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Polymeric Materials for Embolic and Chemoembolic Applications

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Abstract

Percutaneous transcatheter embolization procedures involve the selective occlusion of blood vessels. Occlusive agents, referred to as embolics, vary in material characteristics including chemical composition, mechanical properties, and the ability to concurrently deliver drugs. Commercially available polymeric embolics range from gelatin foam to synthetic polymers such as poly(vinyl alcohol). Current systems under investigation include tunable, bioresorbable microspheres composed of chitosan or poly(ethylene glycol) derivatives, *in situ* gelling liquid embolics with improved safety profiles, and radiopaque embolics that are trackable *in vivo*. This article reviews commercially available materials used for embolization as well as polymeric materials that are under investigation.

Graphical Abstract

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Keywords

Embolics; polymers; drug eluting beads; hepatocellular carcinoma; arteriovenous malformations; uterine fibroids

1. Introduction

1.1 Embolotherapy

The concept of embolization, *i.e.*, deliberate blocking of blood vessels, dates back to the early 1900s. Robert Dawbarn presented “the starvation plan” to treat malignant growths in 1904, discussing how physically stopping blood flow to a tumor via ligation of vessels leads to shrinkage and pain relief for the patient [1]. Attempts to use materials to create a physical obstruction to blood flow dates to the early 1930s by Hamby and Gardener who surgically treated carotid-cavernous fistulas using muscle fragments [2]. Percutaneous embolization as a medical procedure was made possible by the early 1960’s technological development of the fluoroscope, providing the ability to perform an angiogram by viewing X-rays in real-time. Charles Dotter, considered the “Father of Interventional Radiology”, performed the first interventional angiographic procedure in 1964 and was eventually nominated for the Nobel Prize in medicine in 1978. He invented an automated X-Ray Roll-Film magazine capable of capturing images every 2 seconds, as well as various catheters and the J tipped guidewire [3,4]. Later, he introduced the concept of percutaneous transluminal angioplasty and the beginnings of diagnostic coronary angiography. Building off these initial developments, interventional radiology soon advanced to treatment of acute gastrointestinal bleeding, occlusion of arteriovenous malformations and fistulas, to more recent advances in interventional oncology, allowing for chemoembolization of highly vascular tumors such as hepatocellular carcinoma.

The earliest embolizations made use of autologous clots, muscle fragments, or stainless steel pellets. By the 1970s, clinical applications began to drive the development of new materials. Gelatin sponges, first developed as hemostatic materials for open surgery, soon transitioned to use as endovascular embolic agents as a response to failures of muscle fragment embolization. Thomas Speakman reported first use of “mashed” gelatin pieces mixed with saline to a consistency of “good porridge” injected into the internal carotid artery to occlude a carotid-cavernous fistula [5]. The occlusion was successful, eliminating signs and symptoms of the fistula without loss of vision or serious complications in the patient post-operatively.

Embolic materials are classified broadly into mechanical embolic agents or flow-directed embolic agents [2]. Mechanical agents such as metal coils and plugs used to treat focal vascular abnormalities are not within the scope of this review. Flow-directed agents can treat diffuse vascular abnormalities and include particulates, polymers, or *in-situ* gelling biomaterials delivered via catheters positioned within a target’s vascular supply. These materials are subcategorized into permanent or temporary. From a clinical standpoint, the therapeutic goal and intended long-term outcome dictate the choice of materials. Questions typically asked include: “Why is embolization necessary?” and “What lies downstream of

the intended target?” If embolization is required for obstruction of a hemorrhaging vessel, a temporary, fast acting embolic is required. A vascular malformation, however, often requires a more permanent material and more precise administration. Polymers have been successfully designed for a variety of biomedical applications, including as embolic agents [6]. The ability to tailor the polymer structure-function relationship for a defined need provides a unique materials platform. Both natural and synthetic polymers have been used to develop flow-directed embolics [7].

Gelatin sponge made from purified porcine skin is the first example of a successful naturally derived polymer commonly used as a temporary occlusive material [7,8]. The sponge comes in the form of dry sheets that are manually cut into small sections, then mixed with saline and contrast dye just prior to injection. This material provides a fast, low-cost, transient embolic that is used in multiple clinical applications. However, a severe drawback of this agent is lack of precision in delivery, and unpredictable levels of embolization and recanalization [7]. In response, calibrated microspheres (MSs) have been designed using poly(vinyl alcohol) and derivatives to provide improved control [9]. Although with these MSs the level and location of occlusion is highly predictable, they are not degradable and result in permanent vessel occlusion. To address these concerns, degradable materials composed of chitosan derivatives, for example, are being designed for synthesis of microspheres with predictable degradation rates [10]. More recently, biodegradable recombinant silk-elastinlike block copolymers have been used as a liquid embolic [11]. This review highlights these and a series of other embolic materials, first exploring clinical applications followed by material details including physical and mechanical properties, advantages, and disadvantages followed by a discussion of future directions in this field. The article discusses both commercially available agents as well as materials under development.

1.2 Clinical Applications

1.2.1 Vascular Malformations and Hypervascular Tumors—Vascular malformations are often congenital anomalies arising from dysplastic vascular channels. They are characterized by a tangle of thin walled vessels whose abnormal characteristics eventually lead to clinical symptoms requiring treatment. Symptoms vary depending on the category of malformation and location in the body and are classified as either slow-flow or fast-flow [2,12,13]. Slow-flow vascular malformations consist of capillary, venous, or lymphatic vessels. Symptoms range from externally visible port-wine staining of the skin to painful cyst-like formations. Fast-flow arteriovenous malformations (AVMs) consist of an arteriovenous shunt bypassing the capillary bed [14] resulting in high flow into the venous system. AVMs within the central nervous system may be particularly dangerous and require treatment to prevent risk of hemorrhage, stroke, neurological deficit, or seizure. Cerebrovascular AVMs are graded by the Spetzler-Martin scale that classifies malformations according to size, location relative to functionally eloquent cortex, and type of venous drainage [15]. Microsurgery for low grade AVMs can provide an immediate cure with a relatively low risk of complications, but remains an invasive procedure. Embolization therapy can be used as a sole treatment method or in combination with surgery or radiotherapy to eliminate or stabilize an AVM, decreasing the risk for hemorrhage [16]. Embolic microspheres are approved for the treatment of AVMs. More recently, liquid

embolics, particularly non-adhesive ethylene vinyl alcohol (EVOH) or Onyx[®], has been investigated as an improved option [17].

Hypervascular tumors are characterized by tumor induced abnormal vasculature consisting of increased numbers of blood vessels feeding into the tumor. The higher blood flow increases the risk of bleeding during surgical resection. Examples of hypervascular tumors include: head and neck tumors like meningiomas and paragangliomas, hepatocellular and colorectal carcinomas, and subsequent metastases. Embolotherapy is often used to block blood flow to the tumor either as a neoadjuvant method prior to surgery or as a palliative measure if the tumor is inoperable. This treatment not only decreases the risk of bleeding, but also may lead to shrinkage of the tumor thus improving surgical outcome, quality of life, or both. For hypervascular tumors that are operable, neoadjuvant embolization makes use of microspheres, gelatin sponges, liquid embolics or a combination, depending on the clinician or institutional policy. In the past, the primary goal had been solely to block the blood supply of the tumor. In the case of hepatocellular carcinoma (HCC), endovascular therapy has transitioned into a primary treatment involving the co-delivery of chemotherapeutics and embolics. Liver HCC lesions are unique in that they draw blood from branches off of the hepatic artery while the healthy liver parenchyma primarily receives blood supply from the portal system, allowing embolization of the tumor while preserving the surrounding hepatic tissue [18].

1.2.1.1 Transarterial Embolization and Chemoembolization—Transarterial embolization (TAE) will be explained using treatment of a hepatic tumor as an example. The procedure begins with accessing the arterial system via the femoral artery, using Seldinger technique. Once stable access is established, a guidewire and catheter system is maneuvered to the target location under angiographic guidance. A diagnostic angiogram is performed to identify the vascular supply to the tumor along with any potential anatomic variants [19]. After diagnostic angiography, a vessel is selected for embolization [20]. The catheter is used to access the target vessel, and location is confirmed by injection of contrast. The catheter is then used to deliver the selected embolic. The primary procedural endpoint is angiographic evidence of stasis [21]. Classically, the term TAE in reference to HCC treatment refers to bland embolization where the goal is to induce ischemic necrosis in the tumor. The embolic agents used to achieve this clinical outcome are highly variable. If a temporary occlusion is desired, gelatin sponge is used. If permanent occlusion is intended, poly(vinyl alcohol) or trisacryl gelatin particles are used. However, clinical data over the past few decades has confirmed bland embolization does not provide the best possible outcome [18,22]. Since TAE, chemoembolization was developed to improve patient morbidity and mortality. It is a procedure similar to TAE, but with the addition of localized delivery of chemotherapeutics via the same catheter, termed transarterial chemoembolization (TACE).

TACE can be divided into conventional TACE (cTACE) and drug-eluting bead TACE (DEB-TACE). Conventional TACE involves sequential delivery of a mixture of chemotherapeutics and ethiodized oil into the tumor vasculature followed by an embolic, whereas the newer DEB-TACE uses a single delivery system of drug loadable microspheres, which both occlude the selected vessels and provide controlled drug release [23]. A series of clinical studies in the past decade have shown benefits of DEB-TACE over cTACE. The

PRECISION V study, a multicenter randomized trial comparing the short-term outcomes of DEB-TACE and cTACE, demonstrated that DEB-TACE was associated with improved tolerability, with significant reduction in liver toxicity and chemotherapeutic related side effects [24]. The PRECISION ITALIA study randomized patients to cTACE or DEB-TACE, and required a post-TACE follow-up for at least 2 years or until death. This study failed to establish a significant difference between the two groups in terms of overall survival, but did show significantly lower post-procedural pain in the DEB-TACE group [25]. The DEB-TACE group also showed improved objective response, defined as complete or partial response following the modified Response Evaluation Criteria in Solid Tumors (mRECIST) [26].

Finally, the DEB-TACE process provides several more benefits to the clinician and the patient. Handling chemotherapeutics loaded on beads is less complex, and does not require use of ethiodol, which can dissolve certain types of plastic [27]. Lower pain and systemic toxicity post chemoembolization are important benefits to the patient. Some of the current drawbacks of the available DEBs include permanent occlusion, limited selection of drugs that can be loaded onto the beads, and inability to track bead distribution *in vivo*. Development of degradable microspheres may expand treatment options, allowing treatment of organs and tumors that do not have dual blood supplies. New materials that may load more than one drug type will allow for more personalized medicine and combinational chemotherapy.

1.2.2 Aneurysms and Hemorrhage—Aneurysms are outpouchings or dilations of weakened vessel walls. As tension on the weakened wall increases, the risk of rupture and hemorrhage increases. They can occur throughout the body, but are more often associated with the aorta, the renal artery, the Circle of Willis, and AVMs. The type of treatment depends on the shape, location, and cause [9]. Conventional vascular surgery including resection or clipping was the previous standard of care. However, with the development of new tools, endovascular approaches are becoming more common. Before treatment, imaging modalities including multidetector-row CT and Doppler ultrasonography are used to characterize the aneurysm. The liquid embolic Onyx[®] and liquid glue N-butyl cyanoacrylate (NBCA) are commonly being used to treat smaller aneurysms over metal coils or microspheres [9]. A randomized, prospective trial by Simon, *et al.*, followed thirteen patients treated with Onyx[®]. Twelve patients had internal carotid artery aneurysms and one vertebral artery aneurysm [28]. Onyx[®] was shown to successfully embolize the aneurysms in all but one, for whom the treatment was shifted to embolization with metal coils. Eleven of 13 patients (85%) at their 6-month follow-up demonstrated persistent occlusion with no recanalization. One patient (8%) had a complication and required re-treatment. The liquid glue N-butyl cyanoacrylate (NBCA) was more successful in treating hemorrhagic cases because the liquid solidifies much faster than Onyx[®]. One example of an urgent hemorrhagic situation is a hepatic lesion, particularly of the hepatic artery. A retrospective study by Monsignore, *et al.*, investigated eight patients with hepatic hemorrhagic lesions presented in the emergency room who were all stabilized by percutaneous transcatheter embolization with NBCA [29]. Clinical success was achieved in all eight patients, and these patients had no major complications during the hospital stay or during the follow-up period,

except for one who developed a large asymptomatic peritoneal pseudocyst, which resolved spontaneously by the three-month follow-up.

