drug-loaded microspheres, and radioactive microspheres. Hereby, we reviewed the production and clinical and experimental applications of PLGA microspheres in practice.

2. INTRODUCTION

Transcatheter arterial chemoembolization (TACE) was a commonly used medical treatment for solid tumors, especially for hepatocellular carcinoma (HCC). The main purpose of this treatment was to embolize the feeding vessels using an embolic agent delivered through a catheter, causing necrosis of the target site (1-6). The ultimate aim of the treatment was to occlude all arteries supplying the tumor and achieved an ‘inner resection’. However, in clinical practice, there was no evidence to justify such expectations, because multiple factors prevented the complete isolation of the tumor tissues from the blood supply, which made this procedure a palliative treatment for tumors. Among the factors that negatively affect the efficacy of TACE, the nature of the embolic agent was very important. The elasticity and shape of the embolic agent played an important role in its delivery. Irregular embolic agents tended to lodge in larger-diameter vessels more than embolic agents with regular surfaces, resulting in the occlusion of the proximal vascular area (7). In contrast, microspheres were more likely to reach deep areas within the vessels and to achieve complete occlusion. Therefore, embolic microspheres with spherical surfaces and a uniform size on the micron scale, ranging from 40 to 1200 \( \mu \text{m} \), were used to occlude or retard blood flow in feeding vessels or at unexpected sites (Figure 1) (8). Smaller microspheres readily flowed into unexpected organs, such as lung, spleen, and brain, during the operating, known as “nontarget embolization” (9, 10). However, larger microspheres can cause the formation of collateral pathways, when the main trunk was embolized. The collateral pathways could continuously feed the tumor, and failed the purpose of complete occlusion (11).

Based on the degradation characteristics of the different materials used, arterial embolic microspheres...
can be classified into two categories, biodegradable and nonbiodegradable. Nonbiodegradable microspheres included bio-vitreous ion exchange resin, and synthetic polymer microspheres, such as Contour SE®, BeadBlock™, DC Bead™, SAP, Embozene, and Embosphere®, whereas biodegradable microspheres included gelatin, albumin, poly(lactic-co-glycolic acid) (PLGA), starch, chitosan, dextran, sodium alginate, brown algae, hydrogel, and the recently reported Bletilla striata gum. Of all the biodegradable microspheres, PLGA has drawn much attention because of the adjustable degradation rate and hydrophobicity–hydrophilicity (12-30). Most solid tumors required repeated TACE to completely occlude the blood supply, and also need repeated chemotherapy. However, embolization with nondegradable microspheres made it difficult to repeat the procedure because arteries have been permanently blocked and the vascular patterns have changed.

The study and applications of biodegradable materials in medical practice have been progressing for more than 40 years (31-36). Compared with traditional materials, the distinctive advantage of biodegradable materials was their long-term biocompatibility, with no requirement for secondary surgical removal. PLGA was one of the most commonly used synthetic biodegradable polymers in medical practice, and has been approved for use in vivo by a number of national supervising organizations throughout the world, including the U.S. Food and Drug Administration (37). In 1966, Kulkami et al. firstly reported the application of L-polylactic acid (PLLA) in health care in a study that demonstrated that PLLA powder was biologically nontoxic to tissues when implanted in mice and rats (31,38). In 1971, Cutright and Hunsuck reported the use of polylactic acid (PLA) as surgical sutures in orthopedic fixation, which led to the use of this material in medical practice (32). In recent years, the applications of PLGA as an embolic agent has been reported, and hereby we reviewed various studies of the characteristics, the preparation methods, and the different types of PLGA microspheres (39-42).

3. CHARACTERISTICS OF ARTERIAL PLGA MICROSPHERE

Arterial PLGA microsphere was one of the embolic agents used to occlude the blood flow feeding specific areas. PLGA are made of lactic acid (LA) and glycolic acid (GA), both of which are synthetic biodegradable polymers. LA and Ga are nontoxic, biocompatible, with time-controlled degradation, and hydrophobicity–hydrophilicity, and the raw materials are readily available (43,44). Carbon dioxide and water are the final products of their enzymatic degradation, so there was no risk of accumulation in vital organs or vessels, and their degradation rate is linearly dependent on the molecular weight and ratio of the polymers. Shen and colleagues studied the degradation of dl-PLA microspheres in rats and found that the rate of reduction in their molecular weights was fast in the initial period, and then slowed down sharply, especially for larger microspheres (45). Makino’s studies have shown that the release of high-molecular-weight PLGA microspheres
was fast soon after injection, then slowed down, but became fast again later, whereas the release of low-molecular-weight PLGA microspheres was relatively stable (46). Beck et al. designed microspheres with equal relative molecular weights, but composed of different molecular compositions (LA: GA = 100:0, 85:15, 75:25, 65:35, or 50:50). They found that a ratio of 50:50 showed the fastest degradation, with a half-life of 15 days, and that as the GA content decreased, the rate of degradation decreased. LA reduced the hydrophilicity of the material and slowed down the degradation rate because methyl group blocked the hydrolysis of the ester bond. In contrast, GA increased the degradation rate, but the oil solubility of the polymer was reduced when GA exceeds 50%, when the polymer became insoluble in most organic solvents and microspheres cannot be prepared (47). Bae and colleagues found that a molar ratio of 50:50 produced a well-organized porous microstructure, and drugs could be loaded into the pore.

