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Development of Image-able Beads for Transcatheter Embolotherapy

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Abstract

Purpose—To develop and characterize radiopaque embolization microspheres capable of *in vivo* detection with intra-procedural fluoroscopy and CT imaging and to evaluate their spatial distribution inside target tissues during and following transcatheter embolization.

Materials and Methods—PVA hydrogel microspheres were loaded with lipiodol and examined for iodine content, stability of loading, and conspicuity with fluoroscopy and CT *in vitro*. Transcatheter embolization of swine liver and kidney was performed with the radiopaque microspheres and spatial distribution was evaluated with intra-procedural fluoroscopy and CT. *Ex vivo* evaluation was performed using light microscopy and micro-CT.

Results—*In vitro* analyses demonstrated that radiopaque microspheres could be loaded with sufficient iodine content to be detected with routine fluoroscopy and CT imaging and that such loading was relatively stable. Radiopaque microspheres were visible *in vivo* with fluoroscopy and CT during transcatheter embolization. CT imaging during embolization procedures demonstrated a dose dependent relationship in the number and size of visualized embolized arteries. Imaging features of radiopaque microsphere distribution inside target tissues correlated well with *ex vivo* light microscopic and micro-CT evaluation of microsphere distribution.

Conclusions—Radiopaque embolization microspheres are visualized during transcatheter embolization with routine intra-procedural fluoroscopy and CT. These radiopaque microspheres provided the three dimensional spatial distribution of embolic material inside target organs during the procedure, and therefore can provide real-time, intra-procedural feedback for the interventional

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radiologist. These microspheres may be useful for demonstrating the influence of material and technical variability in transcatheter embolization in addition to providing intra-procedural identification of tissue at risk of under-treatment.

INTRODUCTION

Minimally invasive transcatheter embolotherapy is a standard treatment option for patients with benign tumors, such as uterine fibroids, as well as for patients with hypervascular tumors. Chemoembolization for liver tumors such as HCC has been successfully performed for nearly three decades, and although the basic procedural steps remain the same, there is variability regarding injected materials and procedural techniques, with a lack of consensus (1–4). For example, there is variability in the i) embolic agent, ii) chemotherapeutic agent, iii) use of lipiodol, iv) degree of catheter selectivity, and v) optimal embolization endpoint (5–8). Material and technical variability is perpetuated by a lack of fundamental knowledge about the spatial distribution of embolic and chemotherapeutic agents. If this knowledge were readily available during the embolization procedure, the interventional radiologist could modify the technique, in real time, to provide a more personalized therapy.

Pre-clinical and clinical experience with microspherical embolic agents has provided some insight into the spatial distribution of these embolic materials inside target tissues following transcatheter embolization (9–10). When compared to non-spherical embolic agents, calibrated microspheres have reduced aggregation, and potentially, a more uniform and predictable distribution (11–15). More recently, calibrated polyvinyl alcohol (PVA) hydrogel microspheres have been developed to contain chemotherapeutic agent(s). These drug-eluting microspheres deliver a chemotherapeutic agent and a uniform embolic material together in a single step that simplifies the chemoembolization procedure and normalizes drug delivery, all of which may decrease procedural variability (16–17). Non-randomized clinical trials on chemoembolization with drug eluting microspheres have reported lower systemic toxicity and improved survival, especially in patients with advanced HCC, when compared to conventional chemoembolization without drug eluting microspheres (16,18–24).

In conventional chemoembolization, radiopaque lipiodol is used as a drug carrier and an embolic material. The radiographic visualization of lipiodol is often postulated to be a surrogate marker of the spatial distribution of chemotherapeutic agents inside the target organ (25). However, *in vitro* studies demonstrate that lipiodol and chemotherapeutic agents rapidly dissociate (i.e., from minutes to hours) and therefore, visualization of lipiodol may not directly indicate presence or optimal levels of drug (26–27). In contrast, drug eluting microspheres release drug much more slowly and in a reproducible and predictable manner (26,28–29). However, drug eluting microspheres cannot be directly imaged with fluoroscopy, and require iodinated contrast material to be mixed with the microsphere suspension to monitor and indirectly guide transcatheter embolization. Despite improved survival with chemoembolization using drug eluting microspheres (16,18–24), delivery of the embolic agent (and thus the drug) is not directly monitored, which may preclude ideal spatial delivery or dosing. Furthermore, visualization of liquid contrast media in which the microspheres are suspended is also not likely to accurately reflect the terminal location of microsphere and drug inside the target tissue. On the other hand, incorporating the imaging agent into the drug source itself (i.e. a radiopaque microsphere) may lead to more accurate spatial localization of embolic as well as drug.

