An In Vitro Evaluation of Four Types of Drug-Eluting Microspheres Loaded with Doxorubicin

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ABSTRACT

Purpose: To compare in vitro properties of 4 drug-eluting embolic agents loaded with doxorubicin.

Materials and Methods: DC Bead (100–300 μm), LifePearl (200 μm), HepaSphere (30–60 μm), and Tandem (100 μm) microspheres were loaded with 40 mg/20 mL of doxorubicin per milliliter of microspheres. Loading, elution, diameter changes after loading, changes in the amount of doxorubicin loaded over 2 weeks in storage, and time in suspension were evaluated.

Results: All microspheres loaded > 99% doxorubicin within 1 hour. In vitro elution reached a plateau by 6 hours, with 30% ± 5, 21% ± 2, 8% ± 3, and 6% ± 0 of the loaded doxorubicin eluted for LifePearl, DC Bead, HepaSphere, and Tandem microspheres, respectively, with at least 1 statistically significant difference between at least 2 of the products in doxorubicin eluted at every time point. The times to elute 75% of the total released doxorubicin were 197, 139, 110, and 77 min for DC Bead, LifePearl, HepaSphere, and Tandem microspheres, respectively. The average diameters of LifePearl, DC Bead, and Tandem microspheres were reduced after loading by 24%, 20%, and 9%, respectively. After suspension in contrast medium, no changes were observed in doxorubicin loading over 2 wk. After loading, times in suspension were 8.4 min ± 0.2, 6.0 min ± 0.1, 3.1 min ± 0.2, and 2.9 min ± 0.3 for Tandem, LifePearl, DC Bead, and HepaSphere microspheres, respectively.

Conclusions: Although drug-eluting embolic agents universally loaded doxorubicin within 1 hour, the elution amounts, rates of release, diameter shrinkage, and times in suspension varied by product.

ABBREVIATION

HPLC = high performance liquid chromatography

A randomized clinical trial evaluating transarterial chemoembolization for the treatment of intermediate-stage hepatocellular carcinoma (1) showed the benefit of doxorubicin-loaded drug-eluting embolic agents compared with transarterial chemoembolization with Lipiodol (Guerbet, Villepinte, France). Currently, various types of drug-eluting embolic agents are commercially available for use with doxorubicin, including DC Bead (BTG, Farnham, United Kingdom), HepaSphere (Merit Medical, South Jordan, Utah), LifePearl (Terumo European Interventional Systems, Leuven, Belgium), and Tandem (Boston Scientific, Marlborough, Massachusetts) microspheres. DC Bead microspheres consist of a polyvinyl alcohol hydrogel modified with sulfonate groups (2). HepaSphere microspheres consist of a poly(vinyl alcohol-co-sodium acrylate) hydrogel (3). LifePearl microspheres consist of a hydrogel network of poly (ethylene glycol) and 3-sulfopropyl acrylate. Tandem microspheres consist of a hydrogel core made of sodium poly(methacrylate) and an outer biocompatible shell of poly(bis[trifluoroethoxy]phosphazene) (4). For all four types of microspheres, the drug loading mechanism is the ionic interaction of the cationic doxorubicin with the anionic functional groups of the microspheres.

Despite the clinical use of several types of doxorubicin-loaded microspheres, a systematic analysis of the similarities

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T.d.B is a paid consultant for Terumo (Tokyo, Japan). S.P., R.Y., A.S., Y.W., and G.M.C. are paid employees of Terumo.

J Vasc Interv Radiol 2016; 27:1425–1431

http://dx.doi.org/10.1016/j.jvir.2016.05.015

and differences of the commercially available products is lacking. The in vitro characteristics of DC Bead and HepaSphere microspheres loaded with doxorubicin have been reported (2,5); however, the characteristics of LifePearl and Tandem microspheres loaded with doxorubicin have not been reported to our awareness. As such, the purpose of the present study was to compare in vitro properties of DC Bead, HepaSphere, LifePearl, and Tandem microspheres loaded with doxorubicin.

MATERIALS AND METHODS

Materials

Microspheres evaluated in this study included DC Bead (100–300 μm), HepaSphere (30–60 μm dry and 120–240 μm expanded), LifePearl (200 μm), and Tandem (100 μm) microspheres. Generic doxorubicin hydrochloride (Pfizer, New York, New York) at a concentration of 2 mg/mL and nonionic Omnipaque (GE Healthcare, Princeton, New Jersey) 300 mgI/mL were used in this study.