1.2.3 Uterine Fibroids—Synonymous with uterine leiomyomas or myomas, uterine fibroids are benign tumors of the myometrium [30–32]. They are a major cause of morbidity among women of reproductive age, with an incidence of 20–40% [32,33]. Patient symptoms are highly variable; some patients are asymptomatic whereas others may experience symptoms including abnormal uterine bleeding, chronic pelvic pain, urinary incontinence, ureteral obstruction, and fertility issues. Clinical management of uterine fibroids depends on patient symptoms. An asymptomatic fibroid identified during a pelvic exam will remain under observation whereas symptomatic fibroids may be treated medically or surgically[30].

Uterine artery embolization (UAE) is an organ-preserving treatment alternative originally reported in 1995 [34]. Since the first case reports by Ravina, *et al.*, this procedure has been shown to be safe and effective [2,35]. In UAE studies using permanent microspheres as an embolic, fibroid volume decreased by $53.4 \pm 26.9\%$, 3 months after UAE, by $63.7 \pm 28.2\%$ after 6 months, and by $71.8 \pm 26.8\%$ after 12 months [36,37]. Randomized comparative trials of embolics have shown differences in infarction rate between the different commercial products. Trisacryl gelatin microspheres (Embosphere®, Merit Medical Systems) compared to spherical poly(vinyl alcohol) (PVA) (Contour SE™, Boston Scientific) beads showed higher infarction rates and lower treatment failure as detected by magnetic resonance imaging [2,38]. In this prospective randomized study of 53 patients, 8 patients in the PVA microsphere group (29.6%) failed treatment, while only one failed in the trisacryl gelatin microsphere group (3.8%), a significant difference ($P < 0.025$) [38]. Yu, *et al.*, conducted a similar double-blinded randomized controlled trial with 27 patients in the trisacryl microsphere group and 29 in the PVA microsphere group. This trial showed that UAE with trisacryl microspheres was superior in terms of clinical success rate, complimenting the results from the older study [39]. The primary difference between groups was microsphere material.

Uterine fibroids are very sensitive to ischemia. Myomectomy has less of an impact than UAE on overall fertility; therefore women desiring children may choose the more invasive surgical procedure [32]. However, due to the advantages of minimal access techniques, new degradable materials are being developed for transient embolization. Several clinical case studies have shown that transient occlusion using mechanical compression of the uterine arteries up to 6 hours leads to fibroid volume reduction and menorrhagia symptom relief [42,43]. Development of temporary embolic microspheres (< 6hr) will provide for a similar outcome using the faster UAE while preserving fertility. Table 1 summarizes these clinical applications that require embolotherapy.

2. Commercially Available Materials

The number of flow-directed embolic materials is expanding. This review investigates a series of products with extensive original research and clinical data associated with development and clinical outcome for the indications outlined above. Sub-categories of flow-directed embolics will be discussed, including temporary and permanent materials. The

availability and approved indication of use for the marketed materials discussed in this review vary between countries. Structures and features of these materials are summarized in Table 2.

2.1 Gelatin Sponge

Correll and Wise first introduced the gelatin sponge in 1945 as a new biologic absorbable hemostatic sponge [44]. They tested the new material in high blood flow applications to assess effectiveness of hemostatic properties. In a series of tests, canine livers were cut and saline soaked gelatin sponge was placed over the site of bleeding while applying direct pressure for up to ten minutes [45]. Complete hemostasis was achieved without any other interventions. Within one month, minimal sponge material remained and the injury had well-differentiated connective tissue covering it. Indications for use expanded and by 1967 the endovascular use of gelatin sponge in two patients was reported by Ishimori, *et al.*, where Gelfoam® was used to endovascularly treat large carotid-cavernous fistula [46]. A polyethylene tube was inserted into the carotid artery and guided to the mouth of the cavernous portion where tightly rolled Gelfoam®, with gold film used as an imaging marker, was injected until no blood refluxed back through the tube. The level of occlusion was verified postoperatively by radiography. Complete vessel occlusion was achieved by a combination of mechanical blockage and thrombus formation after contact with blood with the gelatin acting as a physical matrix to promote clot formation [47]. Gelfoam® manufactured by Pfizer is prepared from purified porcine gelatin that is processed with nitrogen resulting in a porous sponge-like material. Similar products are available globally from several other manufacturers, but the principles of preparation and application are similar. The sheet form requires preparation prior to use, including cutting the sheet into small sections followed by mixing with saline and contrast. The mixture is converted into an injectable slurry utilizing two syringes and a three-way stopcock [48]. Another approach is the torpedo method where thin sections are cut into columns and compressed into a “torpedo” that is flushed through a catheter. In addition to the sheet form, gelatin sponge is available in powder format. Gelatin sponge provides quick embolization at a low material cost. The flow reduction is temporary, with most vessels recanalizing between 3 weeks and 4 months [7,49]. Recanalization, however, is unpredictable and may not occur at all. Furthermore, reorganization of the Gelfoam® starts immediately after injection, resulting in resumption of flow. Finally, older case reports have described infection associated with use of these hemostatic sponges; implicating long exposure of the porous material to contaminated air [50,51]. However, no recent reports or reviews within the past decade have confirmed these problems, which may be attributed general improvements in sterile technique.

2.2. Degradable Starch Microspheres

Degradable starch microspheres (DSM) developed in the mid 1970s by Pharmacia AB, provide for a highly transient embolic effect [52]. Composed of cross-linked hydrolyzed starch, amilomeres, material half-life is approximately 40 min per the manufacturer (EmboCept® S (PharmaCept) or Spherex® (Magle Life Sciences)) [53]. In practice, the half-life ranges between 25 to 60 minutes [49,54,55]. The microspheres are degraded *in vivo* by plasma α -amylase into oligosaccharides, maltose, and eventually glucose that enters the

normal metabolic cycle. The maximum duration of vessel occlusion is 80 min. Both microspheres average 50 μm in diameter and are intended for temporary embolization of small vessels. DSMs have been used clinically in cTACE in treatment of HCC to provide transient occlusion to reduce blood flow in the tumor bed and increase the amount of time the chemotherapeutic emulsion remains in the cancerous tissue. This material has been used in patients who require multiple chemotherapeutic treatments via cTACE or in patients with decreased hepatic function who cannot tolerate more damage to the surrounding hepatic tissue that may be induced by ischemic necrosis. Pieper *et al.*, evaluated the embolic properties, time to perfusion, and histologic changes in temporary embolization of healthy swine liver using EmboCept[®] DSMs [55]. An average of 6 mL of EmboCept[®] at a concentration of 6 mg/mL was used to embolize an entire hepatic lobe and angiography showed reperfusion in 32 ± 6.1 min. Gross examination of the liver tissue did not indicate signs of hypoperfusion, and histological examination demonstrated no difference between treated and untreated hepatic parenchyma. In one animal specimen, however, some previously embolized hepatocytes had signs of apoptosis. This study demonstrated that temporary transarterial embolization with DSMs will not damage healthy hepatic tissue. A potential application for use of these particles based on these results is injecting non-tumor-supplying vessels with DSMs immediately before injecting chemoembolic material into the tumor vasculature in order to preserve non-diseased liver parenchyma [55–57].

2.3. Liquid embolics and glues

Liquid embolics flow through the vasculature as a fluid, which allows for deep penetration of small diameter target vessels and the ability to conform to the lumen of any vasculature before transitioning into a solid material. Unlike particle systems, with narrow diameter ranges of occlusion, liquid embolics can be used to occlude vasculature of a variety of diameters.

2.3.1. NBCA—The glue, N-butyl cyanoacrylate (NBCA), emerged as an embolic material in the 1980s and was approved for use by the U.S. FDA in 2000 for the treatment of cerebral arteriovenous malformations [58,59]. In its monomer form, NBCA is a clear liquid that polymerizes to form a rigid matrix upon activation by contact with any ionic substances (*e.g.*, blood, saline, ionic contrast media, and vascular endothelium) [59]. Trufill[®] marketed by Cordis Neurovascular, Inc., provides physicians with a glue kit consisting of NBCA, ethiodized oil, and tantalum powder, which are combined on site just prior to use. Recommended concentrations of NBCA range from 25–67% depending on the flow characteristics and anatomy of the occlusion target. Combining NBCA with ethiodized oil slows down the rate of polymerization in static blood from less than 1 second at 67% NBCA to 6 seconds at 25% NBCA [59]. The polymerization of NBCA releases formaldehyde, leading to inflammation of the vessel wall and surrounding tissue, eventually causing a chronic granulomatous inflammation [48,59–61]. Mixtures of NBCA are typically prepared immediately prior to administration, as NBCA will begin polymerization upon exposure to air [48]. Manufacturer recommendations are for only a single injection of NBCA to be administered through a catheter, due to risk of NBCA adhering to the catheter and occluding the lumen. NBCA is commonly used for AVM embolization and off-label for hemoptysis [62], preoperative embolization for partial hepatectomy [63], and acute bleeding in the

gastrointestinal tract [64]. While coagulopathies can prevent embolic coils, particles, or Gelfoam[®] from forming an occlusive thrombus within vessels, the adhesive nature of NBCA allows it to mechanically occupy the intravascular lumen and stop blood flow regardless of blood coagulability [47,48,65].

2.3.2. Onyx[®]—Onyx[®] (Medtronic plc), first described as a potential embolic agent in 1990 for the embolization of intracranial arteriovenous malformations (AVMs), is composed of ethylene vinyl alcohol (EVOH) copolymer dissolved in dimethyl sulfoxide (DMSO) [66]. Onyx[®] functions by forming a solid EVOH precipitate as the DMSO carrier solvent dissipates within the bloodstream. Unique among liquid embolics, Onyx[®] solidifies in an “outside-in” fashion. This results in the almost instantaneous formation of a solid cast around the exterior of the flow, while the interior remains fluid and continues to flow deeper into the lesion. This process is reminiscent of the solidification of a lava flow. Formulations with lower concentrations of EVOH travel more distally from the catheter tip due to lower viscosity, while higher viscosity formulations offer better control in high flow environments. Nevertheless, regardless of the formulation, Onyx[®] completely solidifies within five minutes of injection [67]. Onyx[®] forms a permanent occlusion, which has been shown to be stable for up to 5.25 years [68]. After being initially approved for use in AVM and intracranial aneurysms, Onyx[®] has been used to effectively treat peripheral arteriovenous malformations [69], acute hemorrhages in the cardiopulmonary and gastrointestinal systems [70], preoperative embolization of vascular tumors [71], bleeding from ruptured aneurysms [72], and stent graft-related endoleaks [73–75]. Care must be taken when using Onyx[®] to ensure that it does not contact contrast media or blood prior to exiting the catheter. As such, the use of Onyx[®] requires that special DMSO compatible catheters be used, and flushing of the catheters with DMSO prior to administration. While Onyx[®] is non-adhesive, the catheter may still become trapped if the plug formed around the tip during administration becomes too long (>2 cm).

