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Characterization of a novel intrinsically radiopaque Drug-eluting Bead for image-guided therapy: DC Bead LUMI™

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ABSTRACT

We have developed a straightforward and efficient method of introducing radiopacity into Polyvinyl alcohol (PVA)-2-Acrylamido-2-methylpropane sulfonic acid (AMPS) hydrogel beads (DC Bead™) that are currently used in the clinic to treat liver malignancies. Coupling of 2,3,5-triiodobenzaldehyde to the PVA backbone of pre-formed beads yields a uniformly distributed level of iodine attached throughout the bead structure (~150 mg/mL) which is sufficient to be imaged under standard fluoroscopy and computed tomography (CT) imaging modalities used in treatment procedures (DC Bead LUMI™). Despite the chemical modification increasing the density of the beads to ~1.3 g/cm^3 and the compressive modulus by two orders of magnitude, they remain easily suspended, handled and administered through standard microcatheters. As the core chemistry of DC Bead LUMI™ is the same as DC Bead™, it interacts with drugs using ion-exchange between sulfonic acid groups on the polymer and the positively charged amine groups of the drugs. Both doxorubicin (Dox) and irinotecan (Iri) elution kinetics for all bead sizes evaluated were within the parameters already investigated within the clinic for DC Bead™. Drug loading did not affect the radiopacity and there was a direct relationship between bead attenuation and Dox concentration. The ability (Dox)-loaded DC Bead LUMI™ to be visualized *in vivo* was demonstrated by the administration of into hepatic arteries of a VX2 tumor-bearing rabbit under fluoroscopy, followed by subsequent CT imaging.

1. Introduction

Drug-eluting Bead (DEB) technology has now been available to the physician for more than a decade and allows the delivery of the chemotherapy and the embolization agent concurrently [1,2], simplifying the transarterial chemoembolization (TACE) procedure and allowing for a greater degree of standardization of the treatment (DEB-TACE) [3,4]. Moreover, the controlled and sustained delivery of the drug offers an improved safety profile over conventional TACE due to lowered systemic levels of drug [5,6]. The DEBs are mixed with soluble contrast at the appropriate dilution and to provide a homogeneous suspension that can be delivered *via* syringe through the narrow lumen of the microcatheter without occluding it. The contrast-bead suspension flows into the blood stream and the DEBs are carried by the arterial flow into the tumor vessels until they physically block by virtue of their size. DEBs are provided in a range of sizes for this reason, to allow the physician to select the bead depending upon the vessel anatomy to be embolized. When the first DEB was first introduced into clinical practice (DC Bead™), the most common size range of product selected for treating hepatocellular carcinoma (HCC) was 500–700 μm based upon the sizes of embolic agent that were routinely used at the time for cTACE [6,7]. As clinicians have become more experienced with the product and confident in its performance, it is now more usual to treat with 100–300 μm DC Bead™, or if so desired, to achieve even more distal penetration by using DC Bead*M1*™ (70–150 μm) [8] or this in combination with 100–300 μm DC Bead™ [9]. Smaller beads loaded with irinotecan are recommended when treating colorectal cancer metastases to the liver due to their relative hypovascular nature [10,11].

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Whichever the technique employed in the treatment, the Interventional Radiologist (IR) relies upon monitoring of blood flow reduction by virtue of the contrast media mixed with the beads, in order to judge when sufficient embolic has been delivered to reach the intended end-point. As the beads themselves are not radiodense, there is no way of locating their exact location; but instead, it has become commonplace to use the phenomenon of trapped soluble contrast retention as a surrogate for assessing successful devascularization [12,13]. This requires a CT scan to be performed within the first 6 h of bead administration, as the process of devitalising the tumor means that some of the contrast agent that has been delivered in conjunction with the beads becomes resident in the embolized tissue and its elimination by the usual wash-out mechanisms is retarded. With the adoption of intraprocedural cone-beam CT becoming more widespread, this practice is helping to guide the IR and ensure a greater extent of technical success in the DEB-TACE procedure. Despite this, there remains a desire by the IR to see the DEBs themselves, to provide intraprocedural feedback on treated locations and help avoid off-target embolization and to allow for better evaluation of tumor coverage.

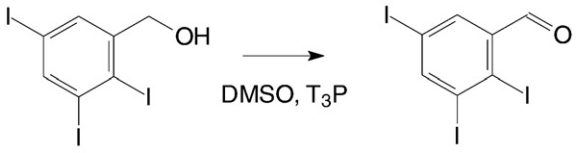
We have previously reported on the use of a lyophilized version of DC Bead™ that can be loaded with Lipiodol® and drug in order to attain a radiopaque DEB [14–16]. This enabled the demonstration of the utility of such a product and provided the proof of concept that bead concentration could be correlated to attenuation to provide a quantitative measure of iodine levels in the bead, and subsequently, that the degree of radiopacity could be correlated to drug concentration (in this case doxorubicin (Dox)) [15]. This finding provides a tantalizing insight into a potential future innovation for radiopaque (RO) DEBs, for which if drug diffusion around the beads can be mapped on a spatial and temporal basis, the concept of DEB dosimetry and dose-painting could become a real prospect to help better quantify and tailor drug delivery to tumors. To this end, we have been developing a more convenient version of a radiopaque DC Bead™ that has intrinsic radiopacity to avoid the need to undertake any contrast loading process. Our initial approach was to couple triiodobenzoyl groups (the basis of most commercial iodinated contrast media) to the preformed beads by various linking groups [17]. This posed some technical challenges, as the presence of such hydrophobic moieties within the hydrogel structure of DC Bead™ resulted in unusual behaviour that was deleterious to the bead properties. These issues were eventually overcome in order to arrive at a RO DEB that can be handled and administered as in normal clinical practice [18,19], has excellent drug elution properties and good levels of radiodensity to be usefully imaged under a range of different imaging modalities. Here we report on the characterization of this novel, intrinsically radiopaque DEB, DC Bead LUMI™.