The *objective* of this study was to develop and evaluate embolization microspheres that could be visualized with clinical fluoroscopy and computed tomography (CT). It was hypothesized that the ability to directly visualize microspheres (either bland or drug eluting) during embolization procedures would provide many technical advantages. First, the ability to directly

visualize microspheres with routine fluoroscopic or CT imaging would define their true spatial distribution inside the target organ *in vivo*. As a research tool, this information could facilitate a better understanding of embolization biology, as well as the impact of specific embolization techniques on microsphere spatial distribution. Furthermore, this information could be acquired without requiring post mortem *in vitro* histological analysis. Secondly, knowledge of embolic spatial distribution within the target volume could provide potentially useful “real-time” intra-procedural feedback to the interventional radiologist during the procedure. This information could help optimize the procedure or better define desirable embolization endpoints. In the case of radiopaque drug eluting microspheres in which the chemotherapeutic agent is contained within the embolic material and released slowly over time, the ability to image the embolic material could provide useful information about microsphere distribution and expected local drug concentration inside the target tissue to guide subsequent procedures. For example, under-dosed tissue could then be specifically targeted. These advantages of radiopaque microspheres may optimize, personalize, or improve chemoembolization technique.

To evaluate some of these hypotheses, PVA microspheres were loaded with Lipiodol to create radiopaque microspheres that could be visualized with routine fluoroscopy and CT. These radiopaque microspheres were evaluated *in vitro* for fluoroscopic visualization and CT attenuation, as well as for contrast distribution within the microspheres using micro-CT. The ability to visualize radiopaque microspheres *in vivo* was evaluated in swine liver and kidney during and following transcatheter embolization and confirmed *ex vivo* with micro-CT and light microscopy.

MATERIALS AND METHODS

Preparation of Lipiodol-loaded PVA microspheres

The 100–300 µm LC Bead™ (commercially available PVA hydrogel microspheres from Biocompatibles UK Ltd., Farnham, UK) was used in this study. All reagents used for Lipiodol® (Guerbet, France) loading were of analytical grade. LC Beads (50 mg) were lyophilized in the presence of an excipient and then mixed with 1 mL of Lipiodol. The loaded microspheres were rinsed with 10 mL of saline five times prior to further analysis or use. The final iodine content in dry microspheres was measured by elemental analysis. For elemental analysis purposes, the Lipiodol loaded microspheres were further rinsed with deionized water and wicked dry with absorbent paper, then vacuum dried completely overnight at 40 °C.

Stability of Lipiodol-loaded PVA microspheres

The Lipiodol-loaded PVA microspheres (dry weight 50 mg before Lipiodol loading) were analyzed for Lipiodol release by incubating the microspheres with 100 mL of phosphate buffered saline in a shaking water bath at 37 °C. At predetermined time points, the saline solution was removed and replaced with 100 mL of fresh saline. The Lipiodol released into the 100 mL supernatant was extracted with 50 mL of dichloromethane three times. After removing dichloromethane by rotary evaporation, the oil residue was washed into a 25 mL volumetric flask with absolute ethanol. The Lipiodol content was measured by a Perkin-Elmer Lambda 25 UV-visible spectrophotometer and compared with a standard curve of Lipiodol in ethanol at the wavelength of 260 nm.

In vitro Fluoroscopic and CT evaluation of Lipiodol-loaded PVA microspheres

One mL aliquots of unloaded 100–300 µm PVA microspheres and the Lipiodol-loaded microspheres were placed in polypropylene tubes and allowed to settle for 1 hour prior to imaging. These samples were imaged with routine fluoroscopy (Philips, FD20, Best, Netherlands) and CT (Philips, Brilliance, 16 slice, Cleveland, OH). In order to determine and

compare the contrast provided by the radiopaque microspheres, a reference standard was created using serial dilutions of Isovue 300, a non-ionic contrast material routinely used for CT and angiographic imaging. CT imaging was performed using 1.25 mm slice thickness and 0.625 mm overlap. These images were then analyzed for CT density measured in Hounsfield Units (HU, CT numbers) using “Image J” image analysis software (NIH, Bethesda, MD).

***In vivo* evaluation of Lipiodol-loaded PVA microsphere distribution**

This manuscript was written in keeping with the Society of Interventional Radiology terminology and reporting standards (4). All animal studies were conducted under an animal use protocol approved by the Institutional Animal Care and Use Committee. The ability to visualize radiopaque microspheres *in vivo* during transcatheter embolization was studied in normal swine liver and kidney with routine fluoroscopy using the abdominal vascular setting available on the fluoroscopy unit (Philips, BV Pulsera, Cleveland, OH) and clinical CT using 3 mm slice thickness and 2 mm overlap (Philips, Mx8000, Cleveland, OH). These studies were performed in domestic swine with weights close to that of human patients (approx 70 kg) to ensure relevant size anatomical structures. Following induction of anesthesia, animals were intubated and maintained under general anesthesia with isoflurane. The carotid or femoral arteries were surgically exposed and a 6 French (Fr) vascular sheath was placed. Subsequently, either the celiac or renal artery was selected using 4 or 5 Fr angiographic catheters under fluoroscopy. If needed, a 2.8 Fr microcatheter was used to select lobar hepatic branches or branches of the main renal artery.