The liquid nature of both Onyx[®] and NBCA provide an advantage over particulate embolics, allowing passage into smaller vessels. By forming a confluent solid mass, these embolics can completely fill a target aneurysm sac or halt flow within an actively hemorrhaging ruptured aneurysm. Onyx[®] can occlude vessels down to 5 µm and NBCA vessels 20 µm in diameter [76]. In further comparison of these liquid embolics, NBCA has a very short working time due to rapid polymerization upon contact with ionic fluid (*i.e.* blood) and can adhere to any material including vessel walls proximal to the intended target, and even the delivery system itself [77]. This can lead to permanent catheter occlusion requiring replacement of the delivery system and increasing procedure times, or even adherence of the catheter itself to the vessel wall. Onyx[®] has a longer working time and is not an adhesive, however, the material is suspended in DMSO that causes vascular inflammation and even angioneurosis if injected rapidly enough to reach high DMSO concentrations at the catheter tip [78]. A study led by Natarajan, *et al.*, analyzing the histopathological changes in resected AVMs after undergoing embolization using Onyx[®] or NBCA identified evidence of inflammatory damage induced by both materials. Of the 32 patients, Onyx[®] was used in 22 and NBCA in 10. Perivascular inflammation was evident in 90.9% of the Onyx[®] tissue samples, and 90% of the NBCA tissue samples. Some inflammation was to be expected as a

response to foreign material within the vessel lumen, but chronic foreign-body giant cells were found in 54% of the Onyx[®] samples and 0% in the NBCA samples. Furthermore, angioneclerosis was observed in 60% of the Onyx injected vessels versus 40% in the NBCA injected vessels [76]. Although the sample size was small in this study, it provides insight into complications with these materials that may be addressed by the next generation of liquid embolics in development.

2.3.3 PHIL[™]—Precipitating Hydrophobic Injectable Liquid or PHIL[™] (MicroVention, Inc.) is a new injectable liquid embolic that is approved for clinical use in Europe for the embolization of lesions in the peripheral and neuro-vasculature, including arteriovenous malformations and hypervascular tumors. Similar to Onyx[®], PHIL[™] is a non-adhesive embolic suspended in DMSO, comprised of the copolymer poly(lactide-*co*-glycolide) and poly(hydroxyl ethyl methacrylate) with triiodophenol incorporated into a portion of the monomers via a covalent linkage, providing radiopacity for visualization instead of utilizing tantalum powder as is used with Onyx[®] [79]. PHIL[™] remains suspended in DMSO until it comes into contact with blood or water, at which time the polymer precipitates into a solid, advancing into the vasculature as a ‘block’ instead of forming layers [80]. The covalently bound iodine component is an advantage over the suspended tantalum powder used in Onyx[®] and PHIL[™] therefore does not require the 20 minutes of mixing required for Onyx[®]. Three concentrations are available: 25%, 30%, and 35% (concentration of polymer by weight). Preliminary clinical data is available for PHIL[™] used in treatment of cerebral AVMs and cranial and spinal dural arteriovenous fistulas [81,80,82]. The study by Kocer *et al.* compared AVMs first treated with a liquid embolic followed by resection [81]. Either PHIL[™] was used alone or in combination with Onyx[®]. In this study, the authors noted PHIL[™] compared to Onyx[®] had better ease of use, faster plug formation, and less CT and MRI artifact at follow-up imaging. However, PHIL[™] was more brittle during vessel dissection and less pliable during surgery in comparison to Onyx[®]. Histopathology showed PHIL[™] caused a moderate vascular inflammatory effect and no angioneclerosis on same-day resected specimens, while Onyx[®] had less vascular inflammation. Samaniego *et al.* reported on successful use of PHIL[™] in treatment of ruptured plexiform AVMs. One major difference of PHIL[™] from other liquid embolics was that no microcatheter occlusion occurred during injection. Furthermore, PHIL[™] offered less resistance to injection allowing for deeper penetration, but increasing the risk of overpenetration and extension into the venous drainage. Finally, Leyon *et al.* described the outcome of PHIL[™] in treatment of dural arteriovenous fistulas (DAVFs) with intent to cure. In the case of DAVFs, venous penetration of the embolic material is important; therefore increased penetration of PHIL[™] when compared to Onyx[®] was advantageous as was the retrograde flow that was used to fill fistula feeders.

All three studies showed favorable outcome for PHIL[™] as a new liquid embolic and strong competitor to Onyx[®]. However, the studies had a limited number of patients, less than ten cases each. Larger studies are required to fully evaluate the long-term safety and efficacy of this new product as well as in comparison to the other liquid embolics previously described.

2.4. Calibrated microspheres

Poly(vinyl alcohol) particles were developed in the 1970s as a widely used and cost effective permanent embolic. The particles are composed of a water-soluble linear synthetic polymer, and synthesized using hydrolysis of poly(vinyl acetate). It was originally marketed as Ivalon® and distributed as a sheet or block material that was subsequently shaved down to irregularly shaped particles prior to use [83]. The polymer material is formulated as foam, which is hardened with formaldehyde then packaged in a dry state. Some of the first case studies using Ivalon® as an embolic were presented by Tadavarthy *et al.*, in 1975, when they embolized the renal artery of dogs and subsequently utilized Ivalon® in patients [84]. This synthetic material proved to be superior to autologous clots in terms of *in vivo* detection and occlusive ability [85]. The PVA material is compressible, but expands back to original size after injection, therefore occluding vessels larger than the internal diameter of the catheter [83]. However, these particles are irregularly shaped with a large size distribution, leading to clumps that can cause unpredictable behavior during embolization. These clinical disadvantages spurred development of calibrated microspheres composed of synthetic polymers in the 1990s and 2000s. Several microspheres (MSs) are approved for use as embolic agents including: trisacryl gelatin Embosphere® (Merit Medical Systems), and PVA based Contour SE™ (Boston Scientific) and PVA hydrogel Bead Block® (BTG). First, the effect of morphology on clinical outcome will be discussed.

In comparative studies between PVA particles and microspheres, geometry and surface properties proved to be important factors for embolic performance and physiologic outcome [41]. Bendszu, *et al.*, compared the efficacy of Embosphere® microspheres to non-spherical PVA in preoperative embolization of meningiomas, measuring devascularization and intraoperative blood loss, along with the inflammatory reaction, the extent of necrotic areas, and the most distal intravascular location of the embolic agent in the resected tissue. Each group had 30 patients and no significant difference existed between groups when comparing devascularization post embolization. However, the mean intraoperative blood loss was significantly higher in the PVA group compared to the MS group. The MSs induced more necrosis, and were found significantly more distally within the tumor vessels compared to the PVA particles. No difference in inflammatory reaction between the groups was observed. These studies corroborate the claims that embolic morphology directly affects performance. Histologic evaluation of the tissues showed that the PVA particles formed aggregates in larger more proximal vessels compared to the Embospheres® that formed chains in smaller vessels located more distally [86]. Similarly, in a prospective study by Chua *et al.*, 355–500 micron PVA particles and 700–900 micron MSs were compared in pre-myomectomy uterine artery embolization for reduction in intraoperative blood loss, with similar results. Under microscopy, non-spherical PVA particles appeared as aggregates of irregularly shaped material, while MSs appeared as isolated or clusters of spherical material with occasional slight deformity. The MSs were found to have penetrated more deeply into smaller caliber tumor vessels, whereas PVA particles were found predominantly in the perifibroid tissues [87].

In an animal study using sheep, the aggregation of the non-spherical PVA particles was demonstrated in embolized ewe uterine arteries. Four size ranges of both PVA particles and

trisacryl gelatin MSs were tested [88]. Histological analysis of uterine tissues post-embolization revealed no difference in distribution of the PVA particles regardless of size, compared to the more precise distribution of MSs. All size ranges of PVA particles were located both proximally in aggregates and distally with a wider range of vessels. Furthermore, the mean number of PVA particles in each occluded artery was higher than that of MSs, 12.2 versus 2.2 respectively, and number of particles in occluded arteries varied more with PVA particles [88]. Aggregation may be attributed both to non-uniform shape (more surfaces and total area for particle-particle interaction) and the highly compressible nature of the PVA fostering accretion [40,41]. Although the particles were able to achieve good vessel occlusion, depth of occlusion and distribution could not be controlled, therefore increasing the risk of non-target embolization and unpredictable level of embolization. These studies provided evidence for a morphological advantage of MSs over PVA, but since the embolic materials tested were different in physical properties, the question remained whether morphology alone could explain the inherent clinical improvements or if the material properties of the polymer itself may have contributed. To address this, multiple clinical studies comparing PVA MSs to trisacryl gelatin MSs have been conducted. Spies *et al.* compared matched particle sizes of spherical PVA MS, Contour SETM, to the trisacryl gelatin Embosphere[®] for uterine artery embolization treating leiomyomas in thirty-six patients; there were no differences in the two treatment groups pre-treatment and patients were followed for three months [89]. A significant number of patients treated with PVA spheres had an incomplete response to treatment, and did not have complete infarction of the lesions. Furthermore, the trisacryl gelatin MSs treated patients had superior outcomes, level of infarction and quality of life score, both for short term and long term measures. Similar results were determined in subsequent, larger prospective studies [38,90,91]. The theory remains that the material properties, high compressibility, of PVA compared with trisacryl gelatin serves to produce early redistribution and recanalization as a result of particle deformation assuming a smaller profile after embolization [89,92], allowing more distal passage when injected, but also partial revascularization of portions of the fibroid tumor tissue [89].

Newer research has been undertaken to identify the effects of material properties on microsphere behavior [40,41,93]. Lewis *et al.*, conducted an *in vitro* characterization study in 2006 comparing several microspheres, including Embosphere[®], Contour SETM and Bead Block[®]. Evaluation of bead compressibility, a characteristic important for flow-directed embolics, demonstrated that the force required to compress Contour SETM was substantially lower than both Embosphere[®] and Bead Block[®], 5 kPa versus 27 kPa and 24 kPa, respectively and illustrated in Fig. 1 [40]. Bead passage through a catheter was evaluated using catheters coiled into a figure “8” to emulate vascular tortuosity and measuring ease of injection and level of aggregation. Using the same catheter type and matched microsphere size range, the degree of catheter occlusion was identified as Contour SETM >> Embosphere[®] > Bead Block[®] [40]. Next, the material composition of the presented MSs will be discussed to explain these results.

The trisacryl gelatin MS, Embosphere[®] (Merit Medical Systems), was originally developed in the early 1990s and is a copolymer of a N-tris-hydroxymethyl methylacrylamide monomer, a diethylaminoethylacrylamide monomer, and a N,N-methylene-bis-acrylamide

monomer [94–96]. The polymerization is followed by a stage of reticulation with gelatin. These microspheres carry an effective cationic charge rendering them hydrophilic and non-aggregating. Suspended in saline and contrast prior to use, they remain uniformly spherical and smooth [97]. Six calibrated size ranges are available, the lowest range being 40–120 μm and highest 900–1200 μm . Contour SETM (Boston Scientific) microspheres are non-ionic materials composed of cross-linked poly(vinyl alcohol) (PVA) synthesized using PVA of molecular weight 75,000 g/mol, which is reacted by acetalization [98]. Unique among these embolics, PVA MSs are macroporous. The surface region is defined primarily by relatively small pores while the interior region is defined primarily by relatively large pores, thus pore size progressively increases from the surface to the center of the particle [98]. This structure renders the periphery of the particle more rigid, while the increasing pore size to the center allows for symmetric compressibility. The macroporous structure explains the high compressibility of PVA MSs and supports the theories presented earlier explaining the further penetration and redistribution of these beads as compared to the trisacryl MSs. As for Bead Block[®] (BTG), these microspheres are composed of a PVA hydrogel cross-linked with acrylic polymer, synthesized following Nelfilcon chemistry that was developed by Ciba-Geigy Corporation (now Novartis) in 1995 [99]. The PVA is modified into a macromer and co-polymerized with 2-acrylamido-2-methylpropane sulfonate salt (AMPS) in a reverse-suspension polymerization [100]. This sulfonate group imparts hydrophilicity and anionic character to the material, driving hygroscopy. The concentration of AMPS monomers affects physical characteristics of the microspheres. In terms of physical properties, Bead Block[®] falls in between Embosphere[®] and Contour SETM for compressibility but has the lowest degree of catheter clogging. Furthermore, Bead Block[®] takes more time to reach suspension for injection, but remains suspended twice as long as its competitors [40]. The hygroscopic nature of the Bead Block[®] may explain the longer suspension time; water molecules surrounding the MS minimize aggregation tendencies. A more comprehensive retrospective study by Abramowitz compared four embolics, non-spherical Contour PVA particles, Contour SETM, Embosphere[®], and Bead Block[®], for uterine artery embolization using post-procedural magnetic resonance (MR) imaging in 84 patients 6-months post-operatively [101]. Pre- and postprocedural MR imaging results were analyzed for the total number of uterine fibroids present and for the percentage of individual fibroid enhancement. The Embosphere[®] and Contour PVA particles significantly outperformed Contour SETM and Bead Block[®]; mean reduction in enhancement by 83.07% and 76.60% respectively, compared with a mean reductions of 49.78% and 52.53%. Partially infarcted fibroids often continue growing after embolization, increasing a patient's risk for future symptom recurrence [101]. These results corroborate to an extent with the Spies study, with Embospheres[®] providing the best clinical outcome. The clinical results of the Contour PVA particles in this study may be attributed to the difference in size range. The MSs were all 500–700 μm in diameter while the PVA particles fell into a smaller range of 300–500 μm in diameter.

