공학석사학위논문

Fabrication of Radiopaque and Biodegradable PLGA Hollow Microparticles Using Microfluidic Devices

미세유체조정장치를 이용한 PLGA 기반 생분해성/조영성 색전 물질의 합성 및 응용

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이 논문을 공학석사학위논문으로 제출함

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조 수 민 의 공학석사학위논문을 인준함

2023 년 5 월

위 원 장 유 웅 열 (인)

부위원장 안 철 희 (인)

위 원 이 명 규 (인)

Abstract

Fabrication of Radiopaque and Biodegradable PLGA Hollow Micro-particles Using Microfluidic Devices

Sumin Cho

Department of Materials Science and Engineering

College of Engineering

Seoul National University

Endovascular embolization is a clinical procedure that selectively occludes the blood vessels that supply oxygen and nutrients to a tumor to induce treatment by blocking blood flow. The demand for endovascular embolization

is growing due to its short procedure time, low blood loss, quick recovery time, and low side effects.

Embolic materials are classified into liquid and solid embolic materials according to their physical state. The application of the liquid embolic material has a large deviation in therapeutic efficacy depending on the operator and has a risk due to its burst release during the procedure. On the other hand, the solid embolic material secures safety and has high usability. Solid embolic materials are injected in the form of dispersed particles in a dispersion medium to block blood flow and occlude blood vessels supplying tumors and are mainly used in the treatment of hyper vascular tumors or cancer.

In the case of solid embolic materials that are currently used in clinical use, there are still difficulties in the procedure due to the lack of materials that are both X-ray opaque and biodegradable. This study aimed to overcome the existing limitations and ensure procedural convenience and safety.

In this study, we developed a solid embolic material for tumor or cancer treatment especially for hepatocellular carcinoma using biodegradable polymer, PLGA. The developed microparticles demonstrate several essential properties, including injectability achieved through density adjustment, radiopacity, sufficient mechanical strength to withstand bloodstream

conditions, and uniform size distribution achieved by utilizing a microfluidic device which is capable of producing hollow microparticles and adjusting density.

Analytical investigations have confirmed to have inherent radiopacity, biodegradability, and injectability through various analytical processes. In addition, the microfluidic device made it easy to adjust the particle size and produce uniform particles.

The biodegradable and radiopaque PLGA hollow microparticles developed in this study using a microfluidic device are expected to be used as solid embolic materials to significantly improve the convenience of the operator and the safety of the patient during intravascular embolization.

Key words: microfluidics, microfluidic device, solid phase embolic material, biodegradable, X-ray contrast, narrow particle size distribution

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1. Introduction

1.1. Endovascular Solid Embolic Materials

Endovascular embolization is a non-surgical treatment that blocks the blood vessels supplying blood flow to the lesion, causing necrosis of the myoma alone. The first embolization using paraffin particles was performed in 1904 to treat head and neck cancer, and the technique of embolization is gradually improving with advances in materials. Moreover, as the technology and materials for imaging become more advanced, it is possible to treat with a short procedure time, fewer side effects during the procedure, quick recovery time, and low risk of infection. Therefore, the demand and possibility of utilization are increasing.

On the other hand, solid-phase embolic materials are actively used in the treatment of tumors and cancers due to their safety and high efficiency, as they can even contain therapeutic drugs inside. Solid-phase embolic material is a flow-directed embolic material, which is based on the movement of solid-phase embolic material with blood flow to block blood flow and selective occlusion of blood vessels supplying the tumor, and is mainly performed within arteries for the treatment of hepatocellular carcinoma. [5-7]

1.2. Procedures for Embolization with Solid Embolic Materials

What is needed to perform embolization is a real-time imaging device, a microcatheter for vascular access, and an X-ray contrast agent that is mixed together for path monitoring purposes to ensure that the catheter reaches the lesion and is positioned in the desired target vessel during the procedure.

The procedure for embolization is as follows: first, the inside of the thigh is punctured for the insertion of the microcatheter, which is then inserted through the femoral artery. A CT contrast agent is injected to monitor its path to the site of the problematic lesion, and the tip of the microcatheter is positioned correctly in the vessel to be treated. The angiographer then injects the embolization material, after the vessel is properly shaped. During this process, it is mixed and delivered with a contrast agent because it does not have contrast properties of its own. After the desired amount of embolic material is injected, an angiogram is performed to confirm that the vessel is completely blocked. In the overall procedure, after blocking the blood vessel, chemotherapy may occur together, in which case the drug loaded inside the embolic material is released, as shown below. The procedure for emboli therapy is illustrated in (Figure1-1).

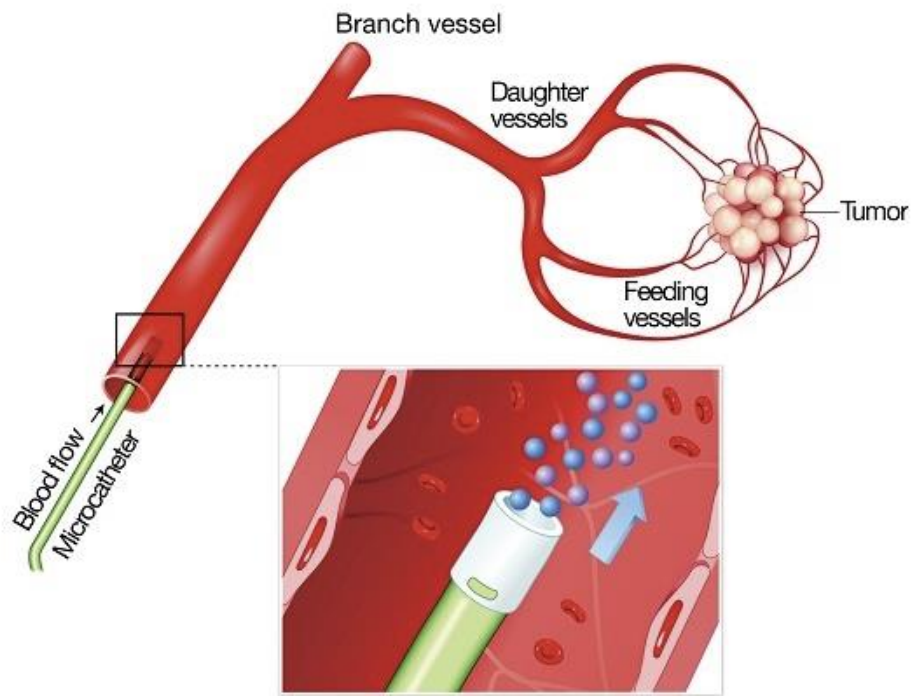


Figure 1- 1. Schematic illustration of endovascular embolization procedure [1].

