Translational Development of Biocompatible X-Ray Visible Microspheres for Use in Transcatheter Embolization Procedures

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Abstract

Embolization is a minimally invasive treatment that specifically blocks the arterial blood flow into a target blood vessel bed, which is usually a benign or malignant tumor. The aim of the procedure is to shrink the tumor and/or to retard its growth. Embolization the injection of embolic particles via a catheter tube, of which the tip has been navigated carefully (under X-ray guidance) into an arterial branch that exclusively feeds the tumor, and no surrounding healthy tissues. Most of the clinical experience with embolization relates to treatment of leiomyomata (benign tumors growing in the wall of the uterus). There is solid evidence that catheter-based embolization of leiomyomata provides a fully acceptable therapeutic alternative for much more demanding surgical procedures (i.e., hysterectomy and myomectomy). Embolization offers much faster recovery, possible options to become pregnant, and considerable cost saving. There are several commercial brands of embolization agents, suitable to treat leiomyomata. We hypothesized, some years ago, that these products are suboptimal, and that embolization of leiomyomata may be improved further through better engineering of the embolic particles. We developed injectable radiopaque, polymer microspheres, which can be monitored during and after the embolization procedure. The embolic microbeads are X-ray traceable, and this has been achieved without compromising other essential properties, such as structural stability and excellent biocompatibility. Herein, we describe new the features of the new embolic microspheres, as observed in preclinical experiments and in the first clinical cases. It is mentioned briefly that this work became an example of successful translation: it has led to a new medical device (Class-IIB) that is now CE-certified and commercially available throughout Europe.

Keywords: Embolization; Radiopacity; X-ray visibility; Polymer microspheres

Introduction

The hallmark of minimally invasive therapy is the endovascular coronary stent, which secures that a coronary atherosclerotic lesion remains open after percutaneous transluminal angioplasty [1]. Stenting has become the preferred revascularization modality in patients with coronary single-vessel or low-risk multivessel disease. The technique is minimally invasive, fast, relatively cheap, and associated with faster patient recovery. Over the years, coronary stenting has seen many technical improvements, in part due to the exploitation of improved biomaterials [2,3]. Recently this has culminated in the development of polymer bio-eroding and drug-eluting vascular scaffolds [4,5].

During the last years, comparable minimally invasive techniques have gained importance in other fields as well. An important example is found in gynaecology, particularly in the treatment of benign tumors that grow in the uterus wall (leiomyomata) [6]. This disease, too, can be treated effectively in a minimally invasive manner, i.e. through controlled targeted injection of embolic particles (diameter around 500 µm) into the arterial vessel tree of each fibroid, via a catheter tube [7-10]. The particles are usually spherical (microspheres), but they can also be irregular [11]. The procedure is known as TACE (TransArterial ChemoEmbolization), and is performed by an interventional radiologist. Embolization is carried out under real-time X-ray fluoroscopic guidance in a dedicated angiouisite, which is comparable to the facility that is used for coronary stenting. There is good evidence that embolization of leiomyomata provides a genuine alternative for the two surgical techniques which are used classically: myomectomy (which is not always possible, depending on shape and location of the myomas), and hysterectomy (which involves radical excision of the uterus and all benign tumors growing therein) [12-15]. Embolization offers significant advantages in terms of patient comfort, and recovery is fast. Psychological burden, which is inevitably associated with hysterectomy, can largely be avoided. Several cases of pregnancy after embolization of leiomyomata have been reported [16,17], but the actual fertility rate after this treatment is still uncertain [18].

It is important to underline that embolization is also rapidly gaining importance in the treatment of malignant tumors, particularly those in the liver or in the kidney. However, embolization of malignant tumors usually stimulates angiogenesis, leading to the formation of new arteries guiding the arterial blood around the embolic obstacles. Hence, embolization of malignant tumors must be accompanied by local or systemic chemotherapy.

Our interest in embolization started when we realized that chemical synthesis in the context of (bio)materials science offers possibilities to add functionalities to the injectable embolizing particles. We (and others) hypothesized that efficacy and safety of TACE for the treatment of leiomyomata can be enhanced when the embolic microspheres would...
be radiopaque (i.e. detectable via X-ray fluoroscopy) [19-26]. Note that X-ray fluoroscopy is used in every procedure anyway, for roadmapping during navigation of the catheter’s tip, and also to determine the procedure’s end point. Note, furthermore, that all existing commercial products for embolization use embolic particles that consist of classical polymers (such as polyvinyl alcohol), which are radiolucent [24]. We reasoned that use of radiopaque microspheres will enable interventional radiologists to actually monitor the synthetic emboli in situ. We discussed this idea extensively with >20 interventionalists/interventional radiologists to actually monitor the synthetic emboli [24]. We reasoned that use of radiopaque microspheres will enable interventional radiologists to actually monitor the synthetic emboli in situ. We discussed this idea extensively with >20 interventionalists/interventional radiologists to actually monitor the synthetic emboli.