2. Materials and methods

2.1. Bead synthesis

2.1.1. Preparation of 2,3,5-triiodobenzaldehyde

In a 50 mL three-necked round-bottomed flask fitted with a thermometer, a nitrogen bubbler and an air-tight seal, (10.2 g, 0.021 mol) of 2,4,5-triiodobenzyl alcohol (Sigma Aldrich, Poole, UK) was dissolved in 100 mL of anhydrous DMSO (Romil, Cambridge, UK) under a nitrogen blanket and stirring conditions. Then, 1.0 mol equivalent of propane phosphonic acid anhydride (Sigma Aldrich, Poole, UK), (T3P), (50% solution in ethylacetate) was added drop by drop over 5 min at 22 °C–25 °C. The reaction solution was stirred at room temperature and monitored by high performance liquid chromatography (HPLC). The conversion finished after 240 min (scheme 1). The yellow solution was poured into 100 mL of deionised water while stirring, yielding a white precipitate which was filtered, washed with the mother liquors and 50 mL of deionised water. The cake was slurried in 50 mL of ethylacetate (Romil, Cambridge, UK), filtered and washed with 50 mL of water again, dried *in vacuo* at 40 °C for 20 h to yield (7.7 g, 75.8%) of a white solid. The structure and purity were confirmed by NMR analysis and HPLC.



Scheme 1. Preparation of 2,3,5-triiodobenzaldehyde.

2.1.2. Preparation of dried PVA hydrogel beads

The beads were produced using a PVA-based macromer (150 g, equivalent to 3.41 mol of CH_2CH_2OH units) that was synthesized by the acid-catalyzed (HCl, pH 1–2) reaction of N-acryloyl-aminoacetaldehyde dimethylacetal (0.104 mmol/g of PVA, 2.49 g) (NAAADA, Ciba Vision GmbH, Großwallstadt, Germany) with the 1,3 diol units on a 67 kDaL PVA backbone (Mowiol® 8–88, Sigma Aldrich, Poole, UK) to form a stable cyclic acetal structures with pendent reactive acrylamide groups. The macromer solution 21% *w*/w (400 g) was mixed with 2-acrylamido-2-methylpropanesulfonate sodium salt (140 g, 0.61 mol) (AMPS, Sigma Aldrich, Poole, UK) and potassium persulfate(5.22 g, 0.019 mol) (one half of the initiator redox couple) in aqueous media stabilized by cellulose acetate butyrate to prevent coagulation and suspended in butyl acetate with stirring. The mixture was stirred (speed of stirring being used to control the droplet size) and heated to 60 °C and then tetramethylethylenediamine (6.4 mL, 0.055 mol) (TMEDA, the other component of the redox couple) was added to the oil phase, where upon free-radical copolymerization was initiated and a water-swollen cross-linked network formed (Scheme 2). The swollen beads were washed with high purity water and then extracted with two sequential boil washes in buffered saline to remove residuals. The beads were then mechanically separated into 40–90 μm, 70–150 μm or 100–300 μm size fractions using a sieve stack with copious washing using purified water. After sieving the beads were dried by washing in acetone and drying in an oven at 40 °C overnight to form a free-flowing powder, approximately 200 g total yield.

2.1.3. General preparation of radiopaque beads from 2,3,5-triiodobenzaldehyde and preformed cross-linked PVA hydrogel beads

The dried PVA hydrogel beads (4 g) were placed in dimethyl sulfoxide (DMSO) and reacted with 2,3,5-triiodobenzaldehyde (6.6 g, 0.14 mol) in an acid-catalyzed (methane sulfonic acid) reaction under nitrogen with stirring, to form stable cyclic acetal linkages with pendent triiodobenzoyl moieties (scheme 3). Consumption of the aldehyde was monitored using HPLC and once complete, the reaction was cooled to room temperature and filtered. The cake of beads was washed thoroughly with copious amounts of DMSO and then water, until free of unreacted aldehyde as determined by HPLC.

2.2. Evaluation of bead physicomechanical properties

2.2.1. Sizing and optical microscopy

Bead size was determined by forming a monolayer of beads within a petri dish and images acquired at × 10 magnification using an Olympus BX50 microscope equipped with a ColorView III camera. Sizing was performed manually on population of at least 200 microspheres randomly throughout the dish using the sizing tool provided in the AnalySIS software package (Soft Imaging Systems GmbH, Berlin, Germany).

2.2.2. Energy dispersive X-ray analysis (EDAX) micro-mapping of bead elemental composition

Beads were embedded in PVA gel (FSC 22) and sectioned using a cryomicrotome (Leica CM1860) to 10 μm thick slices. The sample