1.3. Current Solid Embolic Materials in Clinical Use

Currently, representative solid embolic materials commercialized for clinical use include Contour TM (Boston Scientific, Boston, MA), GelFoam ® (Pfizer, Germany), DC Bead ® (BTG, UK), Embosphere ® (Merit Medical, South Jordan, UT), EmboCept (PharmaCept , Germany), and LC Bead Lumi ® (BTG, UK). (Figure 1-2)

For GelFoam ® (Pfizer, Germany) and Contour TM (Boston Scientific, Boston, MA), made of gelatin and polyvinyl alcohol (PVA) [10], respectively, have the disadvantages of poor mechanical properties and large size, which can cause catastrophic damage to normal tissue due to uncontrollable phenomena such as immediate precipitation at the injection site. (Table 1.)

In the case of DC Bead ® (BTG, UK), Embosphere ® (Merit Medical, South Jordan, UT), EmboCept (PharmaCept , Germany), and LC Bead Lumi ® (BTG, UK), the risks associated with utilizing irregularly shaped solid embolic materials have been greatly reduced by their perfect spherical shape, with a narrow distribution of particle sizes.

In particular, EmboCept (PharmaCept, Germany) and LC Bead Lumi ® (BTG, UK), which are made of crosslinked starch and thus biodegradable[8], respectively, or the particles themselves are radiopaque[9], have added

functionalities that are now being commercialized.

Still, there are no commercially available solid embolic materials that are biodegradable and completely degradable after the procedure, while having contrast properties that provide real-time information on the extent of vessel occlusion or location of the embolic material during the embolization procedure.

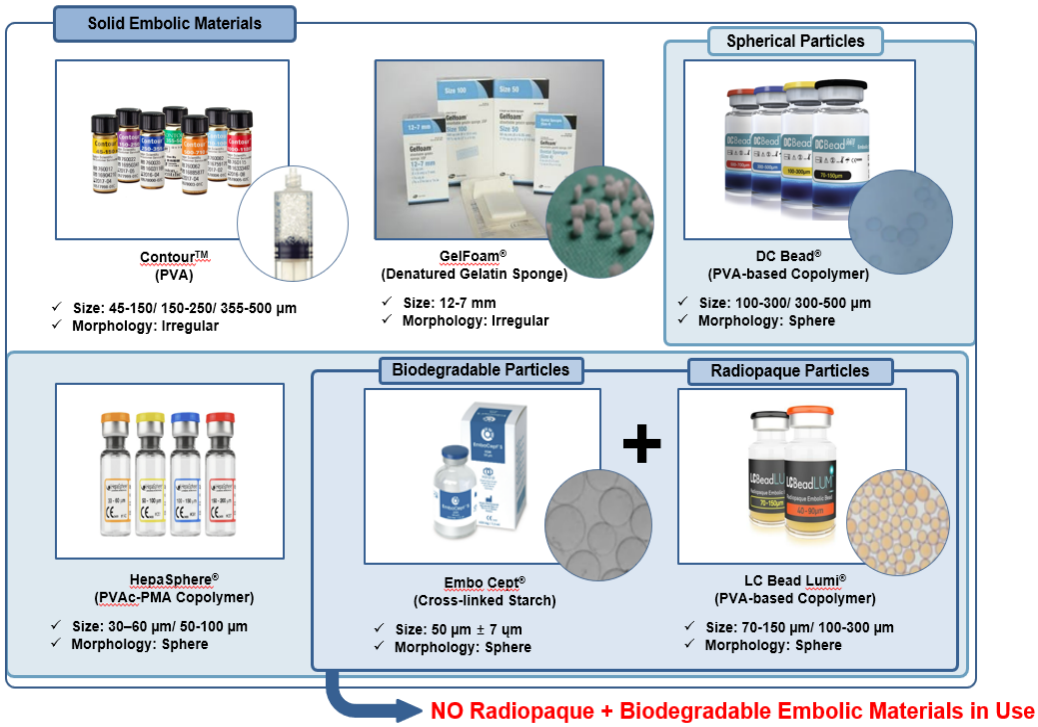


Figure 1- 2. Current Solid Embolic Materials in Clinical Use.

Table 1. Chemical structure and properties of solid embolic materials in clinical use.

Trade Name	Polymer	Particle Shape	Biodegrad-ability	Radio-pacity	Ref.
Contour™	Polyvinyl alchohol	Irregular	X	X	[33]
GelFoam®	Gelatin	Irregular	O	X	[33]
DC Bead®	Polyvinyl alchohol-co-poly(2-acrylamido-2-methylpropane sulfonate	Spherical	X	X	[34]
Embosphere®	Poly(N-acryloyl-2-amino-2-hydroxymethylpropane-1,3 diol)-co-Poly (N-methylene-bis-acrylamide)	Spherical	X	X	[35]
EmboCept®	Cross-linked starch	Spherical	O	X	[36]
LC Bead Lumi®	Polyvinyl alchohol-co-poly(2-acrylamido-2-methylpropane sulfonate	Spherical	X	O	[34]

1.4 Purpose of Research

We aim to develop solid embolic materials that will allow operators to perform embolization procedures more efficiently and safely, by developing a solid-state embolization material that guarantees efficient and safe treatment when practitioners perform embolization.

Conditions of solid embolic materials

While imparting various functionalities to the solid embolic material, it can improve safety and effectiveness, and significantly reduce side effects.

(I) In the case of biodegradable solid embolic materials, after the procedure, the existing blood vessels can be reused, allowing for re-procedures in the event of recurrence.

(II) solid embolic materials themselves have a narrow size distribution, which reduces the risk of particles that are too large or too small being misdirected into normal tissue and causing disruption.

(III) Properly adjust the density of the solid embolic material to improve its injectability. This solves the problem of sinking inside the microcatheter, which prevents accurate determination of the dose.

(IV) By making the embolic material itself X-ray opaque, it is possible to see exactly where the embolic material is moving during the procedure and where it finally settles down, greatly reducing the risk of embolic material being placed where it is not wanted and ensuring that the blood vessel is occluded in exactly the desired area.

(V) The device must be capable of occluding the vessel and have sufficient mechanical strength to withstand the pressure of the solid embolic materials as they flow in under the appropriate pressure, which eliminates the risk of the solid embolic materials being destroyed on its way to the desired vessel.

As there are no commercially available solid-phase embolic materials that have all of the properties of (I) to (V), we tried to develop a solid-state embolic material with all the functionalities mentioned above.

Poly(D,L-lactide-co-glycolide) (PLGA) is one of the most used biocompatible polymers for biomedical applications . In particular, its degradation rate can be adjusted by adjusting the ratio of lactic acid and glycolic acid in the body. Above all, after degradation, the byproducts are also low in cytotoxicity and fast in vitro elimination, so it has many applications as an embolic material due to its good biocompatibility and

biodegradability.[2-4] .