Materials and Methods

Injectability and X-ray imaging

Both kidneys were explanted from a cadaver of a rabbit that was sacrificed in a completely different experiment. The explantation was performed by BSL BIOSERVICE Scientific Laboratories GmbH, Planegg, Germany. This company is certified according to the Principles of Good Laboratory Practice and accredited according to 90/385/EWG 93/42/EWG, and DIN EN ISO/IEC 17025:2005. The study complies with internationally accepted guidelines and recommendations regarding biological testing of medical devices:

- ISO 10993-1:2009 "Evaluation and testing within a risk management process"
- ISO 10993-6:2007 "Tests for local effects after implantation"
- OECD Biological Reactivity Tests, In Vivo, Implantation Test, current version

Nine animals (healthy female New Zealand White Rabbits) were used (three animals per time point, i.e. 7 days, 14 days and 28 days). The rabbits were purchased from Charles River Deutschland (97633 Sulzfeld, Germany). The animals were derived from a controlled full-barrier maintained breeding system (SPF). The animals were bred for experimental purposes, according to Art. 9.2(no. 7) of the German Act on Animal welfare [26].

USP reference standard high-density poly(ethylene) (Promochem GmbH, lot no. 046) was used as the negative control material. The control samples were prepared according to the guideline ISO 10993-6, i.e. the material (film with thickness 1.0 mm) was processed by heating in a validated autoclave (121°C, 20 min). Then, the samples were cut out of the film (circular, 10 mm diameter, volume approximately 80 µL). The test samples were radiopaque iodine-containing microspheres in the diameter range 200-800 micrometers. These particles were also processed by heating in the autoclave (vide supra). In all cases, the

Figure 1: (a) Structural formula of the reactive monomer that contains covalently bound iodine (Figure 1a), and hydrophilicity is introduced through use of the monomer 2-hydroxy-ethylmethacrylate (HEMA).

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trocar was filled up with a quantity of microspheres corresponding to approximately 80 µl. Pre- and post-surgery; the animals were housed in an air-conditioned room. An adequate acclimatization time of at least 5 days was maintained. The animals were housed in ABS-plastic rabbit cages with a floor surface of 4200 cm². The temperature was 18 ± 3°C, and the relative humidity was 55 ± 10 %. The artificial light was automatically switched on and off; 12 h light and 12 h dark. The air exchange was 10 x per hour at least. The animals had free access to autoclaved hay and to Altromin 2123 (maintenance diet for rabbits, which is rich in crude fibre). The animals also had free access to tap water (drinking water, municipal residue control, microbiological controls at regular intervals). The animals were anaesthetized with ketamine (Pharmanovo, lot no. 23116, exp. Date 07/2012), and xylazine (Riemser, lot no. 000660/1, exp. Date 12/2011). The fur on the back of the test animals was shaved on both sides of the spinal column. Care was taken to avoid mechanical irritation and trauma. Then, the implantation area was washed with antisepctic solution. The test items and control material were implanted into the muscular tissue, approximately 2.5 cm. away from the midline, and approximately 2.5 cm. apart from each other. The test items were implanted on the left side of the spinal columns, the control items in the right side. A sufficient number of implant samples was used to yield 10 test specimens and 10 control specimens for assessment. The implantation period was either 7 days, 14 days, or 28 days. Post-implantation, the animals were observed at least once daily. At the end of each experimental period, the animals were euthanized with an overdose of anaesthetic. After examination and macroscopic evaluation, the test and control material implant sites were excised together with sufficient unaffected tissue, to enable the evaluation of the biological response. The tissues were fixed in a 10 % formalin-buffered solution.

Tissue samples were received by BMP Laboratory for Medical Material Testing GmbH (Aachen, Germany). This company is accredited by the Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten ZLG-P-585.00.08). The samples were first cut in three equal parts, and each part was placed in a HistoTec box. The samples were dehydrated in alcohol, and then embedded in paraffin. In total, 90 samples were processed in this way: (15 control samples + 15 test samples) * 3 parts per sample. Sections of each specimen (thickness 4 µm) were cut (microtome), and stained with either hematoxylin and eosin (H&E) or Elastica von Gieson (EvG).