Doxorubicin Loading, Elution, and Loading Stability

Each package of microspheres, containing 2 mL of microspheres, was split into two equal samples to form 1-mL aliquots. Doxorubicin loading of five aliquots of each product was then evaluated in accordance with the manufacturers' instructions. The excess supernatant was removed, and 20 mL of doxorubicin (40 mg) solution was added. The microspheres were agitated on a rocker during the incubation. At 0.25, 0.5, 1, 2, 3, 4, 5, and 24 hours of incubation, 50 μL of the solution was removed and diluted with 1.0 mL of water, and the concentration of doxorubicin was quantified by using a validated high-performance liquid chromatography (HPLC) method. Briefly, doxorubicin concentration was quantified by using an Agilent 1260 Infinity HPLC system with a Phenomenex Gemini-NX 3-μm C18 column (4.6 mm × 50 mm) from 5 to 400 ppm. The mobile phase was 73:27 15 mM $NH_4OH/(NH_4)3PO_4$ (pH 10):acetonitrile at a rate of 1 mL/min. The injection volume was 10 μL, and the wavelength was 234 nm. Mass and percentage loading were calculated from the concentration data.

The diameters of unloaded and doxorubicin-loaded microspheres were measured by using an AxioZoom V.16 motorized stereo zoom microscope with a Plan NEOFLUAR Z 1×/0.25 free working distance 56 mm objective and an AxioCam high-resolution cooled camera (Zeiss, Thornwood, New York). Diameter measurements were calculated by using AxioVision SE64 measurement software (Zeiss). For each measurement group, the diameter of at least 200 μm was determined.

For determination of elution, doxorubicin-loaded microspheres were placed in one of seven flow cells of a CE 7smart USP 4 system (Sotax, Westborough, Massachusetts). Five replicates per microsphere group were evaluated. The Sotax elution system was prepared with a 500-mL sink of 0.9% saline solution per channel at a temperature of 37°C and a flow rate of 8.0 mL/min. Samples, 1 mL per time point, were taken at 0.33, 0.67, 1, 2, 3, 4, 5, 6, and 24 hours after the beginning of the elution. Doxorubicin concentration was determined by using the validated HPLC method described earlier. Mass and percentage elution were calculated from the concentration data.

For determination of stability of the loading over time in storage, doxorubicin-loaded microspheres were placed in 65:35 (volume/volume) contrast agent:water for injection solution or 100% contrast agent. Five replicates per microsphere group were evaluated per aqueous solution. After loading, microspheres were placed in 10 mL of the solution and stored at 4°C. At periodic time points from 1 hour to 10 days, samples were collected and analyzed by using a validated HPLC method with a range from 5 to 400 ppm to quantify the doxorubicin in the fluid. Briefly, with the use of the same equipment and column described earlier, a gradient method with the same mobile phases was used. The injection volume was 10 μL. The mobile phase flow rate was 1 mL/min with 15%–33% acetonitrile from 0 to 2.5 minutes and 33% acetonitrile from 2.5 to 2.6 minutes. Mass and percentage elution were calculated from the concentration data.

Time in Suspension

Time in suspension was evaluated for doxorubicin-loaded microspheres in a manner previously described (6). Six replicates per microsphere group were evaluated. After loading, the supernatant was expressed from the microspheres and replaced with 8 mL of 50:50 Omnipaque 300:water for injection (volume/volume) solution in a 10-mL syringe. The microspheres and contrast agent:water solution was mixed by 15 passes of syringe-to-syringe mixing. At the end of the mixing, all the contents were placed in one syringe, and that syringe was immediately placed vertically on a countertop. The time taken for the microspheres to vacate one third of the syringe was taken as the time in suspension.

Statistical Analysis

Statistical analyses were performed by using Minitab 17 (Minitab, State College, Pennsylvania). Differences in continuous data were assessed by using a one-way Welch analysis of variance. If the analysis of variance indicated a significant difference, a two-tailed t test was performed to identify the specific differences. Statistical significance was accepted at $\alpha \leq 0.05$.