Unique to the Embosphere[®] is a gelatin coating. However, most studies surveyed have not specifically investigated how this coating may affect occlusive ability. Since clinical studies often have small patient numbers and individual clinician's techniques differ, a definitive answer has not been identified as to the superiority of one vs other. For uterine fibroids, the

Embosphere[®] may be more advantageous as a bland embolic. Next, MSs used to treat hypervascular tumors requiring chemotherapy will be explored.

The DC Bead[®] (Drug Capable) also by BTG, has a similar composition as Bead Block[®], but synthesized using a higher content of AMPS, which lowers compressibility, therefore are manufactured at lower size ranges [102]. Further, the higher AMPS increases the total number of sulfonate groups, which allow these beads to be loaded with low molecular weight, positively charged drugs via ion-exchange method (Fig. 2). Drug loading ability establishes another category of embolic agents, namely chemoembolics. Drug loadable microspheres do not have FDA approval in the United States, but are approved by regulatory agencies in many other countries throughout Europe and Asia. Along with the DC Bead[®], the HepaSphere[™] (Merit Medical Systems) is available for drug loading. Similar to the DC Bead[®], the HepaSphere[™] can load small, positively charged molecules. HepaSphere[™] microspheres are synthesized by a polymerization process of vinyl acetate and methyl acrylate monomers, forming superabsorbent beads that are distributed dry. These beads swell up to 64 times their dry volume following hydration [103,104]. Both of these beads have been tested and approved for loading and release of irinotecan or doxorubicin in TACE treatments in the liver. Multiple comparative studies have been released throughout this past decade addressing the benefits of each drug eluting microsphere. A 2010 study by Jordan *et al.* characterized *in vitro* the loadability, physical properties, and release of irinotecan (IRI) and doxorubicin hydrochloride (DOX) from DC Bead[®] and HepaSphere[™] [105]. Loading mechanisms for each bead depends on the bead material. The DC Bead[®] utilizes the ionized sulfonate groups on the polymer chain, exchanging a water molecule for the charged drug molecule when submerged in a drug solution. HepaSphere[™] uses mechanical loading where rapid absorption of water transports the drug molecules into the matrix and charge interactions between the drug and carboxylic groups maintain the drug inside. DC Bead[®] requires 2 hrs for drug loading while HepaSphere[™] requires 1 hr. Both drugs had similar loading profiles for each bead, however, release kinetics into 0.9% saline media under 5 mL/min flow differed as did the changes imparted to the bead mechanical properties. The total percent of doxorubicin released from the DC Bead[®] was 27 ± 7 and 18 ± 2 for the HepaSphere[™]. Time required to reach 75% of the plateau value was 2.2 hrs for both beads. A significant difference between release of doxorubicin from either bead compared to irinotecan has been shown: 102 ± 11 % from the DC Bead[®] and 95 ± 9 % for the HepaSphere[™], with $t_{75\%}$ being 1.1 hr and 0.12 hrs respectively. This indicates the two drugs interact with the microsphere matrices differently. Although both drugs have similar molecular weights (DOX: 580 Da, IRI: 623 Da) and ionic character, the total partial charge and charge distribution are different. The higher positive charge of the primary amine carried by doxorubicin provides stronger binding kinetics with the sulfonate groups in the DC Bead[®] and carboxylic groups in the HepaSphere[™] compared with the tertiary amine of irinotecan. Differences in release kinetics also affect drug distribution into tissue. Biondi *et al.* developed mathematical simulations based on the Langmuir isotherm and the Boyd and Bhaskar models elucidating transport mechanisms governing doxorubicin and irinotecan release from the DC Bead[®], which were confirmed experimentally [106]. In ionic drug loading/releasing systems, mass transport is dictated by the resistance to transport of both the drug and counterion in the liquid film surrounding the carrier. The ion exchange process

is much faster than solute diffusion, therefore drug diffusion within the bulk bead material or across the DEB surface is considered the rate controlling step for release kinetics. Their experimental studies validated the models, showing faster release of irinotecan than doxorubicin. Faster release of irinotecan is dictated by its lower affinity with the DEB, facilitating its removal from the DEB surface making particle diffusion through the bulk the rate controlling step, while the higher doxorubicin affinity for the DEB allows for film diffusion control [106]. These mathematical models may be applied to other systems like the HepaSphere™ as well as other positively charged drugs to estimate release kinetics of a chosen drug prior to clinical application.

In terms of mechanical properties and morphology, understanding the effects of drug loading on the microsphere matrices is important for catheter delivery and appropriate vessel occlusion. The Jordan study investigated both change in microsphere size and mechanical properties pre and post drug incorporation [105]. Both microspheres prior to drug loading suspended in saline and contrast media were measured microscopically. Post drug incorporation, either doxorubicin or irinotecan, diameters of both microspheres significantly decreased. DC Bead® diameters dropped by a factor of 0.80 and 0.67 for doxorubicin and irinotecan, respectively, while HepaSphere™ diameters dropped by 0.91 and 0.78. Both drugs are hydrophobic and they displace water to interact with the polymer backbone. In week long release studies using a saline sink, MS diameters began to return to original size. Doxorubicin was noted to have a slower release profile, particularly in the DC Bead®. This data corroborates with the Biondi mathematical analyses. Finally, change in MS elastic properties was tested. Drug loading increased the elastic moduli. Doxorubicin loaded DC Bead® had a 2.8 fold increase in modulus, while HepaSphere™ increased by a factor of 7.1. Increase by irinotecan was not as high, 1.33 and 1.81, respectively. Increase in rigidity may pose problems clinically since fracturing of particles becomes a risk. In fact, Jordan *et al.* observed fractures in the doxorubicin loaded HepaSphere™. Fractured particles may lead to a change in effective size, causing unpredictable embolization patterns. Finally, the variation in drug release profiles is important to consider prior to clinical use. Incomplete drug elution as seen with doxorubicin loaded DC Bead® may lead to reduced therapeutic efficacy and too fast of elution as seen with irinotecan from either MS system may also lead to reduced efficacy over time. However, *in vitro* release studies do not sufficiently model *in vivo* release kinetics.

In vivo studies have been conducted to answer how release kinetics affect tissue concentration of the drug, and how this changes efficacy in a tumor model. Namur *et al.* investigated doxorubicin release from DC Bead® in a porcine model, evaluating local tissue concentrations post liver embolization [107]. Two size ranges were tested, 100–300 µm and 700–900 µm, loaded with 37.5 mg doxorubicin/mL. Livers were sampled at 28 and 90 days. Infrared microspectroscopy was used to quantify drug amounts within the beads and surrounding tissue. At day 28, the drug content remaining in the beads was 22.5 mg/mL in the smaller diameter spheres and 15 mg/mL in the larger spheres, decreasing to 4 mg/mL and 7 mg/mL, respectively. Tissue changes were also evaluated at these time points. At day 28, the small doxorubicin eluting beads were associated with higher coagulative necrosis as compared with the larger beads, whereas the larger beads were associated with a higher percentage of fibrosis. By day 90, the necrotic tissue had converted to fibrosis in both test

sets. Drug concentration within the tissue corresponded to the state of the tissue. At day 28, small bead group, the doxorubicin concentration was higher in the necrotic tissue, 1.45 μM , than the fibrotic tissue, 0.55 μM . By day 90 these values increased to 2.30 μM and 0.60 μM , respectively. The same trend was observed with the larger beads, 4.80 μM within necrotic tissue, and 1.40 μM within the fibrotic tissue. By day 90, only fibrotic tissue remained surrounding the larger beads, with a mean doxorubicin concentration of 4.80 μM . Doxorubicin levels within normal hepatic parenchyma were below the limit of detection. Based on hepatocellular explant tissue, the IC_{50} of doxorubicin is 0.97 μM , and based on the results from this study, the drug levels were within antiproliferative range. However, microsphere size and location do dictate release kinetics and subsequently tissue drug levels. The larger particles occluded large vessels in proximal portal vessels with high flow adjacent to portal vein branches while the smaller particles travelled more distally into the arterial branches closer to hepatic lobules and sinusoids. A combination of these factors may lead to variable clinical outcome in a tumor model.

In a comparison study of DC Bead[®] to HepaSphere[™] loaded with irinotecan measuring safety and efficacy in a VX2 liver metastases rabbit model, Namur *et al.*, 2015, showed microsphere material affected drug release rates *in vivo*. This study measured plasma pharmacokinetics of irinotecan and its active metabolite SN38 followed by sacrifice of the test animals 3 days post embolization treatment for tissue assessment [108]. Both spheres at the smallest size range available were used. The median drug loaded HepaSphere[™] (HS-I) diameter was 153 μm and 99 μm for the DC Bead[®] (DC-I). Plasma concentrations of the drug were twice as high in the HS-I group than DC-I at all measured time points, ranging 5 minutes to 2 hrs. This trend agrees with the *in vitro* data from the Jordan study [105]. Histologically, both types of drug-loaded microspheres induced significant tumor necrosis as compared with untreated controls. However, HS-I treated animals showed significantly higher rates of tumor necrosis and reduction of surface tumor nodules compared with the DC-I group. This study's use of irinotecan changes the method of drug induced toxicity when compared to doxorubicin. Irinotecan must first be metabolized into its active form, SN38, by hepatic cytochrome P450. Therefore the faster rate of release may not be the only cause of higher necrosis. The HS-I were larger in diameter and less frequently found inside the VX2 tumor nodules than DC-I. This study showed not only the importance of understanding release kinetics from different microspheres, but also understanding the effect of drug loading on microsphere structure and the unique drug mechanism of action. The irinotecan loaded microspheres induced significantly greater necrosis than bland embolization, showing drug efficacy, but the HS-I microspheres were associated with higher necrosis than the DC-I due to faster drug release in normal liver parenchyma (less distal penetration), and ultimately higher conversion to SN38.

These studies comparing the different microspheres show the importance of understanding material differences and how they may affect clinical outcome. Calibrated microspheres have superseded non-spherical PVA particles for bland embolization procedures, providing for more reproducible and complete embolization results. Drug eluting beads have superseded older materials such as Gelfoam[®] for chemoembolic procedures by providing a combination embolic and drug carrier.

3. Research and development of advanced materials

3.1 Degradable/bioresorbable microspheres

Degradable gelatin sponge particles or slurry mixtures composed of porcine proteins have been used since the 1970s. This material, however, does not present the physician enough control with respect to size and therefore vessel selectivity nor rate of degradation. Vessel occlusion depends on aggregation of the particulates followed by thrombus formation. The enzymatic degradation is variable, and rates have been reported on a scale of days to up to 4 months. Furthermore, chronic inflammation is associated with the porcine origin material, along with the formation of foreign body giant cells [109]. The next development in degradable microspheres was the creation of starch MSs. The hydrolyzable starch has a short half-life, minute scale, and the particles have very small diameters, averaging 50 μm , limiting clinical applications. The advancement of degradable embolics has been slower, but several labs are currently developing improved materials [49,110,111]. Treatment of uterine fibroids, for example, requires transient embolization of the uterine artery, particularly in patients who want to remain fertile. Permanent or unpredictably degrading materials lead to chronic inflammation that may lead to consequences for fertility and pregnancy [30,31]. In this application, a resorbable microsphere providing at least 6 hrs of complete occlusion may be well suited [42,43]. In treatment of hepatocellular carcinoma, >90% of patients require re-treatment as new vessels sprout to feed the residual tumor. But, current microspheres are permanent and may induce ischemia, resulting in worsening of hepatic function to the point where further embolic treatments may not be tolerated without inducing complete hepatic failure [112]. Degradable microspheres that occlude and deliver chemotherapeutics over a period of a few weeks up to 1 month may overcome this problem.