4. PREPARATION OF PLGA MICROSPHERE

So far as we know, there were five methods of preparing PLGA microspheres, among which the emulsion-solvent evaporation, phase separation, and spray drying methods are commonly used in practice.

4.1. Emulsion-solvent evaporation method

This was the most commonly used method of preparing PLA or PLGA, which can produce well-rounded microspheres with smooth surfaces. The process was easy, and was applicable for small-scale manufacturing (40,48). An emulsion of two immiscible phases of fluids was first made, with mechanical stirring and ultrasonic emulsification, and the spherical bodies were solidified when the internal phase infiltrating the outer phase was volatilized. Many systems, based on different phases of the solvent, have been used for various purposes. Oil-in-water (O/W) and oil-in-oil (O/O) systems have been used to embed water-insoluble drugs, and water-in-oil (W/O), oil-in-oil (O/O), and water-in-oil-in-water (W/O/W) systems have been used to embed water-soluble drugs. W/O/O systems can achieve high encapsulation efficiency, and W/O/W systems can embed biological substances, such as proteins and peptides, that were damaged by organic solvents. Currently, we were preparing drug-loaded PLGA microspheres, such as 5-fluorouracil (5-FU)-containing PLGA microspheres, generating stable spherical surfaces, as shown in Figure 2 and Figure 3. Moreover, with the increasing popularity of the drug micronization technology, two new methods of emulsification–solvent evaporation have been developed, oil-in-oil-in-solid (S/O/O) and water-in-oil-in-solid (S/O/W). The former was able to preserve the activity of drugs by avoiding the formation of an oil–water interface during ultrasonic emulsification. As well as the advantages described above, S/O/W can resolve the incomplete release of cumulative microspheres and can be used in large-scale manufacturing, such as the manufacture of peptide- or protein-embedded microspheres, because the particles are easily collected and washed in the discrete phase (49).

4.2. Phase separation method

In this method, the drugs and emulsion droplets were firstly dispersed in the PLGA solvent, forming condensation nuclei, and the new phase (the condensed phase) was formed after polycyagulant was dropped into the PLGA solvent. The solubility of the PLGA solvent was reduced and precipitates on the surfaces of the condensation nuclei (50). Fine spherical microparticles can be obtained by repeating the processes of precipitation, dissolution, and precipitation while the mixture was stirred. The current problem with this method was that a large amount of organic solvent must be used as the polycyagulant, which cannot be removed from the final product, causing toxicity, environmental pollution, and residues of organic solvent. Therefore, smaller microspheres cannot be produced with this method, making it unsuitable for large-scale manufacturing.

4.3. Spray drying method

Polymers were dissolved in a low-boiling-point solvent, into which the granulated drug was mixed. The polymer solution was then sprayed from a nebulizer and dried by up-flowing nitrogen gas, forming microspheres. Because there were fewer parameters required and the sterilization process was simple, this method was suitable for preparing drugs, albumin, and peptide-embedded microspheres on a large scale or for industrial purposes (51,52).

4.4. Supercritical fluid method

Because the density of the fluid was close to that of liquid and its viscosity was close to that of gas, the dissolution capacity and diffusibility of the fluid increased when the temperature and pressure of a fluid exceeded their critical points (53). Because the supercritical fluid and the organic solvent were mutually soluble, the supercritical fluid acted as an anti-solvent to separate the polymer when drug-loaded microspheres were prepared. The polymer, which was slightly soluble in the supercritical fluid, can be separated from the organic solvent, generating microspheres. This method was widely used to prepare drug carriers, and had the advantages of less residual solvent, mild reaction, and short period required. The method was used to either implant insoluble drugs into nanoparticles or to embed drugs in polymers, and drug-loaded microspheres with a core–shell structure can be manufactured, allowing controlled drug release (54-56).