Results and Discussion

In vivo biocompatibility

Photomicrographs of microspheres and surrounding muscular tissue are shown in Figure 1b-1d (follow-up 28 days); the embedded microspheres appear as circular regions. Figure 1b shows the mild foreign-body reaction that is observed at the interface of the microspheres and the host tissue. There is some accumulation of histiocytes, T-lymphocytes and some foreign-body giant cells. Connective tissue formation was minimal with some collagen fibers and fibrocytes around each microsphere. Almost no fibrotic reactions were encountered, and no granulocytes or plasma cells were found. The tissue reactions are similar to those observed after 7 days or 14 days of implantation, with one exception: the density of capillaries was larger after 28 days, compared to 7-days and 14-days follow-up. This reflects the flexible and slightly compressible nature of the particles, in vivo. Figure 1c is a photomicrograph of the same slide, now at larger magnification. Cells and fibrotic tissue surrounding the microspheres are clearly seen. In addition, a capillary blood vessel, filled with erythrocytes (arrow) is noted. The formation of capillaries reveals that the microspheres became integrated in the host tissue. Microspheres that are in contact with each other may deform slightly, as is seen in Figure 1b and 1c.

Ex vivo embolization

“Artificial embolization” was achieved with two freshly explanted rabbit kidneys. These were perfused, via the renal artery, with a 1.8 ml of a suspension of the radiopaque microspheres (20 mg microspheres, diameter range 400-600 µm). Immediately thereafter, the kidneys were frozen and stored until X-ray imaging (Figure 2a). This revealed how the microspheres were distributed throughout the vascular beds (Figure 2b). Most of the microspheres are aligned in one of the major arteries, while other microspheres entered side branches. The data provide an example of non-specific embolization.

Preclinical in vivo embolization

Catheter-based embolization of the left kidneys of two living sheep was performed by an experienced interventional radiologist. Both procedures proceeded smoothly. Figure 3a shows a kidney upon perfusion with contrast, but prior to the injection of microspheres. Note that Figure 3a is a digital subtraction image, i.e. it is the difference between the X-ray images before and shortly after (several seconds) injection of contrast [25]. Hence, the image only provides information about the distribution of the injected contrast fluid; all other X-ray absorbing parts of the body are, in fact, eliminated. Numerous arterial branches within the kidney are seen clearly. The organ’s contour is clearly visible as well, and this reveals that the contrast nicely flows throughout the entire organ. Figure 3b is technically the same, although this image was recorded near the endpoint of the embolization procedure. Note that Figure 3a and 3b are markedly different. In Figure 3b, the contrast is seen to accumulate in the larger arteries. The contrast hardly reaches the cortical regions of the kidney, and the organ’s contour is almost invisible now. Furthermore, many small arteries are not discernable in Figure 3b. Apparently, these are no longer perfused with contrast fluid, indicating that embolization was successful.
However, still unclear for which type of embolizations the feature will be most advantageous. We anticipate that the feature will be particularly helpful in pre-operative embolization of cerebral tumors, which can be done to prevent bleeding complications during surgery. Monitoring of the embolic particles may help to prevent non-target embolization, and can help to judge whether or not total embolization has been achieved. The latter is important to maximize the chances for success. The new radiopaque microspheres are used in a new CE-certified medical device for embolization, called X-Spheres. Therefore, this work provides a quite unusual example of translational development of a new medical device, classified within the highest-but-one risk group (IBB), on the basis of a new synthetic polymer biomaterial that was originally developed in an academic laboratory.

Competing Interests

The authors Y.B.A. and J.H.K. declare to have stock in the company Interface BIOMaterials BV (Geleen, The Netherlands). This company is the manufacturer of the new embolization product X-Spheres; this product utilizes radiopaque embolic microspheres.

Acknowledgements

We thank Dr. U. Mueller, Professor B. Klosterhalfen and mr. C. Musters for advice and technical assistance. We thanks prof. P. Lohle (Elsabeth Hospital Tilburg, the Netherlands) for performing the kidney embolizations in the sheep models. This study was financed, in part, by the Interreg IV-A project ‘BioMMedics‘ (www.biommedics.org) (Interreg IV-A 1.2-2010-03/063), The Universities of Maastricht, Liege (Belgium), Hasselt (Belgium) and Aachen (Germany; RWTH and Fachhochschule), as well as several local regional biotechnological enterprises cooperated in ‘BioMMedics’. This particular study was financed through generous contributions of the EU (through Interreg IV-A), the Government of the Province Dutch Limburg, the Dutch National Ministry of Economic Affairs, Agriculture and Innovation, Maastricht University, The Limburg Bank for Industry Innovation (LIOF), and the Company Interface BIOMaterials BV (Geleen, The Netherlands).

References


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