In the study, a biodegradable solid embolic material was developed fabricating PLGA Hollow Microparticle using Microfluidics, and in the process, hydrophilic CT contrast agent was loaded at the core position, inside the particle to have its own contrast, and adjusted the particle to be hollow inside to reduce its density to increase its injectability during the procedure. Ultimately, this made it much easier for the operator to insert a microcatheter into the artery to reach the desired lesion location and inject the embolic material at the desired dose and in the desired location.

2. EXPERIMENTS

2.1 Materials and Equipment

Iopamidol (Pamiray®370, Dongkook Pharmaceutical, Seoul, Korea), PLGA (lactide:glycolide 75:25, M_w 66,000~107,000), Polyvinyl alcohol (PVA; M_w 31,000~50,000, 98~99% hydrolyzed), Span® 80 were obtained from Sigma Aldrich (St. Louis, MO). Chloroform (99%) and dichloromethane (DCM; 99%) were purchased from Sigma-Aldrich (St. Louise, USA).

All microfluidic T-Junction chips[12] used were purchased from Dolomite

Microfluidics (Royston, UK). Fluids were injected into the microfluidic devices by Mitos P-Pump (Dolomite, 3200016) and in-line Mitos Flow Rate Sensors (Dolomite, 3200097 and 3200098) were used to monitor the flow rates. The droplets were tracked in real time under microscope and were focused at the chip junction of the microfluidic chip. [13]

2.2 Methods

Three microfluidic systems are utilized to generate PLGA Hollow Microparticles using the W/O/W method. The types and concentrations of substances within the three systems, as well as the flow rate ratios between the systems, are adjusted to optimize the shape and size of the particles. The T-Junction Chip is a glass microfluidic device designed to facilitate the formation of aqueous droplets when the three fluids meet. The T-Junction Chip, which is the location where droplets are formed, is designed as a double emulsion by sequentially connecting hydrophobic and hydrophilic chips. [14]

2.2.1 Microfluidic System Using Double Junction Chip for PLGA Hollow Microparticles

This approach involves two microfluidic devices arranged in sequence to

generate a double emulsion. First, As in Figure 2-1, the inner fluid generates aqueous droplets using the middle phase (chloroform/PLGA) and forms a W/O emulsion while passing through the chloroform/PLGA with stabilizer, SPAN 80 fluid. The W/O single emulsion continuously passes through a T-Junction chip with a surface-coated inner diameter of 190 μm and 275 μm etch depth (dolomite, 30004) for the hydrophobic channel. It then passes through the channel at the junction connecting the two T-Junction chips and proceeds to the outermost phase (aqueous phase), where it finally forms a W/O/W double emulsion. Overall, this particle fabrication process simplifies complex size homogenization processes like sieving, making size adjustment easier. [15]

The generated PLGA droplets are placed in an outlet containing a PVA solution (5 wt%) and stirred for 1 day at 190 rpm to facilitate solvent evaporation. Afterward, centrifugation and freeze-drying are conducted, and the residual chloroform remaining in the PLGA microparticles is removed by additional vacuum drying for 3 days at 55 °C. This process allows for the collection of solidified particles while preventing potential toxicity issues or side effects that may arise from exceeding the permissible amount of residual solvent during particle fabrication for particles larger than 20 μm in diameter.[16]

2.2.2 Preparation of Iopamidol Loaded PLGA Hollow Micro Particles using Microfluidic Devices

Iopamidol (Pamiray®370) Loaded PLGA Hollow Microparticles are prepared using the water-in-oil-in-water (W/O/W) emulsion method with Microfluidic Devices (Figure 2-1). The three microfluidic systems consist of an innermost water phase, a middle oil phase, and an outermost water phase. Iopamidol (250 mgI/g, 300 mgI/g) is loaded into the innermost water phase, and the PLGA solution (25 wt% in chloroform with 1 wt% ,SPAN80) is used in the middle oil phase with the aim of enhancing the stability of the inner aqueous droplets using SPAN80. Finally, a PVA solution (5% in water) is used in the outermost aqueous phase.

Initially, within the hydrophobic T-Junction Chip[19], the innermost water phase and the middle oil phase form a W/O emulsion. The droplets pass through the channel at the junction and are then transformed into a W/O/W double emulsion by the outermost phase (PVA solution). To stabilize the flow rate of the microfluidic sensor, a register is used to maintain a faster flow rate of the inner phase. The W/O/W double emulsion droplets are collected in a beaker containing PVA aqueous solution (5 wt%). They are then stirred at room temperature at 190 rpm for 1 day.

Finally, the formed Microparticles are washed three times with water

through centrifugation, followed by freeze-drying.

In the process of final confirmation of the remaining solvent, as the chloroform above the limit was confirmed, the freeze-dried particles were recollected for vacuum drying at 55°C for three days. This process successfully removed the entrapped solvent, as confirmed by 1H-NMR (DMSO-d6) 1 H-NMR (CDCl 3 , δ, ppm) = 8.30 .(Figure2-2)

Characterization of PLGA Microparticles

PLGA Hollow Microparticles are optimized for injection by controlling density. The size, thickness, density, and radiopacity of the particles were adjusted by controlling variables such as the flow ratio among three microfluidic systems, PLGA concentration, and contrast agent concentration. For the representative particles used in OM imaging and degradation tests on a double junction chip (with inner diameter of 190 μm and 275 μm), the flow ratios of the innermost water phase, middle oil phase, and outermost phase (PVA solution) were set to 130:40:5 (μl/min). The particle sizes were measured using an optical microscope (OLYMPUS BX51M) at magnifications of 50× and 100×, and ImageJ software was used to determine the average size and standard deviation, which were found to be 102.65 μm ± 12.71 μm and 216.60 μm ± 19.04 μm, respectively. Two types of particles

were used for loading amount, density, radiopacity, and degradation tests. (Figure 2-5)

In the case when the innerphase solution of Iopamidol (Pamiray®370) was prepared at a concentration of 250 mg/I, the mass ratio of Iopamidol to PLGA micro-particles was determined to be 0.0681:1 using 1H-NMR (DMSO-d6), indicating a loading amount of 6.824%. Furthermore, gel permeation chromatography (GPC) was utilized to measure the molecular weight, revealing a degradation time of approximately 9 weeks.

2.2.3 Controlling Particle Size

The size adjustment method is as follows: As the concentration of PLGA increases, the overall size of the particles tends to increase. To maximize particle size, the maximum concentration of the Middle Phase (chloroform/PLGA) applicable in the microfluidic system was 25 wt %. The factors determining particle size are prioritized as follows: PLGA concentration > Aqueous phase (Outer Phase) flow rate > PLGA flow rate. After fixing the PLGA flow rate, the Aqueous phase (Outer Phase) flow rate was adjusted from 100:40 to 200:40 (ul/min), resulting in a decrease in particle size from 216.9 um ± 22.9 um to 197.5 um ± 22.1 um. If the velocity ratio difference becomes extremely larger or smaller than this, the PLGA

droplets rupture, resulting in unstable particle formation.