4.5. Membrane emulsification method

This technique involved the imposition of an external pressure on a discrete phase, causing
permeating on inorganic membrane to obtain a continuous phase. Uniform microspheres were obtained by controlling the pressure and membrane bore diameter (57, 58). Compared with traditional methods, such as emulsion-solvent evaporation method, this method was suitable for the large-scale manufacturing of microspheres. The diameter of PLGA microspheres with the Shirasu porous glass (SPG) membrane emulsification method were generally less than 100 μm. The SPG membrane emulsification method was unsuitable for preparing microspheres with more hydrophilic monomers, such as methyl methacrylate, ethyl methacrylate, etc. The SPG membrane was made of hydrophilic Al₂O₃-SiO₂, so the capsule walls were easily wetted by hydrophilic monomers, causing the formation of unevenly sized droplets (59-61).

5. APPLICATIONS OF DIFFERENT TYPES OF ARTERIAL PLGA MICROSPHERES

5.1. Blank embolic microspheres

There have been many reports of the applications of PLGA as drug carrier in the field of medical materials, but few reports of its use as an embolic agent. Flandroy mixed PDLLA of different molecular weights (65000 and 3500 Mr) to prepare microspheres, and injected it into the renal arteries of rabbits (62). He tracked the degradation of the PDLLA with gel-permeation chromatography, measured its molecular weight in vivo, and found that PLGA microspheres with a 50:50 ratio of LA: GA were the most suitable arterial embolic agent. Ding et al. prepared PLA microspheres of 40–120 μm with emulsion solvent vaporization method to investigate their suspension properties, swelling capacity, and digestion in vitro. They found that PLGA microspheres can be preserved in pepsin solution at 37 °C for more than 72 h, indicating that PLGA was maintained for a long time in vivo and can therefore be used for embolization (63).

5.2. Drug-loaded embolic microspheres

Drug-loaded microspheres played dual roles in cancer treatment, both occluding the blood flow through the cancer tissue and maintaining high concentrations of anticancer agents in the target tissue, because the tumor tissue was more sensitive to chemotherapeutic agents and internal radiation under ischemic and hypoxic conditions. The controlled release of drugs from the embolic agent can also reduce any systemic adverse effects and limit its distribution to unrelated organs. In 1985, Sakatoku et al. reported the results of both animal experiments and a preliminary clinical study that showed that after VX2 tumors in rabbit livers were treated with TACE using PLLA microspheres containing 5-FU, they were significantly reduced in size compared with the control group, and that three of eight patients treated similarly showed beneficial effects without severe complications (64). Unfortunately, no further report from this team has been published. Shen et al. prepared PLGA microspheres of 105–180 μm with the phase separation method, and embolized the renal arteries. After 8 weeks, the majority of embolized renal arteries were recanalized to varying degrees, and no microspheres were detected in the recanalized arteries. 5-FU loaded into the microspheres was sustainably released for 42 days, whereas 5-FU
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Injected into the renal arteries alone decreased rapidly, reaching a rather low level 4 h after injection (45). Liu et al. prepared cisplatin-loaded PLGA microspheres, 115.76 ± 35.94 μm in diameter, with drug content of 37.16%. The cisplatin concentration in the liver tissues of the group treated with drug-loaded microspheres reached 21.55 ± 12.18 μg/g, which was significantly higher than that in the cisplatin infusion group (16 ± 0.04 μg/g). The Cmax of cisplatin and the area under the curve in the group treated with drug-loaded microspheres were lower than those in the control group, implying fewer systemic adverse effects (65).

It is important to track the distribution of microspheres and their interactions with the arteries in various organs in vivo. Bastian et al. investigated the distribution of PLGA microspheres of different sizes in a mouse model of metastatic liver cancer. Fluorescently labeled microspheres (17, 25, 30, or 40 μm) were injected into the hepatic artery, and tissue slices from the liver (including the cancer), lung, and spleen were dissected and observed under fluorescence microscope. Microspheres <40 μm failed to completely occlude the artery and dispersed into the spleen and lung, whereas microspheres >40 μm remained in the liver and few were found in other organs. This study confirmed that microspheres used as arterial embolic agents must exceed 40 μm in size. Chen et al. prepared tanshinone A-loaded PLGA microspheres with the emulsion-solvent evaporation method to investigate their blocking action in liver vessels (66). No peripheral hepatic arteries were seen with angiography 1, 3, 7, 14, 21, or 30 days after embolization, only appearing at 30–42 days, which demonstrates that this sort of microsphere was an ideal embolic agent for cancer interventional therapies. Zhong et al. prepared special PLA microspheres made of iodized oil–fluorouracil–PLA compound with the double emulsion method, which were characterized by their goodroundness, equivalent dimensions, porous surface, mean size of 100 μm, 10.78. ± 0.14.4% drug content, 63.3.4. ± 0.55.5% encapsulation efficiency, excellent radiopacity, and sustained release (67).