Catheter tip position within lobar hepatic arteries or renal arteries was confirmed with fluoroscopy and transcatheter CT angiography (CTA). Prior to radiopaque microsphere embolization, interval CT imaging was performed to ensure contrast washout from hepatic or renal parenchyma and to ensure that stable baseline attenuation was achieved. Instead of a flow-based endpoint (e.g., residual forward flow or complete stasis) traditionally used during transcatheter embolization, a predetermined volume of 1 mL packed microspheres was used as a final endpoint, in an effort to study conspicuity related to microsphere dose. Small volumes (0.2 mL packed microspheres for liver and 0.25 mL packed microspheres for kidney) of radiopaque microspheres suspended in a total volume of 5 mL saline without iodinated contrast material were slowly embolized from the selected artery at a rate of approximately 2.5 mL/minute (including agitation time) under careful fluoroscopic monitoring. Each injection of microspheres was followed by a slow 5 mL saline flush. A fluoroscopic image and CT scan of the targeted organ was obtained directly after each flush. This process was repeated until a total 1.0 mL packed radiopaque microsphere volume was administered and the corresponding imaging was obtained. The axial CT images were evaluated following each injection of microspheres. At the end of the embolization procedure, all CT data were transferred to a post processing work station for three dimensional reformations which more accurately depicted the embolization volume. These were reviewed prior to tissue harvest. At the end of the study, the animal was euthanized and embolized organs were harvested and stored in formalin at 4 °C for *ex vivo* analysis.

***In vitro* and *ex vivo* evaluation of Lipiodol-loaded microspheres with micro-CT**

The distribution of iodine within the radiopaque microspheres was determined with a SkyScan 1172 high-resolution micro-CT (Skyscan, Konitch, BE). The radiopaque microspheres were suspended at a low density in a 1% agarose gel and imaged at 5 µm resolution, 78kV, 127 micro-Amps, using a 0.5 mm Aluminum filter. High resolution three-dimensional spatial distribution of radiopaque microspheres within swine liver and kidney after transcatheter embolization was determined with an Inveon micro-CT (Siemens Preclinical Solutions, Knoxville, TN). The fixed organs were imaged *ex vivo* at a 42 or 49 µm resolution, 80 kV with

a tube current of 380 or 430 micro-Amps and a 2 mm Aluminum filter. Image reconstruction was performed with the Siemens software package using streak and ring artifact reduction.

Analysis of Lipiodol-loaded PVA microsphere distribution

Custom image processing code was written in Matlab (MathWorks, Natick, MA) to isolate and calculate properties of highly attenuating structures (i.e., embolized arteries) resulting from radiopaque microsphere embolization. Liver tissue was isolated from axial CT images with a hand drawn region of interest marked on each slice. The highly attenuating structures were isolated as individual objects in the liver using a consistent, but arbitrary, threshold. Properties of the embolized arteries including number, mean, median and maximum size (approximate length) were calculated for each dose of radiopaque microspheres. These properties may be considered an attribute of the embolic material within each artery.

Confirmation of imaging findings with light microscopic analysis

The location and arrangement of radiopaque microspheres within liver and kidney tissue was documented with light microscopy using a stereomicroscope (Lieca, Bannockburn, IL) equipped with an AxioCam HRc color CCD camera (Zeiss, Thornwood, NY). Micro-dissection of the embolized tissues was performed to isolate microsphere-containing vasculature from tissue parenchyma.

RESULTS

In vitro characterization of Lipiodol-loaded PVA microspheres

The iodine content of radiopaque microspheres was assayed by elemental analysis. Compared to iodine content of pure Lipiodol, which was measured to be 38.2 wt%, the radiopaque microspheres achieved up to 35.7 wt% iodine. Stability of Lipiodol loading was tested by incubating the radiopaque microspheres in phosphate buffered saline at 37 °C over a 27-hour period. As shown in Figure 1, there was an initial rapid release of Lipiodol from the microspheres (8% in the first 4 hours) followed by a more gradual release to a total of 13% over 27 hours. Furthermore, elemental analysis of these microspheres after a 27 hour incubation indicated that iodine content in dry microspheres still remained high (~36 wt%). Therefore, it can be concluded that most of the Lipiodol in the radiopaque microspheres is relatively stable under these very conservative *in vitro* elution conditions.