Controlling Thickness [20]

The thickness of the particles can be adjusted based on the flow rate ratio between the Middle Phase (chloroform/PLGA) and Inner Phase (Aqueous Phase) in the Microfluidic System. As the flow ratio decreases from 40:1 to 40:10, the thickness of the particles decreases from 37.34 um to 27.01 um (Fig 2-6). If the flow ratio exceeds this range or becomes smaller, droplets inside do not form properly, resulting in either non-hollow particles or excessively thin droplets that are not stable.

2.2.4 Controlling Density

The density of the particles is a highly important factor that affects the injectability when using a microcatheter. If the particle density exceeds 1 g/ml, the particles tend to settle down during injection, making it impossible to accurately determine the dose of PLGA micro-particles passing through the catheter. To address this issue, the thickness of the internal PLGA hollow micro-particles, loaded with the iodine compound, was adjusted based on the concentration and flow rate ratio to optimize the density and achieve a density similar to 1 g/ml when dispersing the particles in water.

Specifically, by adjusting the concentration of the iodine compound in the injected Iopamidol (Pamiray®370) from 300 mgI/g to 250 mgI/g, the overall density of the particles was reduced. Additionally, further optimization of the density conditions was achieved through adjustment of particle thickness. The particle thickness can be adjusted based on the flow rate ratio between the Inner Phase (Aqueous Phase) and Middle Phase (chloroform/PLGA) in the microfluidic system. Among the flow rate ratios of Inner Phase (Aqueous Phase) 1-10 (ul/min) compared to Middle Phase (chloroform/PLGA) flow rate of 40 (ul/min), the density of the particles was closest to 1 g/ml at a flow rate ratio of 40:4 (ul/min), indicating a higher likelihood of successful injection.

2.3. Characterization of Microparticles

2.3.1 Elimination of Residual Solvent

After particle preparation, the PLGA microparticles were subjected to vacuum drying at 55°C for 3 days to completely remove the residual chloroform. Chloroform belongs to Class 2 solvents, which should be limited in pharmaceutical products due to their inherent toxicity. It is known that particles larger than an average size of 20 μm are difficult to eliminate residual

solvents through freeze-drying, which can cause side effects such as dizziness, headache, and nausea. The 1H nuclear magnetic resonance (NMR) analysis (Figure 2-2) demonstrated complete removal of residual chloroform after an additional 3 days of vacuum drying of the PLGA micro-particles.

2.3.2 Size Measurement & Surface Morphology of Particles

The overall size of the particles was analyzed using an optical microscope (OLYMPUS BX51M). Droplet images were captured with a magnification of 50× and 100× using a CCD camera (HAYEAR, 5.0MP CMOS). The mean diameter and standard deviation of the microspheres were measured directly from the optical microscope images using ImageJ software (Figure 2-5).

Additionally, the surface morphology of the particles was observed using Field Emission-Scanning Electron Microscopy (FE-SEM 7800). The particles were mounted on double-coated carbon conductive tape (Ted Pella Inc., Redding, CA) attached to aluminum stubs and sputter-coated with Pt for 70 s before imaging with SEM at an acceleration voltage of 20 mV. To observe the thickness of the particles based on the flow rate ratio, the particles were sliced using a cross-sectional approach, followed by Pt coating and imaging with

FE-SEM 7800. The length of the cross sectional of the particles was also measured directly from the SEM images with ImageJ. (Figure 2-5)

2.3.3 *In* vitro Radiopacity/dispersity

The *in vitro* radiopacity of Iopamidol (250 mgI/g) loaded PLGA hollow microparticles was confirmed by comparing their x-ray images with those of contrast agents in clinical use, such as Iopamidol (250 mgI/g, 300 mgI/g). Real-time C-arm x-ray imaging was performed using a Dyna CT device (Siemens, Germany) to evaluate the in vitro radiopacity of the PLGA microparticles.

The dispersity of Iopamidol (250 mgI/g) loaded PLGA hollow micro-particles was qualitatively assessed. An appropriate amount of water was added to a conical tube, and the particles were dispersed. The particle state was compared immediately after dispersion, 5 seconds after dispersion, and 30 seconds after dispersion to determine if the particle dispersion achieved a density similar to 1 g/ml.

2.3.4 *In* vitro loading amount

The actual loading amount of Iopamidol within the PLGA hollow micro-particles was determined through NMR analysis. (Figure2-3) According to the NMR analysis, the loading amount of Iopamidol in the PLGA micro-particles with a diameter of 216.60 um ± 31.06 um was 6.824% (Iopamidol: PLGA MP = 0.0681:1). (Figure 2-2, 2-3) [21]

2.3.5 *In* vitro degradation test

A suspension of 300 mg of PLGA micro-particles was prepared in 40 ml of PBS buffer with pH 6.8 and 7.4, respectively, and placed in a thermostatic bath. The suspension was agitated at 130 rpm and 37°C, and samples were taken at one-week intervals. After collection, the samples were subjected to centrifugation and washed three times with H2O before freeze-drying. The PLGA hollow micro-particles were dissolved in tetrahydrofuran (THF) for gel permeation chromatography (GPC) analysis to measure the molecular weight and assess the degradation rate until complete degradation. (Figure 2-9)

2.3.6 Instruments.

The microfluidic system was performed using Dolomite Microfluidics (Royston, UK), Mitos P-Pump (Dolomite, 3200016) and in-line Mitos Flow Rate Sensors (Dolomite, 3200097 and 3200098). The ^{1}H-NMR analysis was performed using Bruker Advance 300 MHz spectrometer in DMSO-d6. UV / v is spectrometer (UV-2450, Shimadzu, Japan) was used for Dox analysis and the molecular weight of the polymer was measured with GPC (LC-10AD, Shimadzu, Japan).

Optical microscope (OLYMPUS BX51M) and Field Emission-Scanning Electron Microscopy (FE-SEM 7800) was used for measuring size and Surface Morphology. In vitro radiopacity of the sample was evaluated with real-time C-arm x-ray device (Dyna CT; Siemens, Germany).