Laurent and Moine *et al.* have synthesized poly(ethylene glycol) methacrylate (PEGMA) hydrolyzable microspheres for predictable transient embolization that require several hours as compared to minutes to degrade [49,113]. These microspheres were prepared using a suspension polymerization process of poly(lactide-*co*-glycolide)-poly(ethylene glycol) crosslinker and PEGMA monomer. Co-monomers methacrylic acid (MA) and 2-methylene-1,3-dioxepane (MDO) were incorporated to fine tune properties. Minimizing an inflammatory reaction to degradation products was an important factor in design strategy. Materials were chosen that degrade into water-soluble macromolecules post hydrolysis facilitating renal excretion. Microspheres were prepared at sizes ranging from 100 to 1000 μm , similar to commercially available MSs. A series of tests were conducted by Louguet *et al.* characterizing these degradable MSs, determining effects of different mole percents (mol %) of MA, and comparing their properties to either Embosphere® or DC Bead®. Evaluations included measuring swelling, injectability, *in vitro* degradation rates, biocompatibility, and doxorubicin loading. Swelling behavior of these MSs was compared to the Embosphere®. Level of swelling was affected by the mol % of MA incorporated into the backbone, conferred by the ionization of this unit. The microspheres were tested for injectability through a microcatheter, evaluating aggregation behavior, compressibility, and elastic recovery. No resistance was detected during injection, suggesting absence of aggregation and sufficient compressibility. Post injection, the microspheres recovered their original spherical shape. *In vitro* studies resulted in complete degradation within 2 days, and

degradation rate correlated to the level of MA in the polymer backbone. Degradation products were tested in contact cytotoxicity studies using L929 cells. No disruption of cell membranes or change in cell proliferation was observed, indicating good biocompatibility. Next, these microspheres were loaded with doxorubicin and compared to the DC Bead[®]. Anionic groups along the polymer backbone, carboxylic moieties, provided for electrostatic loading of the drug. Loading efficiency of doxorubicin was >90% in 1 hour, similar to DC Beads[®]. Finally, *in vivo* degradation and local biocompatibility were examined after implantation into subcutaneous rabbit tissue. Animals were sacrificed at two time points: 2 days and 7 days and 594 different skin sections were examined. By day 2, degradable MSs were detected in 25% of the sections; however, by day 7 there was complete degradation. These degradable microspheres had similar physical characteristics as the marketed Embosphere[®] and DC Bead[®], but with the added advantage of complete degradation within a week of using a hydrolyzable crosslinker. Based on these results, the microspheres' transient uterine artery occlusion was tested in a sheep model and performance was compared to Embosphere[®]. Six sheep underwent bilateral UAE with either the degradable microspheres or the Embosphere[®] in the 500–700 μm size range until stasis was reached. Animals underwent angiography at day 7 and then sacrificed for histological evaluation. All animals embolized with the degradable MSs had normal arterial blood flow in both uterine arteries at day 7, while 50% of the Embosphere[®] had normal flow and the remaining had reduced flow. Histological analysis revealed the absence of degradable MSs and inflammatory markers, whereas the Embospheres[®] were found in organized thrombi. In the Embosphere[®] group, recanalization was characterized by new endothelium, but surrounded by layers of macrophages, neutrophils, and at times foreign body giant cells. Finally, a similar level of degree of uterine necrosis was detected for both groups suggesting the duration of occlusion by the degradable MSs was sufficient to achieve clinically significant ischemia. Although this was a small study and more comprehensive *in vivo* work remains to be completed, these results are promising, signifying the advantage of a degradable MS for UAE.

The unique properties of natural polymers, like polysaccharides, have been investigated with a recent focus on chitosan derivatives to develop degradable microspheres [10,114,115]. Chitosan is a linear polymer composed of α -1,4-linked 2-amino-2-deoxy- α -D-glucose (N-acetyl-glucosamine), which is derivatized from chitin via deacetylation [10]. Different functionalization methods of chitosan provide for change in water solubility and degradation rates. Weng *et al.* recently developed a set of drug capable biodegradable microspheres. Carboxymethyl was introduced into the chitosan chain for water solubility and this resulting structure was cross-linked with oxidized carboxymethyl cellulose (OCMC) with different degrees of oxidation to synthesize microspheres via an inverse emulsion method. The degradation of these microspheres is contingent upon enzymatic action of the glycosidic bonds between the monomer units by enzymes like lysozyme along with hydrolytic cleavage of the cross-linking bonds. The oxidation degree of OCMC defined as the percentage of CMC units oxidized directly affects degradation rates. Three degrees were investigated: OC-I, 9% oxidation degree; OC-II, 17% oxidation degree; and OC-III, 35% oxidation degree. The resulting microspheres ranged in size from 100 to 1000 μm with mean diameters 485 μm , 620 μm , and 689 μm , respectively. The morphology of these MSs after reconstitution

from a lyophilized form postproduction revealed round particles with a wrinkled surface. The interior of a fractured MS revealed a highly porous, but nonuniform, internal structure. All three groups were tested *in vitro* measuring degradation rates. At 7 days, the weight percentages were 44.2, 78.3, and 85.7, respectively. OC-I MSs degraded completely within 15 days while OC-III remained at 55 weight percent after 4 weeks. Swelling experiments determined a higher degree of oxidation correlated with a higher level of cross-linking and a more rigid internal network, therefore lower swelling ratio. Next, these microspheres were loaded with doxorubicin. The polymer network of these MSs included carboxylic moieties, providing an ion exchange mechanism for drug loading similar to the DC Bead[®]. Release studies were conducted over a period of 24 hrs. Doxorubicin showed linear release from all MS up to 8 hrs, after which release plateaued. Higher release was associated with a lower degree of oxidation: OC-I>OC-II>OC-III. Again, the level of cross-linking affects the release kinetics, offering a level of design control. Finally, the OC-II microspheres were tested *in vivo* in a rabbit renal embolization model to assess embolization and degradation characteristics. During injection, the MSs did not clog the microcatheter and embolization was successful. Three groups of rabbits were followed and sacrificed at days 7, 14, and 73. The embolized and non-embolized kidneys were compared for all gross and histological analyses. At day 7 the embolized kidney grossly matched the non-embolized kidney in size, however, it had demarcated infarction spots as expected. By day 73, the embolized kidney decreased in size by 1/3, a phenomenon seen with permanent embolic MSs. Histologically, the week time point revealed partially degraded microspheres within branches of the renal arteries, designated by “moth-eaten” edges and by day 73, the vessels were patent. Inflammatory cell infiltration was observed at all time points, and sometimes microspheres were found encapsulated within a thrombus but at different stages of degradation and phagocytosis. A more comprehensive animal study was later conducted and rabbits were sacrificed at additional points to sufficiently profile *in vivo* degradation and resultant inflammatory response [110]. Resorbable MSs, size range 300–500 µm, of oxidation degree 25% were used in these studies (in between OC-II and OC-III). The most significant pathological findings included mild foreign body response at all time points from day 0 to day 30, but increasing neointimal hyperplasia and fibrosis from day 7 to day 30. In a third study, these MSs (group II) and a set with lower oxidation degree, 10% (group I), were compared with Embosphere[®], again using a rabbit kidney model. Histological results showed the distribution of group I to be similar to Embosphere[®] throughout the renal arteries indicating similar physical characteristics including compressibility and deformation. Group II did occlude more proximally (larger vessel diameters) than group I, confirming the physical differences due to higher cross-linking and resultant MS rigidity.

The two degradable microsphere systems reviewed have unique advantages. The PEGMA MSs provide for a more transient embolic that may clinically prove advantageous for uterine artery embolization for the treatment of leiomyomas. These MSs histologically did not demonstrate the same level of inflammatory response and fibrosis as the chitosan based MSs, which is an important characteristic for a true transient embolic to a highly sensitive organ like the uterus. The longer degradation time of the chitosan MSs would prove advantageous in TAE/TACE procedures where retreatment is required after several weeks. Both MSs were loaded with the same chemotherapeutic, doxorubicin, which is currently a

widely used drug for the aforementioned treatment of hepatocellular carcinoma. The PEGMA MSs, however, have recently been loaded and tested with two other drugs, sunitinib, an anti-angiogenic multikinase tyrosine inhibitor, and bevacizumab, a monoclonal antibody specific to VEGF-A. This provides a unique and very promising advantage over both the chitosan MS and all commercially available MSs [116]. One of the modes of failure in HCC treatment is initiation of angiogenesis after inducing ischemia. Angiogenesis drives regrowth of vessels to residual tumor nests and ultimately relapse. A degradable MS capable of co-delivering a chemotherapeutic along with an anti-angiogenic may address this fundamental issue. Both systems require further testing and analysis in tumor animal models as well as comparison testing to current medical practice to validate their advantages. A weakness in both systems is the release rates. Neither system showed linear release kinetics over an extended period of time. Further modifications of the polymer matrices may improve these rates.

3.2. Imageable microspheres

As previously described, conventional TACE involves a sequential process of injecting an oily emulsion with a chemotherapeutic drug followed by an occluding material. Typically, the emulsifying agent is an iodinated oil such as Lipiodol® or Ethiodol®. The high iodine content renders the fluid radiopaque. Initially, it was assumed that the opaque oil was an indicative marker for the distribution of the delivered drug. To the contrary, studies have shown ethiodized oil content is a poor marker of local drug concentrations. Gaba *et al.* conducted a study in a VX2 liver cancer model in rabbits testing this hypothesis [117]. Rabbits in this study underwent tumor implantation and were subsequently treated via cTACE with doxorubicin during which a series of computed tomography (CT) images were obtained for calculation of iodine content. Post treatment, with angiographic proof of stasis, the animals were sacrificed and tissue harvested for detection of doxorubicin concentration within the tumor at various locations using liquid chromatography/tandem mass spectrometry (LC-MS/MS). These results found no significant correlation between calculated iodine content and measured chemotherapy level in the treated tumors, indicating the degree of intratumoral oil deposition is a poor predictor of local drug delivery post TACE. Design of a drug capable embolic material with an incorporated imageable agent would provide more accurate spatial localization of the embolic and the drug. This combination material would serve both as a research tool to help better understand embolization biology and also provide real-time feedback to the clinician regarding location and level of occlusion [118]. Sharma *et al.* began addressing some of these points by loading poly(vinyl alcohol) hydrogel microspheres with Lipiodol® [119]. The LC Bead® (same chemical composition as the DC Bead®) was modified by first lyophilizing the MSs in the presence of an excipient, mannitol, and then mixed with 1 mL of Lipiodol®. These microspheres were analyzed for iodine content and then tested in a saline solution to measure Lipiodol® release and stability. The MSs achieved up to 35.7% iodine by weight versus 38.2% iodine content of pure Lipiodol®. During stability testing over a 27 hr period, the cumulative Lipiodol® release remained below 20% and the radio-opacity of these MSs was validated with routine fluoroscopy and CT. Next, visualization of these MSs was characterized *in vivo* in normal swine liver. The MSs were not visible during injection; however, as the MSs began to accumulate within the hepatic artery branches they were

visualized via fluoroscopy or CT. Approximately 0.4 mL of radiopaque MSs was required for fluoroscopic visualization while only 0.2 mL was needed for CT. More sensitive CT imaging provided visualization of how single microspheres packed within the vasculature, providing further insight into the mechanics of how the microspheres occlude. In a second study by the same group, the microspheres were loaded with Lipiodol[®] followed by loading with doxorubicin [118]. A swine model was used, and either the liver or a kidney underwent chemoembolization. Three-dimensional tissue penetration of the radiopaque microspheres was evaluated *ex vivo* with microCT, and drug permeation and tissue coverage was evaluated with fluorescence microscopy. Two size ranges were tested, 70–150 μm and 100–300 μm . The MSs were visualized forming long columns within the vessels, with the smaller MSs penetrating further into the tissue as expected. Deeper tissue penetration correlated with two-fold greater drug delivery. The highest drug concentration was located immediately surrounding the bead within the first 4 hrs post embolization and declined over hundreds of microns from the bead surface. This study showed that the ability to correlate radiopaque MS location and drug distribution might serve as a useful clinical tool. However, sensitive microCT was required to appropriately visualize these MSs *in vivo*, which is not feasible in human patients.