RESULTS

Doxorubicin Loading, Elution, and Loading Stability

All four microsphere types quickly and repeatedly loaded doxorubicin (Fig 1). HepaSphere microspheres

incorporated doxorubicin in less than 15 minutes, as the microspheres were rehydrated by the doxorubicin solution. Loading of more than 99% of doxorubicin in the solution was achieved within 1 hour for all microspheres. The loading of HepaSphere microspheres was significantly greater than that of DC Bead microspheres at 15 minutes ($P = .001$), 30 minutes ($P = .001$), 1 hour ($P = .01$), and 3 hours ($P = .01$). The loading of LifePearl microspheres was significantly greater than that of DC Bead microspheres at 15 minutes ($P = .04$) and 30 minutes ($P = .01$). The loading of HepaSphere microspheres was significantly greater than that of Tandem microspheres at 1 hour ($P = .001$), 3 hours ($P = .001$), 4 hours ($P = .0001$), and 5 hours ($P = .001$). The loading of Tandem microspheres was significantly greater than that of DC Bead microspheres at 30 minutes ($P = .001$), 1 hour ($P = .01$), and 24 hours ($P = .001$). At 24 hours, the loading of Tandem microspheres was significantly greater than that of LifePearl microspheres ($P = .001$).

DC Bead, LifePearl, and Tandem microspheres were separate and spherical before and after loading with doxorubicin, whereas HepaSphere microspheres were not uniformly spherical after hydration and loading with doxorubicin (Fig 2). LifePearl and Tandem microspheres had more uniformity in diameter compared with DC Bead when unloaded and doxorubicin-loaded (Fig 3, Table 1).

The diameters of LifePearl, DC Bead, and Tandem microspheres were reduced after loading by 24%, 20%, and 9%, respectively (Table 1). Because the HepaSphere microspheres were reconstituted from a dry state by the doxorubicin solution, changes in diameter could not be determined. Although the Tandem microspheres did not change diameter appreciably after loading with doxorubicin, approximately 3% of the doxorubicin-loaded microspheres were smaller than 50 μm.

For all types of microspheres, elution reached a plateau by 6 hours, without further elution at 24 hours. LifePearl, DC Bead, HepaSphere, and Tandem microspheres eluted 30% ± 5, 21% ± 2, 8% ± 3, and 6% ± 0 of the loaded doxorubicin, respectively. The times to elute 75% of the total released doxorubicin were 197, 139, 110, and 77 minutes for DC Bead, LifePearl, HepaSphere, and Tandem microspheres, respectively (Fig 4). At 20 minutes, the differences in doxorubicin elution were statistically significant between LifePearl microspheres and DC Bead ($P = .01$), Tandem ($P = .02$), and HepaSphere ($P = .2$) microspheres. At 40 minutes, 1 hour, 2 hours, 3 hours, 5 hours, 6 hours, and 24 hours, the differences were significant for DC Bead microspheres compared with LifePearl ($P = .01$–$.03$), Tandem ($P = .001$–$.01$), and HepaSphere ($P = .001$–$.004$) microspheres, as well as for LifePearl microspheres compared with Tandem ($P = .001$–$.01$) and HepaSphere ($P = .001$–$.01$) microspheres. At 4 hours, the differences were significant for DC Bead and LifePearl microspheres compared with HepaSphere ($P = .001$–$.01$) and Tandem ($P = .01$–$.001$) microspheres.

Mixing doxorubicin-loaded microspheres with 100% contrast agent or 65%:35% contrast agent:water for injection did not result in significant elution of doxorubicin (Fig 5). Approximately 0.5 mg (~1%) was immediately eluted from the microspheres, and no further doxorubicin release was observed over a period of 10 days for all four types of microspheres.

