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Long-term biocompatibility, imaging appearance and tissue effects associated with delivery of a novel radiopaque embolization bead for image-guided therapy

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ABSTRACT

The objective of this study was to undertake a comprehensive long-term biocompatibility and imaging assessment of a new intrinsically radiopaque bead (LC Bead LUMI™) for use in transarterial embolization. The sterilized device and its extracts were subjected to the raft of ISO10993 biocompatibility tests that demonstrated safety with respect to cytotoxicity, mutagenicity, blood contact, irritation, sensitization, systemic toxicity and tissue reaction. Intra-arterial administration was performed in a swine model of hepatic arterial embolization in which 0.22–1 mL of sedimented bead volume was administered to the targeted lobe(s) of the liver. The beads could be visualized during the embolization procedure with fluoroscopy, DSA and single X-ray snapshot imaging modalities. CT imaging was performed before and 1 h after embolization and then again at 7, 14, 30 and 90 days. LC Bead LUMI™ could be clearly visualized in the hepatic arteries with or without administration of IV contrast and appeared more dense than soluble contrast agent. The CT density of the beads did not deteriorate during the 90 day evaluation period. The beads embolized predictably and effectively, resulting in areas devoid of contrast enhancement on CT imaging suggesting ischaemia-induced necrosis nearby the sites of occlusion. Instances of off target embolization were easily detected on imaging and confirmed pathologically. Histopathology revealed a classic foreign body response at 14 days, which resolved over time leading to fibrosis and eventual integration of the beads into the tissue, demonstrating excellent long-term tissue compatibility.

1. Introduction

Embolotherapy is a procedure for introducing a variety of agents into the circulation in order to occlude blood vessels for therapeutic intent, for instance to prevent bleeding, to arrest flow through abnormal connections such as arteriovenous malformations (AVMs) or to devitalise a structure, organ or tumorous mass by inducing ischemic necrosis. Embolic agents come in many different forms including microparticles, pellets, glues or metallic coils [1,2]. These embolics are administered to targeted tissues through a catheter inserted and manoeuvred through the vasculature into the desired location. One common application of this technique is the use of microparticles, most usually microspheres/beads to treat tumors in the liver where the intention is to physically occlude the vessels feeding the tumour in order to induce localised ischemic necrosis of the malignant mass. However, perfect tumour targeting of microparticles is not possible and embolization of normal adjacent healthy liver parenchyma is inevitable. The embolization of healthy liver parenchyma can induce significant local tissue changes, including elevated serum liver enzymes and tissue damage, but these side-effects typically resolve over time [3]. However, there should be no chronic material-related inflammatory reaction or clinical sequelae. The microparticles themselves may disappear over time if bioresorbable, or if non-degradable, should be sufficiently bioinert and well-tolerated in tissue where they reside.

Embolotherpy with microparticles is conducted under X-ray

Abbreviations and acronyms: RO, Radiopaque; TAE, transarterial embolization; CT, computed tomography; DSA, digital subtraction angiography; IV, intravenous; H&E, Hematoxylin and Eosin stain.

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based image guidance where injection of iodine-based liquid contrast agent, a radiodense material, is used to create a roadmap of the network of blood vessels to be embolized. Some embolic devices such as coils are inherently radiopaque which enables them to be easily located during and post procedure. Liquid embolics such as Glue or Onyx® are often mixed with radiodense materials (such as Lipiodol® oil or tantalum powder) prior to use in order to impart radiopacity [4,5]. Microparticles are usually composed of synthetic and natural polymers that are radiolucent and therefore cannot be directly seen during delivery which requires the addition of iodine-based liquid contrast agent to monitor their delivery. However, only the degree of blood flow cessation indicates when sufficient embolic agent has been delivered to achieve the desired flow-based embolization end-point. It has been demonstrated using embolic bead devices that there is a degree of trapped residual soluble contrast agent retention at the site of embolization that dissipates over the next several hours post-procedure [6,7]. CT imaging within a 6 h time frame post-delivery therefore, provides contrast retention as a surrogate marker of bead location and some degree of comfort that the correct blood vessels have been embolized. The exact bead location, however, remains unknown.