Due to the high density of Lipiodol (1.3g/cc) compared with water (1g/cc), the initial Lipiodol-loaded microspheres were significantly “heavier” or less buoyant in suspension when compared to LC Bead™, and a durable uniform suspension of radiopaque microspheres in saline was difficult to achieve. However, this did not preclude their delivery through a 2.8 Fr microcatheter when dilute suspensions were used, especially with frequent and gentle agitation of the syringe. Although not used for the *in vivo* imaging studies, addition of iodinated contrast resulted in a more durable and uniform radiopaque microsphere suspension. To address suspensibility issues, the loading method was altered to reduce the amount of Lipiodol loading resulting in microspheres with approximately 18 and 24 wt% iodine. These subsequent generations of radiopaque microspheres, with lower iodine content, were more buoyant and easier to deliver through the catheter. There is a balance between conspicuity and suspensibility. Decreasing Lipiodol content had the benefit of improving suspensibility but at the cost of decreased conspicuity with fluoroscopy. Despite reduced visibility with fluoroscopy, the microspheres remained highly visible with CT and microCT.

Radiopaque microspheres were assayed for detectability *in vitro* with routine fluoroscopy and CT using clinical imaging equipment. As shown in Figure 2, radiopaque microspheres are easily visualized with both radiographic techniques. In fact, these microspheres saturated the

dynamic range of the CT image in all lipiodol loading conditions. Using a standard curve generated from serial dilutions of iodinated contrast shown in Figure 2, the iodine content of the initial Lipiodol-loaded microspheres was estimated to be greater than 38 mg/ml of iodine, a finding which is consistent with results from elemental analysis.

Radiopaque microspheres were also analyzed with micro-CT to determine the distribution of Lipiodol (i.e., iodine) within single microspheres. Single raw data projections are presented for general appearance while reconstructed data are presented to visualize the attenuation within the radiopaque microspheres. Figure 3 shows a micro-CT image of 100–300 μ m radiopaque microspheres suspended in an agarose gel. A single raw data projection is shown in Figure 3A and magnified in Figure 3B to demonstrate the spherical appearance of the radiopaque microspheres. A line profile across a single microsphere is presented in Figure 3C and suggests that more Lipiodol is preferentially concentrated in the outer portion of the microsphere due to the steep increase in the line profile upon entering the microsphere. This distribution is confirmed in the axial reconstructions shown in Figure 3D that demonstrated greater attenuation in the outer portion of the microsphere. It is important to note that Lipiodol does penetrate into the center of the microsphere, albeit at a lower concentration than is present on the microsphere surface.

***In vivo* characterization and visualization of iodine loaded PVA microspheres**

The ability to visualize radiopaque microspheres *in vivo* during and after transcatheter embolization procedures was studied in normal swine liver and kidney using clinical fluoroscopy and CT units. After appropriate catheter tip positioning was achieved and hepatic or renal parenchymal contrast washed out, baseline fluoroscopic and CT images were obtained prior to transcatheter embolization. Small aliquots of radiopaque microspheres (diluted 1:20 – 1:25) were embolized in saline only (i.e., without iodinated contrast material) under careful and continuous fluoroscopic monitoring. Fluoroscopic and CT images were obtained after each aliquot was embolized as shown in Figures 4 through 7. Although radiopaque microspheres could not be visualized leaving the catheter tip with real time fluoroscopy as is observed for iodinated contrast or conventional chemoembolization using Lipiodol, their accumulation in branches of the hepatic and renal arteries was easily visualized with fluoroscopy after approximately 0.4 mL of radiopaque microspheres and after approximately 0.2 mL with more sensitive CT imaging. The attenuation provided by the radiopaque microspheres persisted for the duration of all experiments (~4hrs) with no decrease in conspicuity.

As shown in Figure 4, 1 mL of 100–300 μ m radiopaque microspheres is seen with fluoroscopy in large hepatic and renal arteries. These radiopaque branching vascular structures grew in number and length with each additional embolization. These observations are better visualized with CT imaging. As shown in Figures 5 and 6, radiopaque microspheres are extremely dense and appear to occupy the hepatic and renal arteries following transcatheter embolization. Figures 5 and 6 also demonstrate a dose dependent accumulation observed following embolization with successive aliquots of radiopaque microspheres. The three dimensional spatial distribution of the radiopaque microspheres and the overall dose dependent effect is perhaps best depicted in Figure 7 which shows a surface shaded display image of the data shown in Figure 5. Note the appearance of a vascular cast of the hepatic lobar arteries instead of a homogeneously dispersed appearance of single microspheres. Most of the embolic material appeared as long columns that grow in length with embolization of each microsphere aliquot. These images are shown adjacent to an image obtained from a transcatheter hepatic CTA obtained just prior to embolization using dilute iodinated contrast. Interestingly, the liquid contrast opacified the arterial arborization in an anatomically central (closest to the catheter tip) to anatomically peripheral (furthest from the catheter tip) fashion whereas the radiopaque microspheres opacified the arterial arborization in a peripheral to central fashion,

demonstrating the discordance between observed location of liquid contrast media and eventual microsphere location.