Investigating a different approach to develop a magnetic resonance imaging (MRI) visible, drug capable microsphere using iron-oxide nanoparticles was evaluated. Poly(lactide-co-glycolide) served as the polymer platform for the microspheres co-encapsulated with a ferrofluid of iron-oxide nanoparticles and loaded with the tyrosine kinase inhibitor sorafenib, an FDA approved drug for treatment of hepatocellular carcinoma [120]. These MSs were synthesized using a double emulsion/solvent evaporation method, with both drug and ferrofluid directly incorporated. Characterization of these new embolics described spherical particles with a smooth surface and average diameter of 0.98 μm , a much lower size scale than commercial MSs. The percent of sorafenib by weight was 18.6% and for ferrofluid, 0.54%. These particles were successfully detected via MRI and *in vitro* cytotoxicity studies using a rat HCC cell line, and demonstrated significant reductions in cell proliferation with increasing doses of the sorafenib-eluting beads. Based on these results, preliminary animal studies were conducted to assess *in vivo* visibility and therapeutic effectiveness in a rat HCC model. Rats implanted with orthotopic tumors were treated via chemoembolization once the tumors measured 2.5–8.1 mm in diameter visualized using MRI. T₂-weighted images acquired immediately post embolization displayed microspheres deposited within the tumors. Microsphere location was later histologically confirmed using a Prussian blue stain specific to iron and compared with the MRI localization. Animals were sacrificed 3 days post embolization and response to the drug was also assessed by measuring changes in micro-vessel density, an angiogenic response induced by ischemia. Results indicated no significant difference between the sorafenib treated group and the control group. Although clinical response was not significant, the microspheres accomplished the goal of *in vivo* tracking. The small size of the MSs may have been a contributing factor, carrying less drug than would a 300–500 μm MS. Another set of MRI visible drug eluting MSs using alginate, an anionic polysaccharide has been developed [121]. Alginate is composed of two uronic acid monomers: (1–4)-linked β -L-guluronic and α -D-mannuronic acids, and the monomers are linked via glycosidic bonds. A cross-linked system is formed

with the addition of divalent cations like calcium, leading to a rapid gelation process that allows encapsulation of a drug or imaging moiety [122]. The drug incorporated was amonafide, an investigational cancer drug that is part of the families of topoisomerase inhibitors and DNA intercalators. Magnetic drug clusters were first synthesized, approximately 42 nm in size, using a high temperature hydrolysis reaction. Subsequently, the combination alginate MSs were fabricated using a microfluidic gelation process. Microfluidic chips with channel dimensions of 130 μm in depth and width were used in a two-step droplet gelation process. The collected MSs were microscopically characterized measuring dimensions and observing morphology. Average diameter was 34 μm with a variation of 8%. The magnetic drug clusters were visualized via confocal microscopy and fluorescent images showed homogenous distribution of clusters throughout the alginate matrix. *In vitro* release studies showed a more linear drug release, with only 47% cumulative release at 24 hrs. These microspheres were used for *in vivo* studies using the rat HCC model to test visibility levels. Immediately post embolization the animals were moved to the MRI scanner and T₂ weighted images were taken. Microsphere deposition was observed as punctate reductions to the signal intensity. Increasing concentration of total magnetic clusters within the microspheres or increasing the overall size of the microsphere in future directions may increase MR signal intensity and improve visibility. Following a similar path and using the same microfluidics system, the Shen group has developed alginate microspheres with radiopaque barium sulfate nanoparticles for CT imaging [123]. Their microspheres were larger in diameter, but polydisperse, ranging from 149–380 μm . Different weight percentages of barium sulfate content were tested. A minimum of 5% by weight was required for CT visibility and 10% for fluoroscopy. The highest content achieved was 9%, however, these MS easily ruptured and led to irregularly shaped particles.

Based on the current literature, imageable microspheres require further development before they are ready for use in clinical applications. Their mechanical properties must be verified and compared with approved products. The size ranges capable of being fabricated remain too small to prevent non-target embolization and associated adverse effects currently seen with the smallest commercially available microspheres, <50 μm [124]. Finally, long term biocompatibility tests and efficacy studies are required to provide insight into their long-term stability and ability to treat intended tumors like liver HCC.

3.3. In-situ gelling liquids

Liquid embolic agents under investigation are primarily targeted towards the treatment of aneurysms, AVMs, and HCC. The administration of currently available liquid embolic polymers, NBCA, Onyx[®], and PHIL[™] are associated with the introduction of toxic compounds [76,60,61]. Hence, many groups are investigating new *in situ* gelling polymer systems that avoid the use of toxic byproducts or solvents, summarized in Table 3. There are several mechanisms to induce *in situ* gelling including: polymerization, physical gelation, and ionic cross-linking, which are outlined in Fig. 3. Key parameters for the development of a liquid embolic system are viscosity, *in situ* setting time, toxicity, swelling behavior, biocompatibility, biodegradability, mechanical properties, and viscosity.

Liquid embolic polymer systems have been developed using poly(propylene glycol) diacrylate (PPODA) and pentaerythritol tetrakis (3-mercaptopropionate) (QT) [125], as well as poly(N-isopropylacrylamide-*co*-acrylic acid) (NiPAAM) [126]. Both groups of polymers are non-degradable, non-adhesive, and have been considered for use in treating both intracranial aneurysms and AVMs [127,128]. By achieving a higher fill volume in aneurysms, the use of these polymers may prevent recanalization that is observed when using coil embolization. In addition, these material platforms do not produce toxic byproducts like NBCA or use hazardous organic solvents like DMSO.

Both PPODA and QT monomers are liquid at room temperature, but when combined in aqueous environments with nucleophilic activators they chemically react via Michael-type addition to form a hydrogel matrix [127,129]. Proof of concept was confirmed *in vivo* using a canine model with surgically induced aneurysms in the lateral wall of the carotid artery [130]. PPODA and QT were combined in a 2:1 molar ratio to create a hydrophobic monomer solution. Prior to administration, the monomer solution was mixed at a 3:1 ratio with Conray (iothalamate meglumine, Mallinckrodt, Inc.), a high osmolarity ionic contrast dye, at a pH of 11.0 for 2 minutes [129,131]. The addition of the high pH contrast initiated the polymerization, while providing a mechanism for monitoring the administration of the embolic. PPODA-QT had a gel time of 10 minutes, although this can be reduced via manipulation of PPODA/QT ratio, adjusting the pH, and increasing the mixing time of the monomer and initiating solutions [127,131]. A drawback of this system is the toxicity of the activating solution. Conray, particularly at high pH, is cytotoxic; however, co-administration with a polymer matrix partially ameliorates the toxicity [131]. This study showed that administration of the polymers was feasible for use in aneurysms, but the low number of samples and lack of controls prevented any comparison of efficacy to other polymeric embolic systems.

NIPAAM polymers show a low critical solution temperature (LCST) of 32°C in aqueous environments, below which they are soluble and above which they precipitate out of solution [132]. This allows the polymer to be delivered in a liquid form and then be entropically driven out of solution to form a solid hydrogel after injection. NIPAAM based polymers have been functionalized to create degradable [133] and radiopaque embolic versions [134]. A key innovation was the copolymerization of NIPAAM with other polymers to create a liquid-solid transitioning platform where physical and chemical gelling occur simultaneously under physiological conditions [135,136]. The dual gelling systems combined polymers of NIPAAM-*co*-cysteamine (NC), which contained a thiol, and either NIPAAM-*co*-hydroxyethylmethacrylate-*acrylate* (NCHA) or NIPAAM-*co*-cysteamine-vinylsulfone (NCVS), both of which contain vinyl groups. Mixtures of NC-NCHA or NC-NCVS contain thiol and vinyl groups that undergo Michael-type addition at pH 7.4 to chemically cross link the polymers at body temperature [137]. In an *in vitro* model, the dual gelling system displayed adhesive properties and became loosely affixed to the catheter, which is not ideal for clinical usage due to the potential for entrapment of the delivery system [136]. Live/dead staining and cell proliferation studies with T3T mouse fibroblasts showed that both systems have a high degree of biocompatibility [136,138]. As a proof of concept study, a swine model with a surgically induced aneurysm was successfully treated with the NC-NCHA system. This study validated the ability for the system to be delivered

via catheter and solidify within an aneurysm under physiological conditions [136]. However, recanalization of the aneurysm was observed shortly after the procedure.

Alginate, derived from polysaccharides extracted from brown seaweed (phaeophyceae), is comprised of a copolymer of β -D-Mannuronic acid and α -L-guluronic acid. The absence of toxic monomers, initiators, bi-products, or solvents in alginate's gelation process makes it of interest as a liquid embolic agent [139–141]. Hydrophilic alginate polymers are negatively charged when dissolved in aqueous solutions, but immediately begin forming a nonadhesive hydrogel upon contact with divalent cations, such as calcium [140]. If alginate is combined with calcium prior to injection, it is injectable, but may solidify or form an “alginate coil” within the catheter. To preclude catheter blockage, specialty double-lumen catheters are used to administer the alginate and calcium solutions separately and have them combine *in situ* [139,142,143]. Alginate gels have been extensively explored for the controlled release of bioactive compounds [144–146], but only bland embolization has been attempted with alginate as a liquid embolic. A solution of alginate lyase and ethylenediaminetetraacetic acid (EDTA) has been communicated by Barnett and Gailloud for being able to selectively remove alginate emboli from occluded vessels *in vivo* [147]. EDTA chelation of calcium ions and enzymatic hydrolysis of the polymer backbone by alginate lyase causes the hydrogel matrix to be rapidly broken down into soluble non-toxic components. The ability to occlude and then selectively reopen vessels is a novel therapeutic treatment modality not available with current commercially available embolic agents.

Algel (Neural Intervention Technologies), an investigational alginate based embolic, was shown to be effective in embolizing aneurysms and AVMs in swine models [148,149]. A high degree of filling (93%) was achievable using Algel by injecting it into the aneurysm sac while the neck was blocked with a temporary balloon. Follow up angiograms of the study animals (n=8) showed that the parent vessel remained patent while the aneurysm remained occluded after 90 days. Control animals (n=2), whose aneurysms were partially filled, survived only 6–8 days [148]. A swine model of AVM was used to demonstrate stability of Algel *in vivo*. Occlusion was stable in all animals at 1 month (n=3) and in 5 of the animals in the 6 month group (n=6) [148]. Histological analysis of tissue samples from both the aneurysm and AVM models showed no significant histological response at either endpoint. Gore and Associates acquired Neural Intervention Technologies and launched a phase 1 clinical trial with the Gore Embolic Liquid (GEL), previously known as Algel, for the pre-surgical embolization of AVM in 2006, however, the results of this trial have yet to be reported [141]. Safety concerns with alginate gels include efflux of material from the aneurysm cavity during administration, inadvertent embolization of non-target vessels, and fracturing of the alginate mass after the reintroduction of flow [150].

Fibrin glue is widely used as a hemostatic in surgery, but has also been explored as an embolic agent. Formed from the catalytic action of thrombin on fibrinogen, fibrin is a protein polymer responsible for the structural formation of hemostatic clots in response to tissue damage [151,152]. Fibrin glue harnesses this natural pathway in order to create a sealant that can be applied selectively during surgery or other procedures to stop bleeding. For use as an embolic, a double barrel syringe is loaded with fibrinogen and aprotinin, an antifibrinolytic agent, in one half, and thrombin, calcium chloride, and visualizing agent in

the other. The contents are then mixed in the tip of the syringe as they are injected. The polymerization begins immediately and the solution must be injected rapidly and continuously or the catheter will occlude. Time to occlusion ranges from 55 seconds in a 110 cm 3F catheter to 3 minutes 10 seconds in a 27cm 5F catheter [153]. Critical to the use of fibrin glue is the injection rate. If injected too slowly the catheter will occlude, and if injected too rapidly the glue will fail to solidify within the target vessel and may pass distally into the lungs, liver, spleen, or heart [153].