3. Results and Discussion

3.1 Characterization of PLGA Hollow Microparticles

PLGA hollow microparticles, using the water-in-oil-in-water (w/o/w) emulsion method with Microfluidic Devices, were fabricated. Iopamidol (Pamiray®370), a water-soluble contrast agent, was loaded into the microspheres to provide radiopacity. Iopamidol was loaded into the inner core

of the microparticles. Iopamidol (Pamiray®370) was chosen for its liquid phase, high water solubility, non-toxicity, and high iodine content of 0.7552 g/ml. [22] PLGA solutions with 20 wt% and 25 wt% concentrations in chloroform were used. (Table 2), This minimized the density and viscosity differences between the inner Iopamidol solution and the chloroform (density of 1.5 g/ml) in the microfluidics, facilitating droplet formation. After particle formation, centrifugation, freeze-drying, and additional vacuum drying for 3 days were performed to ensure complete removal of entrapped and residual solvents. Subsequently, PLGA hollow microparticles were dissolved in DMSO for NMR analysis, confirming the complete removal of chloroform [23](Figure 2-2). Additionally, to assess the possibility of Iopamidol evaporation during the 3-day vacuum drying, the particles were dissolved in DMSO, and the solution was analyzed using a UV/Vis spectrometer (UV-2450, Shimadzu, Japan) to compare the amount of loaded water-soluble dye before and after vacuum drying. This ensured the maintenance of radiopacity in PLGA MPs.

3.2 Adjusting the size and cross-sectional length

According to Figure 2-5, the size of PLGA microparticles could be adjusted

based on the PLGA concentration, the flow rate of the outer phase in the microfluidics, and the flow rate ratio between the outer and middle phases. Specifically, increasing the PLGA concentration from 20 wt% to 25 wt% resulted in an overall increase in the average particle diameter. When the flow rates of the outer, middle, and inner phases were 130 ul/min, 40 ul/min, and 5 ul/min, respectively, increasing the PLGA concentration from 20 wt% to 25 wt% led to an increase in particle diameter from 213.3 um ± 26.1 um to 216.6 um ± 19.0 um. Furthermore, gradually increasing the flow rate of the outer phase from 100 ul/min to 200 ul/min at PLGA 25 wt% resulted in an overall decrease in particle diameter from 216.9 um ± 22.9 um to 197.5 um ± 22.1 um. Lastly, the particle size decreased from 219.5 um to 212 um as the flow rate ratio between the outer and middle phases changed.[24]

The thickness of the shell could be adjusted by varying the flow rate ratio between the middle and inner phases while keeping the flow rate of the outer phase constant at 130 ul/min. When the flow rate ratio between the middle and inner phases increased from 40:1 to 40:10 by increasing the flow rate of the inner phase, the shell thickness decreased from 37.34 um to 27.01 um, based on a particle diameter of 216.6 um ± 19.0 um.

(Figure2-5) Optical Microscopy (OM) Image and the particles used for SEM

Image shows particles produced using chips with diameters of 190 um and 275 um, resulting in particle sizes of 102.65 um ± 12.71 um and 216.60 um ± 19.04 um, respectively.

In Figure 2-5, The thickness of microparticles increases from 30.04 um to 36.70 um if the inner phase is changed from 5 ul/min to 3 ul/min, which demonstrates that using a 275 um chip, PLGA 25 wt%, and flow rates of 130 ul/min, 40 ul/min for the middle and inner phases, respectively, the particle thickness increases from 30.04 um to 36.70 um.

3.3 In vitro density for injectability

To ensure injectability, the density of the particles was adjusted. This prevents particles from settling within the catheter, which would hinder accurate dosing. To adjust the particle density, the flow rate ratio between the middle and inner phases was modified while maintaining a thin shell thickness. Despite the thinning of the shell, the increase in the Iopamidol (IOPA) content within the particles was minimal. Therefore, the loading solution of IOPA was reduced from 300 mgI/g to 250 mgI/g to achieve a density similar to 1 mg/ml. The particles were dispersed in water and their

state was observed immediately after dispersion, and at 5 s, 10 s, and 30 s. The optimal flow rate ratio for injectability was determined to be 40 ul/min for the middle phase and 5 ul/min for the inner phase, using 5 wt% PVA and 25 wt% PLGA. (Figure 2-7)

3.4 *In vitro* IOPA loading amount for radiopacity

The loading amount of IOPA in PLGA hollow microparticles was determined to be 6.824% through H NMR analysis. The mass ratio of IOPA to PLGA microparticles was 0.0681:1. (Figure 2-8).

3.5 *In vitro* degradation test.

The biodegradability of micro particles is a crucial factor for their use as solid embolic materials, ensuring vascular occlusion. Without proper biodegradability, the treated blood vessels cannot be reused.[26-28]

In the case of PLGA microparticles, their biodegradability allows for subsequent reuse of the treated site, enabling repeated procedures. Additionally, the degradation rate of PLGA can be adjusted by selecting an L/G ratio of 75:25. To observe the in vitro degradation of hollow

microparticles, particles of two different sizes were utilized, with chips measuring 190 μm and 275 μm, corresponding to particle sizes of 102.65 μm ± 12.71 μm and 216.60 μm ± 19.04 μm, respectively.[29]

As shown in Figure 3, degradation progresses rapidly from 3 to 11 weeks until complete degradation is achieved. Larger particles exhibit slower degradation rates, with a time of 12 weeks required for complete degradation in the case of particles sized 216.60 μm ± 19.04 μm. (Figure 2-9)

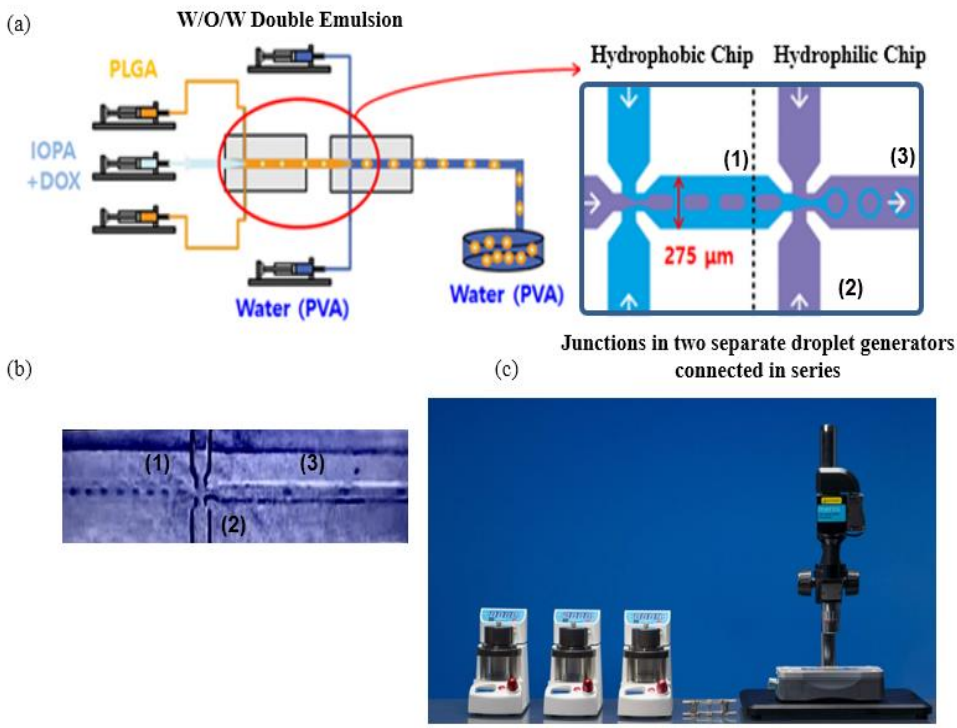
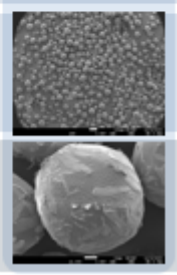
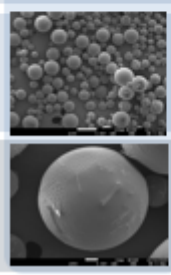
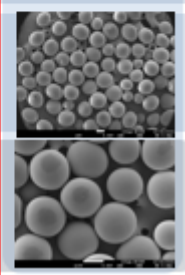
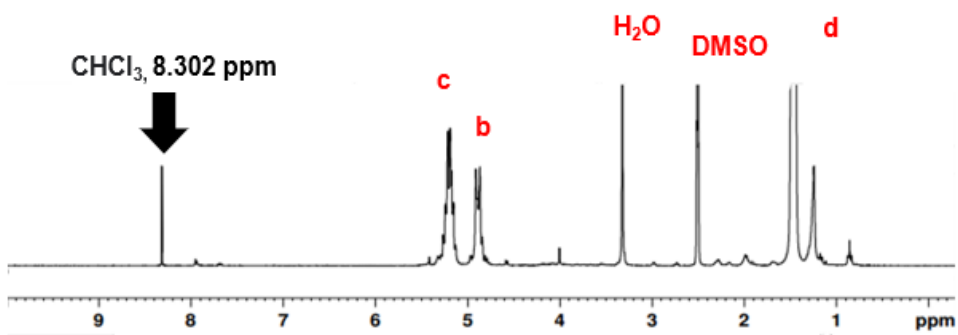