The concept of intrinsically radiopaque embolic beads has been explored for many years [8] and multiple experimental studies are available in the literature. In some cases the beads have been made radiodense by the incorporation of metallic components such as tantalum [9] or barium [9,10]. This can have significant effect on the handling and administration of the beads as the increased density induces rapid sedimentation [11]. There is also concern for the long-term fate of the entrapped contrast material and the potential for leaching into the surrounding tissue over time. The incorporation of iodine-containing species into polymers has therefore been a more widely studied approach, resulting in materials useful as bulking agents [12], in bone cements [13,14], for nucleus pulposus replacement [15,16] and as microparticle emboli [17,18]. The radiopacity can be introduced by means of an iodine-bearing monomer at the polymerization stage [17–19] or by chemical attachment of an iodinated species with reactive functionality to preformed polymer microspheres [20]. Compounds based upon iodinated benzyl groups are convenient starting materials for either of these approaches as they provide for synthetic flexibility and enable high iodine content per unit mass. It is for these reasons that such compounds are the basis for most of the commercially-available soluble contrast media (Fig. 1 (1)). Radiopaque beads have been prepared based upon incorporation of 2,3,5 triiodobenzyl moieties (Fig. 1, (3 & 5)) [17], but whilst they possessed high unit iodine content in the 25–30 wt% region, this somewhat compromised the hydrophilicity and softness attributes that are desirable for the handling and microcatheter delivery of embolization beads. Horák et al. tried to address this problem with the synthesis of 3-(methacryloylamido-acetamido)-2,4,6-triiodobenzoic acid (MABA, Fig. 1 (4)) and its subsequent copolymerization with HEMA in the presence of additives to induce porosity to the microspheres [21]. They found it necessary to incorporate at least 27 wt% iodine for adequate radiopacity but they experienced issues with irregular particle formation and agglomeration during the polymerization. Others have attempted to increase the hydrophilicity of the system by utilising the mono-iodinated 2-[4-iodobenzoyl]-oxo-ethyl-methacrylate monomer (4IEMA, Fig. 1, (2)) copolymerised with hydrophilic comonomers such as hydroxyethyl methacrylate (HEMA) or 1-vinyl-2-pyrrolidinone (NVP) [19]. Whilst this did enable the synthesis of some microsphere formulations that were water-swellable in nature, only those with low water content and iodine contents of ~20 wt% were sufficiently radiopaque to be useful in practice.

While it has been difficult to establish a balance between the appropriate physicochemical properties (e.g., water content, density, sphericity, dispersibility) and useful levels of radiopacity, it has been demonstrated that the materials based on the triiodinated chemistry display good biocompatibility [23]. *In vitro* cell-based analyses show no indications of cytotoxicity or effect on cell proliferation, while *in vivo* implantation studies show they are well-tolerated with no signs of adverse tissue reactions. We have therefore recently reported on efforts to modify LC Bead®, well-characterized polyvinylalcohol-based hydrogel beads for embolization of hypervascular tumors and AVMs. Approaches were developed to activate the bead chemistry towards triiodinated species (Fig. 1, (5)) to render them radiopaque whist maintaining their hydrogel nature [20]. We have since optimized this chemistry and have a process for manufacture of intrinsically radiopaque beads (RO Beads) that have water contents in the region of 60–70% with iodine contents in the range 189–258 mg/mL true bead volume (133–177 mg Iodine/mL sedimented beads, equivalent to >60 wt% iodine on a dry mass basis) [22]. This provides for an excellent degree of radiopacity coupled with the benefits of a hydrogel performance (Fig. 1, (scheme 6)). The additional visual information provided by these beads may provide tools for standardization and reproducibility of end points and treatment effects in addition to offering better conspicuity to determine target and non-target embolization [22,24]. Furthermore, the durable imaging appearance of the beads may also aid in the guidance and evaluation of the embolization procedure. Intra-procedural identification of tissue at risk for under-dosing or under-treatment can better inform the physician of options to immediately target this tissue with additional therapies rather than waiting for the outcome of follow-up scans [25]. In this study we present the outcome of the biocompatibility testing of the optimized product, LC Bead LUMI™, and investigate the long-term effects up to 90 days post embolization in a swine liver. We also concurrently examine the bead location with X-ray fluoroscopy and computed tomography (CT) as well as the associated tissue changes resulting as a consequence of their embolization.

2. Materials and methods

2.1. Materials

LC Bead LUMI™ used in this study was prepared and characterized as described previously [22]. Briefly, sulfonate-modified acrylamido-polyvinylalcohol beads were made using a reverse suspension polymerization process [26]. Triiodobenzyl groups were coupled to the PVA chains of the preformed beads using a proprietary process (scheme 6) to yield beads that were sieved into different size fractions, dispensed into vials and steam sterilized. The 70–150 μm size range of RO Beads was selected for the biocompatibility and embolization studies as these provide a high challenge given the large number of beads and high surface area for a given volume. Visipaque™ 320 was used to make the bead suspension. Each vial of beads (2 mL volume was mixed with 18 mL of contrast agent taking care to eliminate any air bubbles from the mixture.

2.2. ISO10993 biocompatibility studies

The biological evaluation of the RO Beads was based upon the principles of ISO 10993-1: Biological evaluation of medical devices-Part 1: Evaluation and testing within a risk management process (ISO 10993-1: 2009). The biological evaluation took into account the anticipated nature and duration of contact with RO Bead, which is a permanent implanted device in contact with blood. All biocompatibility testing studies (*in vitro* and *in vivo*) were