5.3. Radioactive PLGA microspheres

Because malignant liver tumors currently have a poor prognosis, chemotherapy combined with radiotherapy has become a popular option for enhancing the clinical effectiveness. Therefore, microspheres loaded with radioactive nuclides were developed to both block the blood supply and lower the tolerance of cancer cells to ischemia and hypoxia. Vente et al. used a pig animal model to investigate the action of radioactive holmium-166 PLLA in the hepatic artery in a series of animal experiments. They found that, except for a temporary loss of spirit and appetite in the initial period after injection, the pigs seldom displayed overall toxicity and they found atrophy of the liver lobe on magnetic resonance imaging (MRI). Furthermore, if the absorbable radiation dose in the liver was within 100 Gy, multiple related adverse effects could be avoided (68). In their next study, single-photon emission computed tomography, computed tomography, and MRI were used to detect the distribution of radioactive embolic agents in the liver of the porcine model, demonstrating that a quantitative analysis can be used in practice (69). Smits et al. reported a phase I dose-escalating clinical trial with holmium-166-loaded PLLA microspheres for unresectable liver metastasis, and after data from 15 patients were collected and analyzed, concluded that this method was a practical and safe technique under imaging guidance in clinical practice, and that the maximum tolerable radiation dose was 60 Gy in the liver (70). That study laid the groundwork for the clinical application of holmium-166-loaded PLLA microspheres.

6. SUMMARY

In summary, many kinds of microspheres were under investigation to improve the clinical effectiveness of TACE. An ideal chemoembolization agent should (1) occlude the blood flow completely and effectively; (2) be biocompatible; (3) be soluble to both hydrophilic and hydrophobic drugs; (4) not clog the catheter; (5) have a controlled degradation rate, subject to different needs; (6) allow controlled and sustained drug release to increase the exposure time of the tumor to chemotherapy; and (7) minimize the systemic plasma concentration and any subsequent undesirable adverse effects and toxicity to normal tissues (71). PLGA and its derivatives have a long history of utility as medical materials, and several large-scale experiments with PLGA microspheres have been successfully put into practice. However, no industrial production has yet been reported to our knowledge. One reason for the absence of a commercial product is the lack of a proper large-scale sterilization technique. Even low-temperature sterilization, such as ethylene oxide sterilization, can change or impair the configuration and properties of PLGA microspheres. Our future work will focus on the clinical sterilization of PLGA microspheres.

Drug-loaded microspheres have demonstrated their efficacy in patients with HCC. Drug-eluting beads (DC Bead, BioCompatibles Ltd., Farnham, UK) are a PVA-based microspherical embolic agent made of N-acrylamido acetaldehyde and 2-acrylamido-2-methylpropane sulfonate, and are the most commonly used drug-loaded microspheres currently on the market. Pooled data from six clinical trials have shown high local response rates, ranging from 52% to 81% (81). We conducted a meta-analysis of all published papers concerning drug-eluting beads and drew the conclusion that there was no difference in the disease control or complication rates between drug-eluting-bead TACE and conventional TACE. However, we observed greater clinical efficacy with the drug-eluting beads in...
patients with advanced HCC (72). However, DC beads were hydrophobic, therefore cannot be dissolved in oil-soluble anticancer drugs. Drug-loaded microspheres are promising embolic agents for HCC. PLGA microspheres are soluble to all kinds of anticancer drugs and allow the controlled release of the drugs and their own controlled degradation, making them an ideal embolic anticancer agent. Therefore, drug-loaded microspheres of PLGA deserve further investigation.

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8. REFERENCES


28. Li G, Chen W, Feng H, Zheng C. Flameless atomic absorption spectrometric...


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DOI: 10.1016/j.colsurfb.2004.03.018

DOI: 10.1016/j.jconrel.2008.09.006

DOI: 10.1016/j.jconrel.2008.09.006

DOI not found.

DOI: 10.1016/S0142-9612(02)00084-4

DOI: 10.1016/S0142-9612(00)00115-0

DOI: 10.1081/DDC-100107331

DOI: 10.1016/j.supflu.2010.05.010

DOI: 10.1385/MB:22:3:263

DOI: 10.1016/S0896-8446(01)00064-X

DOI: 10.1016/S1369-7021(05)71036-1

DOI: 10.1111/j.2042-7158.1996.tb05995.x

DOI: 10.1016/j.colsurfb.2007.08.011

DOI: 10.1016/S0927-7757(98)00460-9

DOI: 10.1016/j.memsci.2007.11.029

DOI: 10.1002/jps.22272

DOI: 10.1016/S0168-3659(96)01518-0

DOI not found

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Doi not found


DOI: 10.1007/s00259-008-0747-8

DOI: 10.1007/s10544-009-9291-y


DOI: 10.1016/j.ejps.2011.06.013

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Abbreviations: TACE: transcatheter arterial chemoembolization; PLGA: poly(lactic-co-glycolic acid); HCC: hepatocellular carcinoma; PLLA: l-polylactic acid; PLA: polylactic acid

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