Time in Suspension

The time in suspension, ie, the amount of time for the microspheres to vacate one third of the volume of a 10-mL syringe, was greatest for Tandem, followed by LifePearl, and further followed by DC Bead and HepaSphere microspheres. Differences in the times in suspension were not significant between DC Bead and

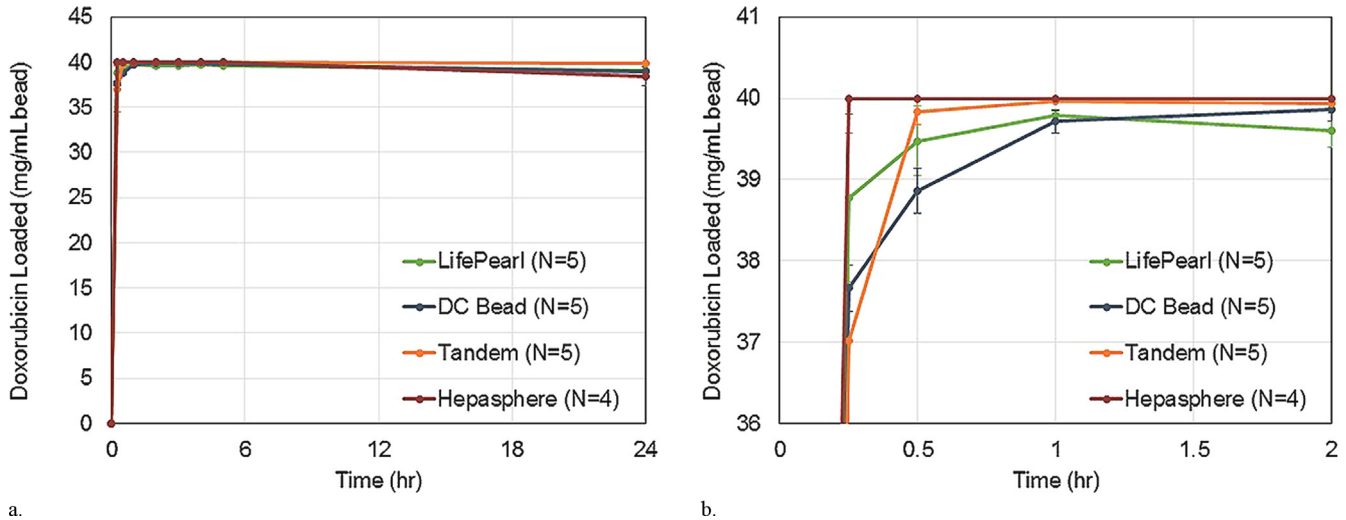

Figure 1. Doxorubicin loading of DC Bead, HepaSphere, LifePearl, and Tandem microspheres: **(a)** entire duration and **(b)** reduced time range to illustrate the differences in loading.