Figure 2- 1. (a) Summary of the design considerations to generate Hollow PLGA Microparticle with microfluidic device and (b)image of the structure of the microfluidic device used for the production of water-in oil-in-water emulsions. (c) three microfluidic Devices and microscope for detecting droplets

Table 2. Candidates for Contrast Agents

	TDS	TIBEE	IOPA®
Physical Phase	Liquid	Solid	Liquid
Solubility in MC	Soluble	Not Soluble	Not Soluble
Solubility in H_2O	Not Soluble	Soluble	Soluble
* Morphology of HMS			

(a)



(b)

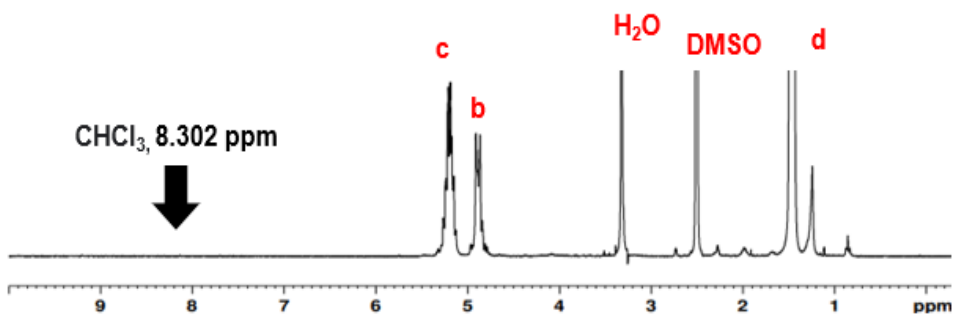
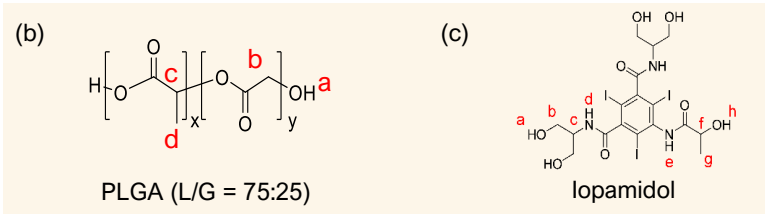


Figure 2- 2. 1H NMR Spectra of Hollow PLGA Microparticle (a) before and (b) after removal of residual $CHCl_3$ of Vacuum Drying for 2 d, 55˚



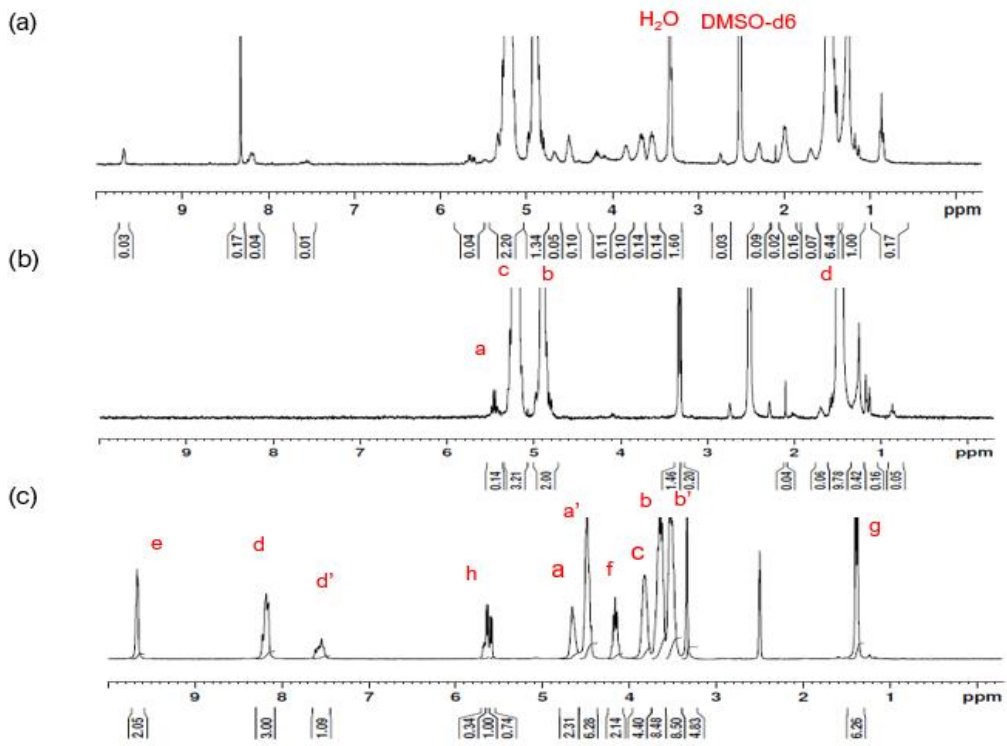


Figure 2- 3. 1H NMR Spectra of (a) PLGA MPs and (b) PLGA (c) Iopamidol

Particle	Diameter (µm)	IOPA: PLGA MPs	Loading Amount	Concentration of IOPA Sol.
IOPA Loaded PLGA MPs (190 chip)	102.65 um ± 12.71 um	0.0681: 1	6.824 %	250 mgI/g
IOPA Loaded PLGA MPs (275 chip)	216.60 um ± 19.04 um			