As an example of the utility of radiopaque microspheres for investigating embolization technique and providing real time feedback, the highly attenuating structures in the embolized liver were analyzed for number and size with each successive injection of microspheres, as shown in Figure 8. This technique is useful in addressing the fate of embolic material with successive injection of radiopaque microspheres. For example, with injection of additional aliquots of radiopaque microspheres, do the additional microspheres embolize to the same arteries or to new arteries that were not previously embolized? Image analysis suggests that initially, the number and size of continuous embolized objects increased but then the number of embolized objects reached a plateau as the size of these objects continued to grow (0.6 to 0.8 mL). Then in contrast, potentially additional new arteries were embolized (0.8 to 1.0 mL) indicated by an increased object number with a decreased average size of all the embolized objects.

Ex Vivo visualization of Lipiodol-loaded PVA microspheres with micro-CT

Clinical CT and fluoroscopy are not capable of resolving individual radiopaque microspheres following embolization. Therefore, the embolized liver and kidney tissues were imaged *ex vivo* with micro-CT to obtain higher resolution images and determine the final location of individual radiopaque microspheres with single microsphere resolution. As shown in Figure 9, micro-CT was able to detect radiopaque microspheres within the arteries of the liver and kidney. Because it is possible to resolve single microspheres with micro-CT, (resolution ~50 μ m) these data are considered to be the true location of each radiopaque microsphere. An approximate comparison between clinical CT and micro-CT of the same tissue suggests that for optimal clinical CT visualization, embolization of the artery must pack at least 2–3 microspheres across its diameter. Similar to clinical CT, radiopaque microspheres appeared as columns of stacked microspheres when imaged with micro-CT; however, micro-CT demonstrated that the microspheres are aligned in a single file as well as packed by two or more across the arterial diameter. Additionally, single microspheres within distal renal and hepatic arteries were infrequently identified with micro-CT.

Confirmation of imaging findings with light microscopy

The location of radiopaque microspheres inside embolized liver and kidney tissue was confirmed with stereomicroscopy. As shown in Figure 10, images of dissected tissue confirmed the *in vivo* image findings. Radiopaque microspheres are tightly packed within the hepatic and renal arteries. In larger, more central arteries, numerous radiopaque microspheres are seen filling the diameter of these vessels completely, whereas in smaller, more peripheral arteries, they are seen in a single file arrangement filling and occluding the vessel diameter. Further, careful micro-dissection of the embolized tissues was performed to isolate and follow peripheral branches of these arteries. An example of the most distal embolic event at a branching point is seen in Figure 10C. An isolated artery can be appreciated in Figure 10D. Figure 11 illustrates the close correlation between the three-dimensional spatial distribution of radiopaque microspheres detected with high resolution clinical CT imaging and the true distribution revealed with light microscopy following micro-dissection of swine kidney.

Discussion

Transcatheter embolotherapy is a standard treatment option for patients with hypervascular benign and malignant tumors (4,30–32). However, there is a lack of understanding of the spatial and temporal distribution of embolized material(s) inside the target tissue. In order to address this shortcoming, we developed Lipiodol-loaded, radiopaque PVA embolization microspheres

that are easily visualized with routine fluoroscopy and CT which can be detected *in vivo* during transcatheter embolization procedures to reveal the true spatial distribution of embolic material inside a target tissue. Radiopaque microspheres provide useful intra-procedural feedback that could potentially help to define optimal embolization endpoints, leading to more personalized, patient-specific treatment. They may also facilitate a better understanding of embolization technique and biology.