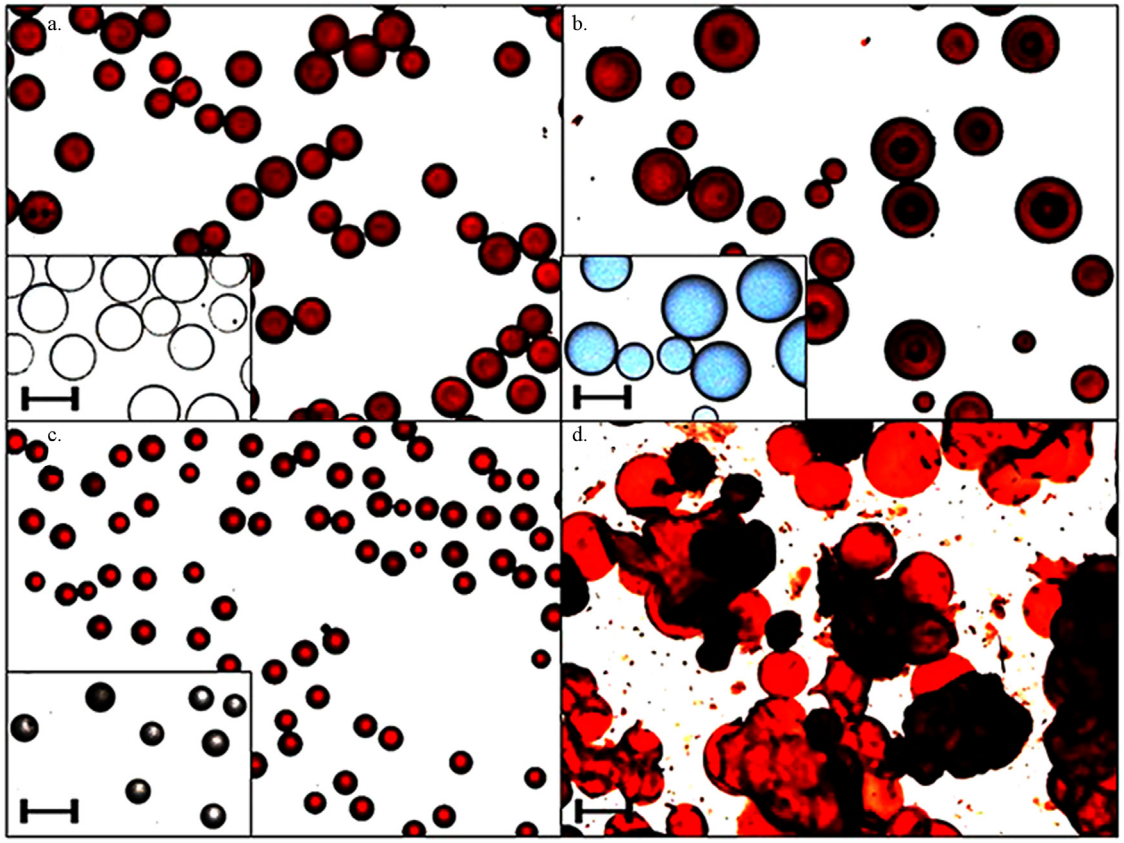

Figure 2. Photomicrographs of doxorubicin-loaded LifePearl **(a)**, DC Bead **(b)**, Tandem **(c)**, and HepaSphere **(d)** microspheres. Inset images in the lower left corners are photomicrographs of unloaded microspheres. (Scale bars: 200 μm.)

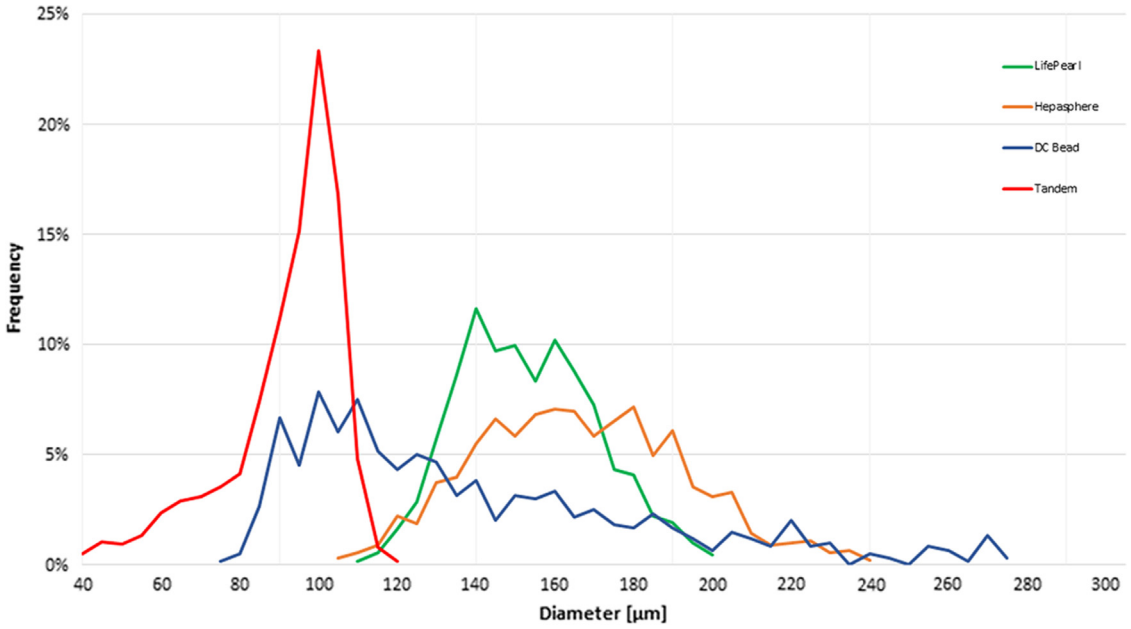

Figure 3. Diameter distribution histograms of doxorubicin-loaded DC Bead, HepaSphere, LifePearl, and Tandem microspheres.

HepaSphere microspheres ($P = .21$). For each product, the time in suspension did not differ for immediate suspension upon mixing with contrast agent or for as long as 30 minutes after mixing (Table 2).