Figure 2- 4. Mass ratio of IOPA: PLGA MPs and loading amount MPs

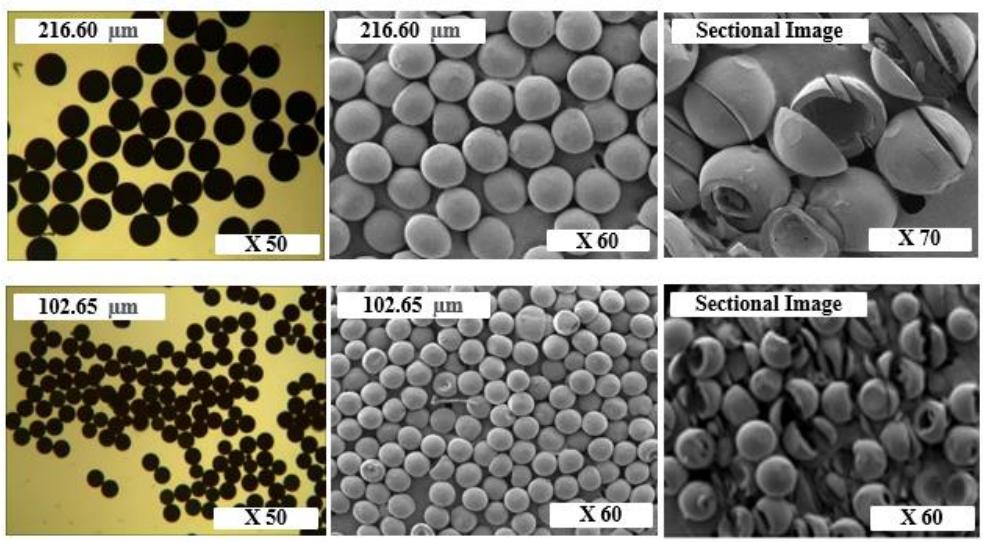


Figure 2-5. (a) OM, SEM image of hollow PLGA MP with a size of 216.60 µm, (b) OM, SEM image of hollow PLGA MP with a size of 102.65 µm

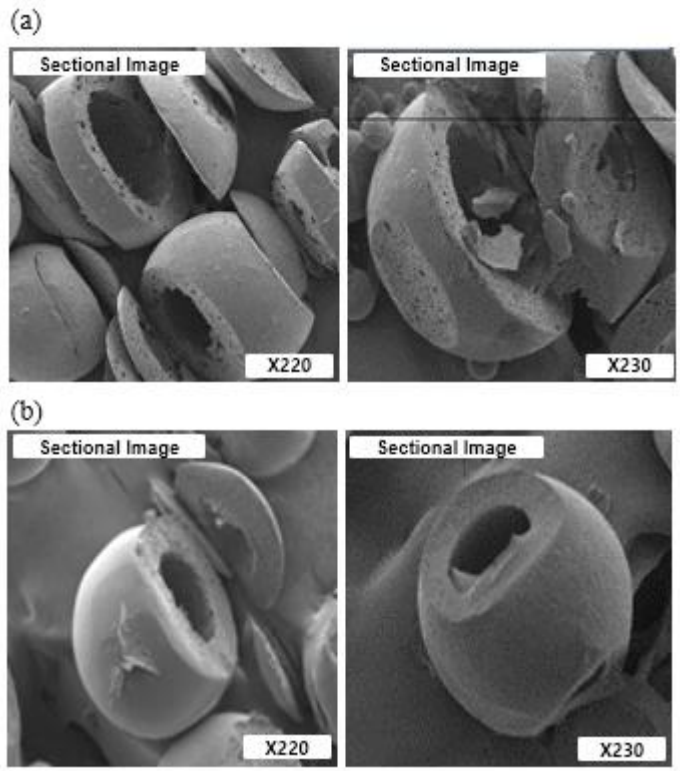


Figure 2-6. Sectional image of Hollow PLGA MP with a size of 216.60 μm with SEM. (a) 40:5 (middle phase: inner phase flow ratio) (b) 40:3 (middle phase: inner phase flow ratio)

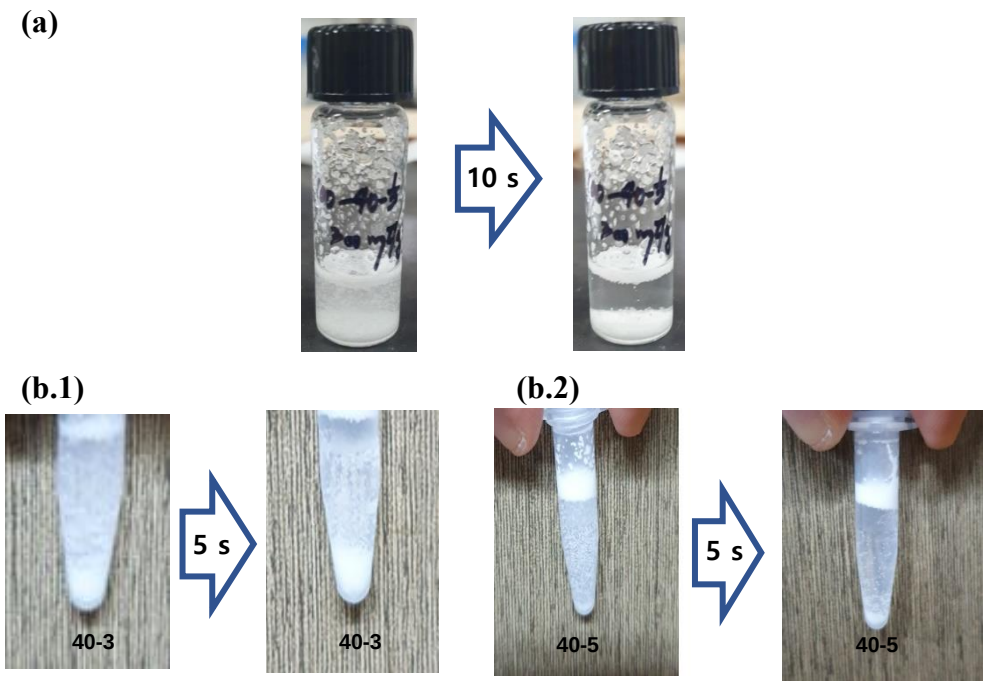


Figure 2-7. (a) Dispersion test with 300 mgI/g of IOPA loaded PLGA MPs (b) dispersion test with flow rate ratio 40:3, 40:5 (middle phase: inner phase) of microfluidic devices