The length of the catheter, the rate of injection, and concentration of fibrinogen, thrombin, and Ca^{2+} determine the viscosity of the fibrin glue as it leaves the catheter [154]. Fibrin glue has only been used clinically as an embolic agent in a study for the preoperative embolization of intracranial meningiomas [155]. The final concentration of components in the fibrin embolic system used in this study was 33.25 mg/ml fibrin (Tissucol Duo S), 3.333 KIU/ml Aprotinin (Trasylol, Beyer), ipromide 139 mg I/ml (Ultravist, Schering), 0.55 mg/ml CaCl_2 , and 62.5 IU/ml of thrombin. The embolization was successful in all 80 of the study patients. However, every patient developed a headache during the procedure and two patients (2.5%) experienced neurological defects including paresis and hypoesthesia from the embolization. The effects of the embolization were mixed. While necrosis was observed within 24 hours of embolization, this was only beneficial if it was within the central mass of the meningioma. In cases where necrosis was observed on the tumor's periphery, the division between the meningioma and surrounding brain tissue became indistinct, increasing risk for compromising healthy perilesional tissue [155]. Unlike other embolics, fibrin glue is derived from human blood and carries a risk of an allergic reaction and transmission of blood borne pathogens [156,157].

Cellulose derivatives have been explored previously as liquid embolics and in the creation of embolic microspheres in part due to their high degree of biocompatibility and biodegradability [158–161]. Whereas previous cellulose based liquid embolics involved the precipitation of cellulose acetate from DMSO similar to Onyx[®], the Golzarian group combined oxidized carboxymethyl cellulose (OCMC) and carboxymethyl chitosan (CCN) to form a chemically crosslinking injectable hydrogel [115,162]. Cross-linking occurs via Schiff base formation as an amine on the OCMC acts as a nucleophile and attacks a carbonyl carbon in one of the aldehyde groups on CCN [162]. Gelation at 37 °C in DI water and PBS occurred in 1.53 and 1.30 minutes respectively. However, when gelation was evaluated in citrated human plasma the rate of gelation slowed to 4.28 minutes, likely due to interactions between the polymer and proteins in the blood. Incorporation of iodine contrast reagent at 20% by volume bestows radiopacity and only mildly impeded gel formation. An MTT assay showed no significant difference between controls and cells incubated with primary human umbilical vein endothelial cells indicating a lack of cytotoxicity. The polymer gel degraded *in vitro* in the presence of lysozyme due to the enzymatic degradation of chitosan and the hydrolysis of the Schiff base. Only 22.1% of the polymer remained after 21 days in a 10 µg/ml lysozyme solution, indicating that this system is not appropriate if permanent occlusion is desired. Evaluation of the release of doxorubicin and tetracycline from the matrix exhibited peak releases of 31.3% after 50 hours and 65.8% after 24 hours, respectively. *In vivo* comparison of the embolic OCMC/CCN demonstrated deeper penetration into the vasculature of rabbit kidneys than did bioresorbable microspheres (100–

300 μm) [115]. This novel embolic shows promise as potential agent for TACE procedures, but may not be appropriate for situations where long-term embolization is desired.

Our lab has recently developed an *in situ* gelling silk-elastinlike protein (SELP) polymer embolic with treatment goal for HCC. Like fibrin and cellulose derivatives, SELPs are synthesized from naturally occurring units, specifically motifs from silk and elastin proteins using recombinant techniques [163,164]. SELP combines the strength of Bombyx mori (silkworm) silk [165] (GAGAGS) and the flexibility of mammalian elastin (GVGVP) [166] via genetic recombination. The silk domains form crystalline structures via long-range physical interactions between polymer chains to provide mechanical strength. Elastin blocks make the polymer soluble in water and separate silk complexes to create a porous matrix structure. By varying the number and sequence of silk and elastin monomer blocks properties such as swelling ratio, gelling rate, drug release profile, and mechanical strength can be controlled with molecular precision [167]. Being composed of amino acids that occur naturally in the body, the degradation products of SELP are nontoxic and bioresorbable. SELP production follows three distinct phases: 1) design, construction, and cloning of the polymer gene, 2) expression of the gene in an *E. coli* host, and 3) extraction, purification, and processing of the polymer product. During the processing, the polymer is solubilized in water and can be combined with therapeutic agents directly. SELP, once injected into the body, rapidly makes a sol-to-gel transition *in situ* at body temperature. These unique features allow for the *ex vivo* loading of multiple bioactive agents into an injectable polymer. A series of studies identifying an optimal SELP formulation for embolization were conducted (Fig. 4) [11]. Two SELP compositions were tested, i.e., SELP-47K and SELP-815K. The polymer compositions differed in the lengths of silk and elastin blocks, which ultimately dictated the material properties. A series of design criteria were established that qualified the SELP embolic including injectability through a commercially available microcatheter and occlusion of an *in vitro* arterio-capillary network simulation of a HCC tumor. Formulation development began with testing the two different compositions at different weight percentages, 16, 14, and 12, via rheological characterization. Initial formulations consisted of reconstituting a calculated amount of SELP in PBS. However, all samples failed to meet the design criteria. A shear processing step was incorporated into the manufacturing procedure, resulting in SELP formulations with highly improved physical properties, including gelation time and final gel stiffness that reached acceptable values to create a successful liquid embolic. One formulation was chosen for further testing, namely the 12% w/w SELP-815K. Occlusive ability of this candidate was confirmed *in vitro*, and continued into *in vivo* feasibility studies using a rabbit model. The liquid embolic was easily injectable through a commercially available microcatheter. The hepatic artery branches were embolized, with angiographically proved stasis. Hepatic and pulmonary tissues from the sacrificed animals were analyzed for evidence of the liquid embolic and any potential non-target embolization. The SELP material was located within the arterioles of portal triads, which was the expected target. The liquid embolic filled the entirety of the lumen, a potential advantage over microspheres. Pulmonary samples lacked evidence of SELP emboli, indicating the embolic material did not wash into the venous vasculature. Smaller microspheres penetrate further into cancerous tissues allowing for higher infarction rates and higher area coverage for drug delivery [118], but beads smaller in diameter than 50 μm have

adverse events due to non-target embolization [124]. A liquid embolic like SELP has the advantage of deeper vessel penetration while reducing the risk of non-target embolization. Furthermore, a SELP embolic has the advantage of incorporating a variety of drugs/bioactive agents, versus small charged molecules that are incorporated into the current drug eluting microspheres.

4. Conclusions and Future Directions of Polymeric Embolics

In the past 50 years, polymers have been designed, modified, and improved into a variety of embolic agents to address new clinical needs, especially in treatment of vascular malformations and hypervascular tumors such as benign uterine fibroids or malignant hepatocellular carcinomas. The move from irregularly shaped particulate embolics to precisely calibrated microspheres has resulted in improved clinical outcomes, by changing the morphology and surface properties of the embolic material. Precise calibration permits reproducible and controllable embolization, while more precise microsphere size ranges results in more consistent and predictable levels of obstruction. Following development of microspheres of different polymer compositions, drug delivery became a clinical possibility. A number of calibrated microspheres are now commercially available and beyond those discussed in this review [168,169], i.e., trisacryl gelatin Embosphere® (Merit Medical Systems), and PVA based Contour SE™ (Boston Scientific) and Bead Block® (BTG). In treatment of uterine fibroids, the Embosphere® has increasing clinical evidence for superiority. Its material properties render it more stiff than competitors requiring more injection force, but these microspheres deform within the vessels and conform to vessel shape serving as a better embolic to induce complete infarction.

Unlike a benign tumor, hepatocellular carcinoma of the liver requires localized injection of a chemotherapeutic. The original method of intra-arterial drug infusion, then liquid drug infusion followed by particulate embolization is being replaced by more effective drug eluting microspheres like the DC Bead® or HepaSphere™ in TACE procedures. The archetypical degradable materials like gelatin sponge allow transient vessel occlusion, but similar to permanent particulate PVA, the irregularity in shape and unpredictable degradation rates are less than optimal. Furthermore, conventional TACE does not allow for controlled release of the chemotherapeutic into surrounding tissue. Clinically, the two drug eluting beads discussed have similar clinical outcomes with doxorubicin, however, the differences in their polymer structures affect release kinetics of different drugs as a result of changes to microsphere structure post drug loading. Irinotecan, for example, induced more necrosis in animal models of HCC when delivered via HepaSphere™. Therefore, as new drugs are developed, they must be tested in comparative studies to determine which drug delivery system will be appropriate.

In the development arena, degradable/bioresorbable microspheres are being designed to overcome the disadvantages of gelatin particles by utilizing polymers with consistent degradation rates while maintaining the advantages of calibrated drug eluting microspheres. These technologies are the most evolved and may be commercialized within the next decade. Polymers have been identified based on biocompatibility of degradation products as well as mechanical properties of the primary polymer backbone. Fitting these criteria include

poly(ethylene glycol) methacrylate and chitosan derivatives, both of which have been fabricated into degradable microspheres that can be loaded with drugs. Applications for these materials are broader than permanent microspheres since degradation time can be controlled based on polymer backbone additives. With regard to surgical procedures, real-time feedback is a fundamental tool, especially to the practice of interventional radiology. However, injection of contrast does not satisfactorily correlate to the location of the embolic within the vasculature. Though still in the infancy of research and development, imageable microspheres have the potential to provide not only real-time feedback to the clinician by indicating location and distribution of embolic material, but also showing the anticipated drug distribution within cancerous tissues.

Finally, liquid embolic materials that solidify *in situ* are increasingly being explored. They are attractive for their ability to deeply penetrate vasculature and completely fill vascular spaces. Fueling future development is the desire for improved efficacy and reduced toxicity. Whereas the first generation of liquid embolics, Onyx[®] and Trufill[®], have toxic byproducts, most of the next generation materials exhibit little to no cytotoxicity. Future developments in liquid embolics will be the ability to locally deliver one or more therapeutic agents to the target lesion while restricting blood flow. Bioactive agents that may be investigated are chemotherapeutics or gene therapy vectors for the treatment of hepatocellular carcinoma. For the treatment of aneurysms, the co-delivery of growth factors with the occlusive agent is potentially attractive to promote the growth of vascular neointima to seal off the aneurysmal sac. Another area of future interest is *in situ* gelling liquids that can be selectively re-solubilized or which degrades passively over time to produce transient occlusive effects. Liquid embolics able to produce predictable transient embolization have the potential to expand into many novel applications including pre-surgical embolization in surgical procedures where blood loss is of concern or reduced circulation would be beneficial. Table 4 provides a quick reference summary of the material presented in this review.

Customization of polymers allows for the development of diverse embolic materials. Embolic agents will continue to evolve as clinical data provides insight into problems and unmet needs. Future directions in designing multi-functional embolics will need to follow key design criteria such as improved biocompatibility, biodegradability, radiopacity, intrinsic bioactivity, and multi-drug loading. Like most medical devices, clinical applications will dictate new design parameters.

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	PVA Particles	Contour SE™	Embosphere®	Bead Block®
	Polyvinyl alcohol Non-spherical	Cross-linked PVA Calibrated MS	Trisacryl gelatin Calibrated MS	PVA cross-linked with acrylic Calibrated MS
Compression Force
	Low	5 kPa	27 kPa	24 kPa
Elastic Recovery	NA	150ms <100% recovery	< 100ms 100% recovery	< 100ms 100% recovery
Material Properties	Advantages: -Inexpensive -Compressible -Less material to occlude Disadvantages: -Aggregation -Catheter clogs -Unpredictable -Recanalization	Advantages: -Higher compressibility -Less time for suspension Disadvantages: -Deformation due to water expulsion -Recanalization	Advantages: -Elastic recovery -Bead packing, complete occlusion in UAE Disadvantages: -More force to administer	Advantages: -Elastic recovery -Least degree of catheter clogging Disadvantages: -More time required for suspension

Figure 1.
Schematic comparing physical properties of four commercially available embolic microspheres used in bland embolization procedures [40,41,93].