DISCUSSION

In the present study, the in vitro performance of four types of microspheres loaded with doxorubicin was evaluated. Although there are many similarities among

the four types, each type has a unique chemistry that imparts unique properties to the microspheres. This study demonstrates the similarities and differences among the four microsphere types.

All four types of microspheres loaded doxorubicin in less than 1 hour. The loading of doxorubicin in DC Bead and HepaSphere microspheres has been previously evaluated (2). Loading was slower in the previous report than observed in the present study, with almost complete loading between 1 and 1.5 hours (2). This difference may be a result of the diameter of microspheres that were evaluated in the two studies. In the present study, relatively small microspheres (100–300 μm in diameter) were used, whereas previous studies evaluated larger microspheres (500–700-μm DC Bead and 400–600-μm HepaSphere microspheres) were evaluated (2). The effect of microsphere size on doxorubicin loading may be a result of the increased surface area of the smaller microspheres, resulting in greater area exposed to the doxorubicin solution. Although the doxorubicin loading of LifePearl and Tandem microspheres has not been previously investigated to our knowledge, the loading of idarubicin, a chemical analogue of doxorubicin, into DC Bead and Tandem microspheres has been evaluated (4). In that study (4), idarubicin loading was > 99% complete within 10 minutes, a result of reduced quantity of drug to be loaded (5–15 mg idarubicin compared with 40 mg of doxorubicin in the present study).

Table 1. Diameters of Unloaded and Doxorubicin-Loaded Microspheres

	Diameter (μm)	
Microsphere Type	**Unloaded**	**Doxorubicin-Loaded**
LifePearl	199 ± 24	151 ± 18
DC Bead	173 ± 53	138 ± 46
Tandem	98 ± 15	89 ± 15
HepaSphere	NA	165 ± 28

Note–Values presented as means ± standard deviation.
NA = not applicable.

Visually, in the present study, DC Bead, LifePearl, and Tandem microspheres were separate, spherical, and uniformly colored by doxorubicin. HepaSphere microspheres were clumped, somewhat spherical, and not uniformly colored by doxorubicin. This study confirms the visual appearance of DC Bead and HepaSphere microspheres as previously reported (2). The appearance of LifePearl and Tandem microspheres after loading with doxorubicin has not been previously reported to our knowledge.

Although the reduction in average diameter of the microspheres after loading with doxorubicin is a well-known effect of water displacement from the microsphere as a result of the ionic loading process (2,4), the effects on the entire distribution of microsphere diameters has not been previously reported to our knowledge. Although the reduction in the average diameter is important, another important piece of information is the diameter of the smallest microspheres, in view of the concern about shunting from the hepatic artery (where microspheres are intended to be injected) toward the hepatic or portal veins, which can result in major complications such as liver or pulmonary infarction. A previous report (7) described three fatal pulmonary embolisms as a result of 40–120-μm microspheres passing through hepatic shunts. The same center has not seen this complication when using 100–200-μm microspheres. In the present study, only Tandem microspheres had individual microspheres smaller than 50 μm in diameter.

The present study confirms previous reports that doxorubicin is eluted in differing amounts depending on the microsphere. The 21% release for DC Bead and