The ability to visualize radiopaque microspheres depends on three main factors: 1) inherent contrast provided by the Lipiodol-loaded microspheres (i.e., iodine content), 2) distribution volume of the microspheres following transcatheter embolization, and 3) sensitivity of the imaging method and post-processing image analysis. The high amount of iodine content in the dry radiopaque microspheres (35.7 wt%) as compared to Lipiodol (38.2 wt%) is due to the high loading capacity of the PVA hydrogel structure. Unloaded microspheres contain ~95% water when hydrated. When lyophilized, a porous structure is created into which the Lipiodol oil can permeate, occupying space that would otherwise be filled with water when rehydrated (hence a decrease to ~35% water content post-loading of the oil). Although there is higher attenuation and presumably more Lipiodol at the outer region of the microsphere structure, this higher iodine density may reflect the structure of the PVA hydrogel and/or the ability of Lipiodol to fully penetrate the hydrogel homogeneously. It may be possible to gain even greater attenuation if Lipiodol had higher iodine content or if another, more radiopaque, agent were used. However, with 35.7 wt % iodine, microsphere material density (g/cc) is high, which cause microspheres to rapidly settle out of suspension, thus making the embolization procedure more challenging by requiring nearly continuous agitation. It is conceivable that this increased mass of the radiopaque microspheres may lead to preferential localization in dorsal arteries when in the supine position due to gravity. Due to this high density, the iodine content of the microspheres was reduced from 35.7 wt% to 24wt% and 18wt% in subsequent generations. This yielded microspheres with acceptable suspensibility and buoyancy while maintaining adequate visual attenuation. This balance between microsphere handling and attenuation is challenging and may require further refinement of materials.

In contrast to conventional chemoembolization or chemoembolization with drug eluting microspheres, where Lipiodol emulsion or iodinated contrast is visualized leaving the catheter tip, individual radiopaque microspheres (suspended in saline only) cannot be visualized leaving the catheter tip. Ideally, individual radiopaque microspheres leaving the catheter tip could be made visible to the interventional radiologist, but given their small size and limitations of physics and current clinical imaging technology, visualization of single microspheres during a procedure may not be possible with iodine-based contrast. Approximately 0.2–0.25 mL of radiopaque microspheres could be visualized with CT in their terminal location in arteries of liver and kidney following transcatheter embolization. At approximately 0.4–0.5 mL, these microspheres were also visualized with fluoroscopy. It is important to note that the lower limit of detection was not specifically evaluated in this study. This limit is partially dependent on the volume of distribution, i.e., the smaller the distribution volume, the easier it is to visualize a fixed dose of microspheres. In hypervascular tumors that can sequester and effectively concentrate embolic particles, a relatively smaller volume of radiopaque microspheres may be needed for visualization (although this is speculative).

It is interesting to compare the pre-embolization CTA with images of the final microsphere distribution. With progressive embolization with up to 1 mL of packed microsphere volume, the microspheres fill the arterial arborization growing from peripheral to central distribution as opposed to contrast which fills the arterial arborization from central to peripheral distribution (defined with respect to the catheter tip). Given that embolization is a flow-directed process, the peripheral to central accumulation of microspheres is not unexpected; however, this observation raises some questions about the evolution of flow during the embolization

procedure with increasing degrees of embolization. Once the terminal microsphere reaches its final destination in a peripheral artery (largely determined by the microsphere size and compliance relative to luminal diameter at this location), how is residual flow altered and how does this change in flow dynamics lead to the observation of stacked microspheres? For example, is there residual flow through/around the microspheres or is there sustained flow through more proximal collateral branches? Furthermore, imperfect packing of microspheres may not provide substantial resistance to flow due to large gaps and require gradual thrombus formation to cause stasis at the level of the embolized artery. This durable residual flow emphasizes the influence of technique on eventual microsphere locations and, in the case of drug eluting microspheres, the potential importance of degree of stasis upon drug location.

Several groups have investigated embolization microspheres detectable by magnetic resonance (MR) imaging to study the distribution of embolic material inside target tissues (33–36). Collectively, these studies have shown that microsphere size can greatly affect spatial distribution inside target tissues following transcatheter embolization in both normal and tumor-bearing organs (34–35). In addition, MR-based techniques have been developed and refined for monitoring embolization with emphasis on characterizing altered perfusion and defining optimal embolization endpoints (6,37). Presently, the wide spread adoption of MR-based embolization microspheres and imaging techniques is not possible due to the lack of availability of this technology, with the exception of a few academic centers. As an alternative to MR-based techniques, we pursued development of materials that can be imaged with currently widely available imaging techniques including fluoroscopy, rotational or cone beam CT, and standard clinical CT.

In vivo images of the spatial and temporal distribution of radiopaque microspheres inside target tissues are shown in Figures 4–7. As expected, radiopaque microspheres are present within arteries inside the target tissue. However, their spatial distribution occupying long, continuous branching columns with large areas of parenchyma between embolized arteries as opposed to numerous small groups of microspheres homogeneously distributed within the embolization volume was quite striking and somewhat unexpected. Even with microsphere sizes in the 100–300 μm range and a slow delivery of a dilute suspension, the location of the embolic material was fairly proximal and spatially infrequent or sparse. The visualized distribution cannot be explained by over-aggressive embolization as the microsphere dilution and the time/rate of embolization were more conservative than what is commonly used in clinical practice. The lack of “central vs peripheral” concordance between liquid contrast and eventual microsphere location suggests that contrast injection may not be an adequate surrogate for drug delivery, and that the operator may misinterpret where the microsphere or drug actually resides.