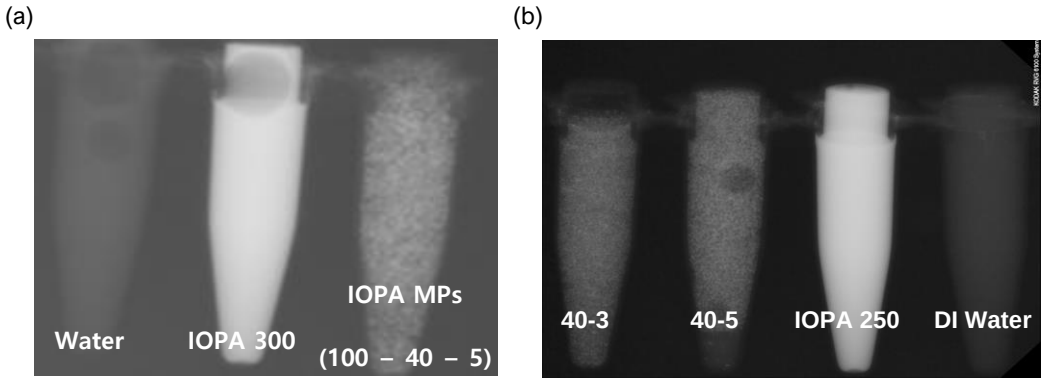


Figure 2-8. In vitro x-ray image of IOPA loaded hollow PLGA MPs and contrast agents, IOPA 300 (300 mgI/g) and IOPA 250 (250 mgI/g)

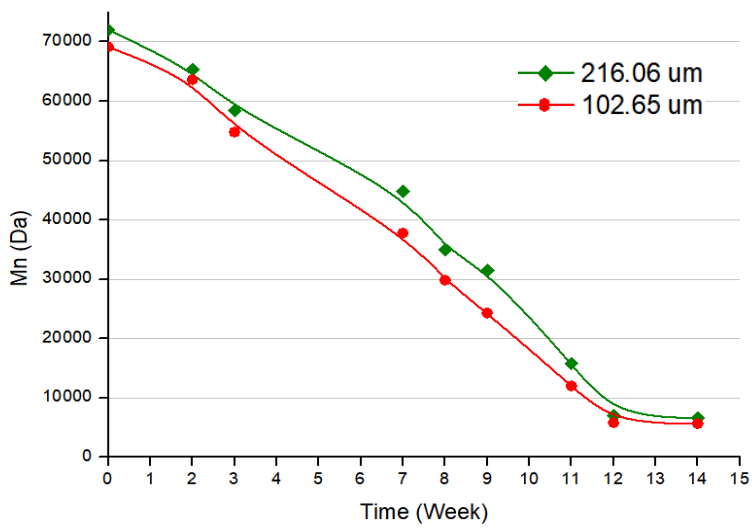


Figure 2- 9. *In vitro* degradation of PLGA MPs with different particle size, monitored by GPC analysis.

4. Conclusion

In this research, radiopaque and biodegradable PLGA Hollow Microparticles with Microfluidic Devices are fabricated for solid embolic materials via w/o/w emulsion method.

By achieving a successful loading of the hydrophilic contrast agent, IOPA, at a rate of 6.82% within the PLGA particles, these particles possess both radiopacity and biodegradability. This allows for repeated procedures in the same location. Furthermore, when PLGA hollow microparticles are used within a microcatheter, they maintain an appropriate density without sedimentation during injection, ensuring dispersion by dispersing the particles in water for 30 seconds.

The microfluidic device's double junction chip, along with adjustments in the flow rates of the three microfluidic phases and the chip's inner diameter, as well as the concentrations of PLGA and PVA, enable precise control over particle size. Moreover, the use of microfluidics results in narrow size distribution of the particles, a significant advantage of microfluidic devices. Through degradation tests, it was observed that the degradation rate varies according to particle size, with a range of 3 to 11 weeks for accelerated degradation. [23] Ultimately, complete degradation occurs after 14 weeks.

In conclusion, PLGA hollow microparticles exhibit high potential for the application of IOPA due to their radiopacity and biodegradability.

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국문 요약

혈관 내 색전술이란 종양에 산소와 영양분을 공급하는 혈관을 선택적으로 폐색하여 혈류 차단을 통해 치료를 유도하는 시술이다. 개복 없는 짧은 시술 시간과 그 과정에서의 적은 출혈량, 빠른 회복 시간과 낮은 부작용으로 혈관 내 색전술에 대한 수요와 활용 가능성은 더욱 높아지는 실정이다.

색전 물질은 상태에 따라 액상, 고상 색전 물질로 분류된다. 액상 색전 물질은 시술자의 역량과 경험에 따라서 치료 효과의 편차가 크고 시술 과정에서의 burst release로 인한 사고 발생 위험으로 활용에 한계가 있다. 이에 반해, 고상 색전 물질은 비교적 안전성이 확보되고 높은 활용성을 가진다. 고상 색전 물질은 입자가 용매에 분산된 상태로 주입되어 혈류를 차단하고 종양을 공급하는 혈관을 폐색하며 주로 과혈관성 종양이나 암 치료에 활발하게 사용된다.

현재 상용화되어 시술에 사용되고 있는 고상 색전 물질의 경우, X선 조영성과 생분해성이 동시에 확보된 물질이 없어 시술 과정에서 여전히 어려움이 존재한다. 본 연구는 기존의 한계를

극복하여 시술 상의 편의와 안전성을 확보하는 것을 목표로 수행되었다.

본 연구는 미세유속조정장치를 이용하여 고분자 PLGA를 활용한 생분해성, 밀도 조정을 통한 주사 가능성, X선 조영성, 혈류에도 유지 가능한 적절한 기계적 강도, 그리고 구형 입자의 균일한 크기 분포를 가진 간암 치료 목적의 고상 색전 물질을 개발했고, 미세유속조정장치를 통해 내부가 비어 있는 형태의 미세구를 제작하여 밀도 조정이 용이하게 하였다.

제작된 미세구는 다양한 분석 과정을 통해 적절한 조영성과 생분해성, 주사가능성이 확보됨을 확인하였다. 또한, 미세유속조정장치를 통해 제작하면서 입자 크기 조정이 용이하였고 입자가 균일하게 제작 가능했다.

본 연구에서 개발된 미세유속조정장치를 활용한 생분해성과 조영성이 확보된 PLGA 미세구는 고상 색전 물질로서 혈관 내 색전술이 진행될 때, 시술자의 편의성, 환자의 안전성을 대폭 향상시키는 데 긍정적인 기여를 하여 그 활용도를 높일 것으로 기대된다.

주요어: 마이크로플루이딕스, 미세유속조정장치, 고상 색전 물질, 생분해성, X선 조영성, 좁은 입자 크기 분포

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