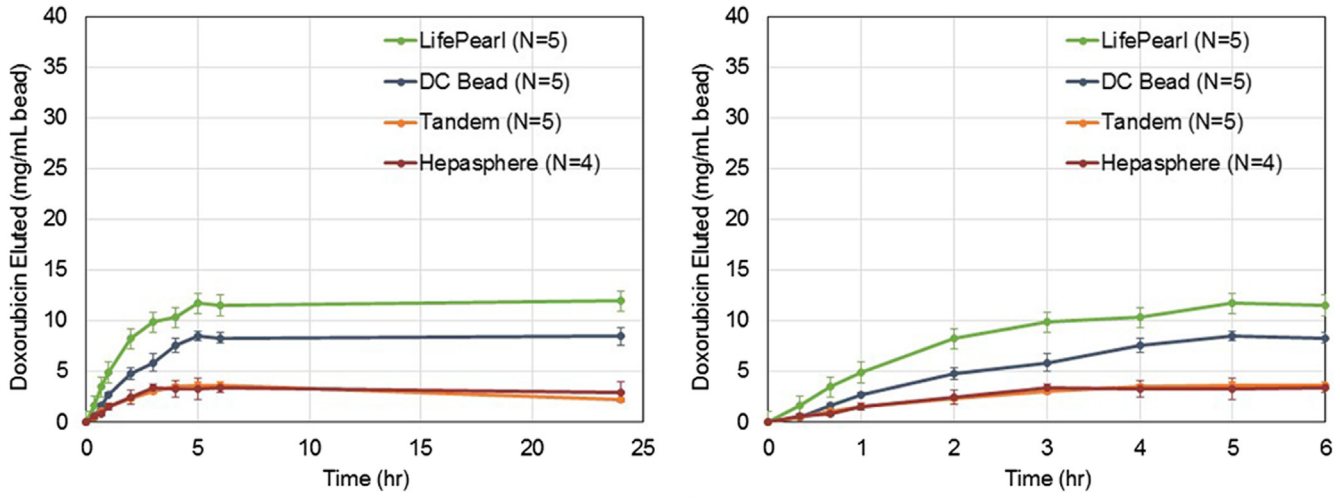

Figure 4. Doxorubicin elution in a dissolution apparatus of DC Bead, HepaSphere, LifePearl, and Tandem microspheres: **(a)** entire duration and **(b)** reduced time range to illustrate the differences in elution.

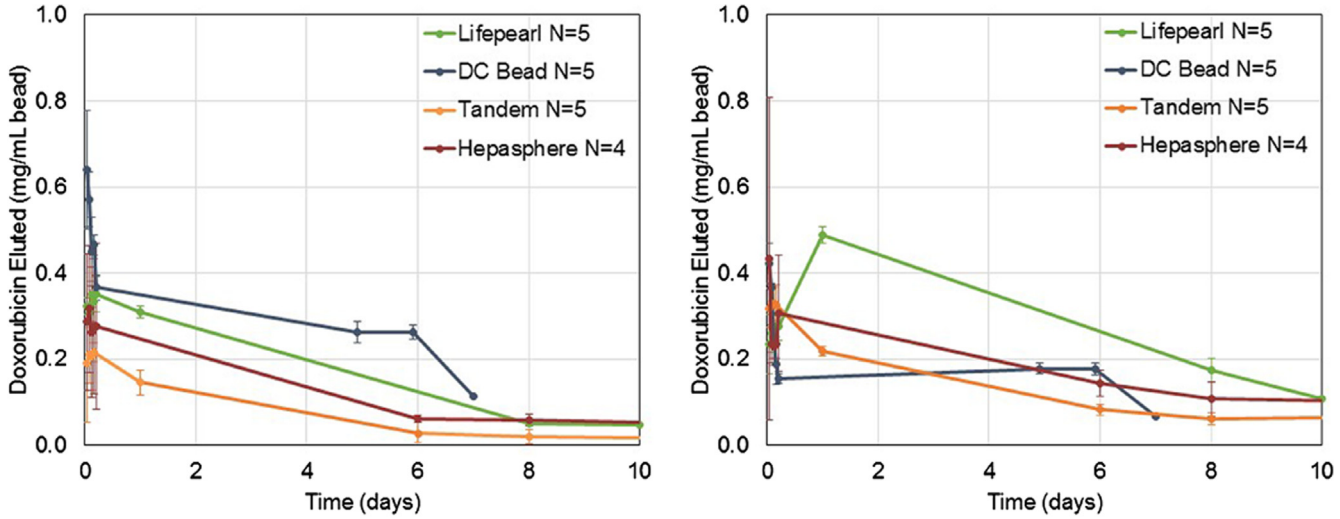

Figure 5. Stability of doxorubicin loading of DC Bead, HepaSphere, LifePearl, and Tandem microspheres in 65:35 contrast agent:water for injection **(a)** and 100% contrast agent **(b)** over a period of 10 days at 4 °C.

Table 2. Time in Suspension for the 4 Types of Microspheres

Microsphere Type	Time in Suspension (s)
LifePearl	357 ± 7
DC Bead	185 ± 11
Tandem	504 ± 12
HepaSphere	172 ± 20

Note–Values presented as means ± standard deviation.