With regards to drug delivery from drug eluting microspheres, it should be noted that the sparse columnar distribution of embolic material in the arterial arborization is not an effective or efficient arrangement for homogeneous delivery to all cells inside a target tissue, and could potentially contribute to residual tumor burden or recurrence. In the context of normal tissue embolized here, this sparse distribution may even provide a protective element. On the other hand, a more uniform distribution with smaller distances between adjacent microspheres would produce a more homogeneous spatial distribution of drug in a diffusion-dominated local environment (38).

A major limitation of the current study is the use of a normal swine animal model rather than a tumor bearing animal model. It will be interesting in future studies to compare the spatial distribution observed here with the distribution found in a tumor, where hypervascularity may lead to microsphere sequestration within and around the tumor. A tumor model could show the physician exactly when the assumptions made from arterial contrast flow actually translate into final drug eluting microsphere location

It is currently unclear how well spatial distribution of embolic may be controlled in the setting of a hypervascular tumor, but these findings demonstrate the utility of radiopaque microspheres as a research tool to begin evaluating the influence of material and technical variability in the practice of chemoembolization. The effect of microsphere size and dilution, catheter tip positioning, and degree of stasis, on embolic distribution and subsequent local drug delivery are important questions that may be answered with this tool. By overlapping the final location of radiopaque drug eluting microspheres with the targeted volume, this tool may also provide intra-procedural identification of tissue at risk of under-treatment. In the future, it is conceivable that radiopaque microspheres could facilitate the personalization of chemoembolization with drug eluting microspheres, where the drug, dose, microsphere size or technique will be tailored to a specific patient's tumor histology, size or physiology. Furthermore, radiopaque microspheres may indicate achievement of tumoricidal drug levels, and therefore, identify regions of undertreated tissue requiring further treatment including local (ablative) and regional (infusional) oncologic therapies. Such personalization could have important implications for chemoembolization efficacy.

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ABBREVIATIONS

CT	computed tomography
Fr	French
CTA	computed tomography angiography
MR	magnetic resonance
HU	Hounsfield units

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Figure 1.

In vitro analysis of lipiodol content in radiopaque microspheres. Cumulative lipiodol release in phosphate buffered saline, 37 °C (n=2).

Figure 2.

In vitro detection of radiopaque microspheres with routine fluoroscopy and CT. The image on the left shows iodine loaded PVA hydrogel microspheres which were allowed to settle for 10 minutes in a microfuge tube. The image in the middle was acquired with a clinical fluoroscopy unit and shows the same tube of radiopaque microspheres, adjacent to a saline control. The image on the right was acquired using a clinical 16 detector row CT scanner and shows radiopaque microspheres adjacent to microfuge tubes containing serial dilutions of Isovue 300 contrast material (the numbers above each tube depict the Iodine concentration in that tube). Based on the serial dilution standard, packed radiopaque microspheres contain greater than 38 mg/ml iodine.

Figure 3.

Micro-CT detection and evaluation of radiopaque microspheres. Panels A and B are a single raw data projection. Panel A is an image obtained with a micro-CT showing 100–300 μm radiopaque microspheres suspended in agarose gel. Panel B shows the radiopaque microspheres at higher magnification. A line was drawn through the center of a radiopaque microsphere and the density values of pixels along this line were calculated and used to trace the “line profile” shown in Panel C. This demonstrates that the iodine is preferentially located on the surface of the radiopaque microspheres. Panel D shows a composite of axial reconstructions of individual radiopaque microspheres obtained with a micro-CT. These confirm the findings that iodine is preferentially located on the surface.

Figure 4.

Fluoroscopic detection of radiopaque microspheres during transcatheter embolization in swine liver and kidney. The top left image was obtained from a selective hepatic angiogram with the catheter tip in a lobar branch and showing contrast opacification of peripheral hepatic artery branches. The top right image was obtained after embolization of this lobar branch with 1.0 mL of 100–300 μ m radiopaque microspheres. Arrows depict radiopaque microspheres packed within peripheral hepatic artery branches. The bottom left image was obtained approximately 10 minutes after selection of the renal artery. Iodinated contrast is seen in the urinary collecting system. The bottom right image was obtained after embolization of the renal artery with 1.0

mL of 100–300 μ m radiopaque microspheres. Arrows depict radiopaque microspheres located within peripheral branches of the renal artery.

Figure 5.

Detection of radiopaque microspheres with CT during transcatheter embolization of swine liver. Representative axial CT images of swine liver at the same table position acquired with a clinical 16 detector row CT scanner during transcatheter embolization with radiopaque microspheres. The pre-embolization image demonstrates baseline attenuation of the swine liver. The dense structure adjacent to the left side of the vertebral body is the catheter, located within the descending aorta. Following transcatheter embolization of 0.2 mL increments of radiopaque microspheres from a catheter with its tip in a lobar branch of the hepatic artery, there is a progressive increase in the number and size of dense vascular structures within the liver. A dose dependent effect is seen following embolization with additional radiopaque microspheres.

Figure 6.

CT detection of radiopaque microspheres during transcatheter embolization of swine kidney. Representative axial CT images of swine kidney at the same table position acquired with a clinical 16 detector row CT scanner during transcatheter embolization with radiopaque microspheres. The dense material seen on the pre-embolization image is iodinated contrast excreted into the urinary collecting system. The excreted contrast was allowed to wash out prior to radiopaque microsphere embolization. The other images demonstrate progressive accumulation of radiopaque microspheres inside renal arteries, which are located within the renal cortex (more peripheral than the central urinary collecting system). Note the dose dependent accumulation following embolization with additional volumes and numbers of radiopaque microspheres.

Figure 7.

Detection of radiopaque microspheres with CT during transcatheter embolization of swine liver. Representative three-dimensional surface-shaded display (SSD) images of swine liver reconstructed from data acquired with a clinical 16 detector row CT scanner during transcatheter embolization with radiopaque microspheres. The image labeled “Contrast” is from a pre-embolization transcatheter CT angiogram using dilute contrast. The other images were obtained following transcatheter embolization of 0.2 mL increments of radiopaque microspheres from the same catheter tip location within a lobar branch of the hepatic artery. This sequence shows a progressive increase in the number and size of hepatic arterial branches in the embolized volume of liver. A dose dependent effect is easily appreciated confirming the findings seen with real time fluoroscopy and axial CT images. Note the central vessel filling of contrast versus the peripheral first filling from radiopaque microspheres.

Figure 8.

Spatial and temporal distribution of embolic microspheres inside the target tissue. Custom software was used to analyze post embolization axial CT images from swine liver to isolate highly attenuating structures (i.e., embolized arteries). The number and size (approximate length) of the embolized arteries calculated for each embolized microsphere volume. This technique is useful in addressing the fate of embolic material in the embolized volume with each additional embolization. These results suggest that initially, the number and size of embolized arteries increased, but the number of embolized arteries reached a plateau as the size continued to increase (0.6 to 0.8 mL). At this point, as new arteries were embolized (0.8 to 1.0 mL), the average size of the embolized arteries decreased.

Figure 9.

Visualization of radiopaque microspheres with micro-CT. Embolized liver and kidney tissues were imaged ex vivo with micro-CT to obtain higher resolution images and determine the final location of individual radiopaque microspheres. The kidney coronal and sagittal maximum intensity projection (MIP) images on the left demonstrate the spatial distribution of radiopaque microspheres within the arterial arborization of kidney. The MIP image on the right shows liver tissue. Because it is possible to resolve single microspheres with micro-CT (resolution $\sim 50 \mu\text{m}$), these data are considered to be the true location of each microsphere. Similar to findings obtained with clinical CT imaging, columns of stacked microspheres were seen; however, micro-CT demonstrated that the radiopaque microspheres are aligned in a single file as well as stacked by two or more across the arterial diameter. Additionally, infrequent single radiopaque microspheres within distal renal and hepatic arteries are also identified with micro-CT.

Figure 10.

Confirmation of radiopaque microsphere location with stereomicroscopy following transcatheter embolization in swine liver and kidney. Radiopaque microsphere location within renal (a) and hepatic (b, c, and d) arteries detected with fluoroscopy and CT imaging was confirmed after microdissection and examination with a stereomicroscope equipped with a color CCD camera. a) Shows radiopaque microspheres located within larger, more central renal arteries as well as in smaller, more peripheral branches. b) Shows a large hepatic artery located adjacent to portal vein and bile duct. The artery is tightly packed with radiopaque microspheres. Note that several radiopaque microspheres are required to fill the large arterial diameter. c) Shows a short segment of a small hepatic artery dissected free from surrounding hepatic parenchyma. Note the terminal microsphere located at a branch point in the artery. d) Shows single file radiopaque microspheres located within a small hepatic artery completely dissected free from surrounding parenchyma.

Figure 11.

Correlation of *in situ* location of radiopaque microspheres with CT imaging. The figure on the left shows radiopaque microspheres located within renal arterial branches following transcatheter embolization. This *in situ* location of radiopaque microspheres closely correlates with the distribution depicted by *in vivo* CT imaging obtained during transcatheter embolization as shown in the figure on the right.