8% release for HepaSphere microspheres is consistent with previous reports (2). For all four microsphere types, doxorubicin is loaded via ionizable functional groups on the hydrogel material, a carboxylate for HepaSphere (3) and Tandem microspheres (8) and a sulfonate for DC Bead (2) and LifePearl microspheres. The negatively charged carboxylate or sulfonate interacts with the positively charged doxorubicin during loading. The interaction is disrupted during elution as the sodium ion replaces doxorubicin as a result of the increased ionic strength of the phosphate-buffered saline solution. Because carboxylic acids have higher pKa values than sulfonic acids, the use of carboxylic acids require a higher pH for the acid to be deprotonated and able to participate in ionic interactions. If ionic interaction is the major factor governing doxorubicin loading and elution, the different acidities of carboxylic acids and sulfonic acids would not cause significant differences in doxorubicin loading and elution because the experiments were performed at a pH at which carboxylic and sulfonic acids are fully deprotonated. The present study shows that ionic interaction is the major interaction affecting doxorubicin loading, as carboxylic and sulfonic acid–based microspheres loaded equally.

However, significant differences in doxorubicin elution were observed from microspheres containing carboxylic and sulfonic acids, indicating that other mechanisms besides ionic interaction are involved in doxorubicin elution. Structurally, doxorubicin contains five hydroxyl groups, which could potentially participate in hydrogen bonding to microsphere materials. Additionally, doxorubicin is known to aggregate and form dimers, trimers, and higher-order aggregates (9). These aggregates possess multiples of positive charges and therefore bond more strongly with the anionic materials and require greater ionic strength to elute relative to single molecules of doxorubicin. As the four types of microspheres evaluated in the present study are composed of different materials, the degree of hydrogen bonding and doxorubicin aggregation inside the microspheres may vary significantly, leading to differing elution rates of doxorubicin.

The present study confirms a previous report of doxorubicin stability with DC Bead microspheres over a period of 14 days (10) and further demonstrates the stability of HepaSphere, LifePearl, and Tandem microspheres loaded with doxorubicin, which has not been previously reported to our knowledge. The decrease in the amount of doxorubicin in the solution over time may be a result of degradation of doxorubicin. Previous reports have shown that the stability of doxorubicin is greatest at low pH and that some degradation and adsorption of doxorubicin occurs at neutral pH (11,12).

Time in suspension is an important characteristic of drug-eluting embolic agents because it impacts the ease of use of the product. Microspheres with longer times in suspension will allow for a smoother embolization procedure without the need for an interruption to resuspend the microspheres. Despite its impact on ease

of use, time in suspension appears to be largely unstudied in doxorubicin-loaded microspheres. In the present study, Tandem microspheres remained in suspension the longest. This may be partially explained by Tandem microspheres having the smallest microsphere diameters of the four groups in the study. Smaller microspheres are known to go into suspension quicker and remain in suspension longer than larger microspheres as a result of their increased buoyancy.

There are limitations to the present study. First, the microsphere types did not have identical diameter ranges. For example, the LifePearl microspheres ranged from 150 to 250 μm in diameter, whereas the Tandem microspheres ranged from 75 to 125 μm in diameter. The effect of the diameter on the performance of the microsphere is unknown and may preclude direct comparison. However, previous reports have shown that loading and elution of doxorubicin is more rapid with microspheres with smaller diameters (5).

Second, doxorubicin elution was measured by using a Sotax dissolution apparatus. This equipment is the standard for elution studies, as several previous studies have used it to study elution from drug-eluting embolic agents (2,4). Although the phosphate-buffered saline solution used in the Sotax equipment accurately reflects the osmolarity and pH of blood, the in vivo milieu is far more complex than pH and osmolarity. The correlation between in vitro elution in phosphate-buffered saline solution and in vivo elution in the liver vasculature is unknown; however, a previous study (13) investigated the differences between in vitro and in vivo elution of doxorubicin.

In conclusion, the present study identified the similarities and differences of four commercially available microspheres loaded with doxorubicin. For all four microsphere types, doxorubicin loading was rapid and nearly complete. However, in vitro doxorubicin elution was slow and incomplete, with differences in total elution among the microsphere types. Currently, it is unknown whether these differences have an impact in vivo.

ACKNOWLEDGMENT

This study was funded by MicroVention (Tustin, California).

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