Figure 2. Schematics demonstrating loading mechanisms of two commonly used drug eluting microspheres.

Figure 3.

Mechanisms of *in situ* gelation for liquid embolics. There are three processes by which liquid embolics transition from a liquid solution to a solid matrix, namely: polymerization, physical gelation, and ionic crosslinking. Polymerization occurs as an activating agent (e.g. free radical, anion, enzyme, photon, etc.) induces monomer or pre-polymer units bind to each other forming progressively larger molecules as part of a chain reaction. The resulting matrix is a network of large molecules that are chemically bound (e.g. NBCA, PPODA/QT, Fibrin Glue, etc.). Physical gelation occurs as the environment around a preformed polymer changes (e.g. temperature, solvent state, etc.) causing the polymer or portions of the polymer to change from a soluble to insoluble state. The polymer thus forms a solid spongy mass composed of entangled polymers that are bound together by intermolecular forces (e.g. Onyx[®] and SELP). Ionic crosslinking occurs with charged polymers as the introduction of multivalent counterions creates bridges between polymer strands via electrostatic interactions (e.g. Alginate).

Figure 4.
A) Poursaid *et al.* identified an optimal liquid embolic comprised of silk-elastinlike protein (SELP) by rheological testing of candidate formulations assessing viscosity, gelation kinetics, and final stiffness. SELP-815K at 12% w/w with shear processing met design criteria and continued to an *in vivo* feasibility test. **B)** *In vivo* results showing SELP filling the lumen of an arteriole branch from the hepatic artery. Both hematoxylin/eosin (H&E) and immunochemistry (IHC) staining methods were used to identify the solidified embolic. Figures modified from [11].

Table 1

Summary of clinical applications requiring embolotherapy

Clinical Application	Treatment Goal	Polymer Embolic(s) Used	Future Directions	Ref.
Vascular Malformations/Aneurysms	Prevention of hemorrhage; permanent occlusion for symptom control	Liquid embolics: NBCA glue, Onyx [®] , PHIL [™]	Non-adhesive liquid embolics with non-toxic diluents	[12–17]
Malignant Hypervascular Tumors	Induction of infarction / local delivery of chemotherapeutics to prompt tumor necrosis	Drug eluting calibrated microspheres, PVA particles, gelatin sponge particles, starch particles	Multi-drug capable MS; “Imageable” MS; degradable MS to allow re-treatment	[18–27]
Uterine Fibroids	Induction of infarction; temporary occlusion to prevent blood loss during surgery	Calibrated microspheres, PVA particles	Degradable MS with predictable degradation rates for transient occlusion	[30–43]

Table 2

Summary of commercially available embolic agents

Material	Polymer Structure	Trade Names	Material Description	Degradable (Y/N)	Drug Incorporation	Ref.
Denatured Collagen		GelFoam® (Pfizer, inc.)	Hemostatic sponge, Comprised of a natural protein polymer (Note X and Y represent amino acid side chains)	Yes (1 month)	None	[142]
Cross-Linked Starch		Spherex® (Magle Life Sciences) EmboCept® (Pharmacept)	Microsphere, hydroxyl groups can be functionalized	Yes (Minutes to Hours)	None	[53]
Poly(n-butyl-hyanoacrylate)		Trufil® (Cordis Neurovascular, Inc.)	Glue	No	None	[58]
Poly(vinyl alcohol)-co-Polyethylene		Onyx® (Covidien)	Precipitating liquid embolic	No	None	[17]
Poly(N-acryloyl-2-amino-2-hydroxymethylpropane-1,3 diol)-co-Poly(N-methylene-bis-acrylamide)		Embosphere® and Embogold® (Merit Medical Systems, Inc.)	Microsphere, Matrix is impregnated with porcine gelatin, and Embogold® has 2% elemental gold incorporated into the sphere	No	None	[94]
Polyvinyl acetate-co-Polymethylacrylate		QuadraSphere® and HepaSphere™ (Merit Medical Systems, Inc.)	Microsphere	No	Yes (Doxorubicin)	[103]

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Material	Polymer Structure	Trade Names	Material Description	Degradable (Y/N)	Drug Incorporation	Ref.
Poly(methyl methacrylate) and Poly (bis(trifluoroethoxy) phosphazene)		Embozene [®] and Oncozene [™] (CelNova BioSciences, Inc.)	Microsphere	No	Yes (Doxorubicin and Irinotecan)	[168] [169]
	Contour [™] (Boston Scientific)	Irregular Particles	No	No	[142]
Polyvinyl alcohol-co-Poly(2-acrylamido-2-methylpropane sulfonate)		DC Bead [®] , LC Bead [®] , and Bead Block [®] (Biocompatibles, Inc.)	Microsphere	No	Yes (Doxorubicin and Irinotecan)	[99]
Polyhydroxyethyl methacrylate and Polylactide-co-Polyglycolide with bound Triiodophenol		PHIL [™] (MicroVention, Inc.)	Precipitating Liquid Embolic	No	No	[79] [81]

Table 3

Investigational liquid embolic materials

Material	Liquid Structure	Gel Structure	Gelation Mechanism	Ref.
Poly(propylene glycol) Diacrylate and pentaerythritol tetrakis (3- mercaptopropionate)			Polymerization	[125]
poly(N-isopropyl acrylamide)-co-cysteamine and poly(N-isopropyl acrylamide)-co-hydroxyethyl methacrylate-acrylate			Polymerization and Physical Crosslinking	[126]
Alginate			Ionic Crosslinking	[140]
Fibrin Glue			Polymerization	[151] [152]
Oxidized carboxymethyl cellulose and carboxymethyl Chitosan			Polymerization	[162]

Material	Liquid Structure	Gel Structure	Gelation Mechanism	Ref.
Silk Elastin-like Protein Polymer 815K	<p>Amino Acid Sequence: MDPVVLQRRDWENPGVTQLNRLAAHPPFASD PM[GAGS(GAGAGS)₆](GVGV)₄GKGVY(GVGV P)₁₁(GAGAGS)₆GA)₆GAMDPGRYQDLRSHHH HH</p>		Physical Crosslinking	[11]

Table 4

f commercially available embolics and materials in development

Commercial Product		Clinical Applications		Advantages		Disadvantages		Next Generation in Development		Ref.
•	Gelfoam® (Pfizer)	•	Hemorrhage control or prevention of hemorrhage during surgical removal of hypervascular tumors	•	Gelatin sponge sheets and powder form available	•	Multi-step preparation process for embolic use: cutting the sheet into small sections followed by mixing with saline and contrast	•	Focus for embolic materials has shifted towards development of improved, multifunctional microspheres (see below)	[8, 44, 46, 57] [47, 49–51]
	Surgiform® (Ethicon)			•	Low cost, simple preparation					
		•	Embolization of hypervascular tumors in cTACE post injection of Lipiodol+drug mixture		Highly hemostatic, fast hemorrhage control	•	Risk of infection due to increased preparation time in open air			
						•	Irregular shapes of particles and large size distribution lead to clumps that can cause unpredictable behavior during embolization			
•	EmboCept® S (PharmaCept)	•	Transient occlusion of small vessels to reduce blood flow, particularly in the tumor bed during cTACE	•	Hyper transient blood vessel occlusion	•	Depending on the application, the minute time scale for degradation may not be sufficient and the degradation rate is variable as seen in case studies	•	Laurent and Moine <i>et al.</i> have synthesized poly(ethylene glycol) methacrylate (PEGMA) hydrolyzable microspheres for predictable transient embolization that require several hours as compared to minutes to degrade, 7 days for complete degradation	[49, 52–55] [113–115]
	Spherex® (Magle Life Sciences)	•	Uterine Fibroid embolization		May be used to protect surrounding healthy from exposure of locally injected chemotherapeutics		Only available in 50 µm average diameter, not useful in occluding larger vessels			

Commercial Product	Clinical Applications	Advantages	Disadvantages	Next Generation in Development	Ref.
•	Trufill® (Cordis Neurovascular, Inc.)	• AVM embolization	• Injectable liquid, allows embolization of small vessels	• Multi-step preparation prior to used	[47–48, 58–65]
	• Hemoptysis	•	• Fast polymerization (seconds scale) for immediate occlusion	• Short working time	
	• Preoperative embolization (<i>ie</i> partial hepatectomy)	•	• Mechanically occupies the intravascular lumen	• NBCA glue releases formaldehyde, leading to inflammation of the vessel wall	
	• Acute bleeding in the gastrointestinal tract	•	• May be used in patients with coagulopathies	• Risk of glue adhering to the catheter and occluding the lumen	
•	Onyx® (Medtronic plc)	• Intracranial and peripheral AVMs	• Injectable liquid, allows embolization of small vessels	• Material suspended in DMSO, which has been shown to cause vascular inflammation and angioneurosis	[66–78] [79–82] [126–136] [139–147] [115, 162] [11, 163–164]
•	PHIL™ (MicroVention, Inc.)	• Aneurysms	• Non-adhesive	• Onyx® requires multi-step preparation with specialty equipment	
	• Preoperative embolization	•	• PHIL™ supplied ready to use in single syringe, inherently radiopaque	• Catheter entrapment can occur	
	• Acute hemorrhage	•			
	• Stent graft-related endoleaks	•			
				•	Golzarian <i>et al.</i> have developed combined cellulose and chitosan derivatives to form a chemically crosslinking injectable hydrogel
				•	Vernon <i>et al.</i> have designed NIPAAm based polymers to create a liquid-solid transitioning platform where

Commercial Product	Clinical Applications	Advantages	Disadvantages	Next Generation in Development	Ref.	
Non-degradable particulate embolics				physical and chemical gelling occur simultaneously under physiological conditions		
				Cappello and Ghandehari <i>et al.</i> have identified an optimal aqueous based liquid embolic comprised of silk-elastinlike recombinant protein that transitions into a gel network at body temperature with no volume displacement		
	PVA Foam Embolization Particles (Cook Medical)	Permanent embolization of vessels feeding uterine fibroids	Low cost Permanent Compressible, occluding vessels larger than the internal diameter of the catheter	Irregular shapes of particles and large size distribution lead to clumps that can cause unpredictable behavior during embolization	Focus for embolic materials have shifted towards development of improved, multifunctional microspheres (see below)	[40,41, 83–89]
	Contour™ PVA Particles (Boston Scientific)	Pre-myomectomy uterine artery embolization for reduction in intraoperative blood loss		Low control over depth of occlusion and distribution Require preparation with contrast dye prior to injection		
Calibrated microspheres	Contour SE™ (Boston Scientific)	Permanent embolization of vessels feeding uterine fibroids	Multiple size ranges available to suit target vessel size Better penetration into vessels as	Require preparation with contrast dye prior to injection As a particulate system, risk of	Wood and Dreher <i>et al.</i> began developing imageable beads by	[40–41] [93–101] [118–123]
	Embosphere® (Merit Medical)					
	Bead Block® (BTG)					

Commercial Product	Clinical Applications	Advantages	Disadvantages	Next Generation in Development	Ref.
<ul style="list-style-type: none">LC Bead® (BTG)Embozene® (CellNova Biosciences, inc.)	<ul style="list-style-type: none">Treatment of hypervascular tumors via TAE or cTACEAVMs	<ul style="list-style-type: none">• compared to PVA particles• Less aggregation than PVA particles and reduced clogging of catheters	<ul style="list-style-type: none">• catheter clogging still exists, though improved from irregular particles	<ul style="list-style-type: none">loading the LC Bead® with Lipiodol®Larson <i>et al.</i> have designed poly(lactide-co-glycolide) microspheres co-encapsulated with a ferrofluid of iron-oxide nanoparticles and loaded with sorafenibShen <i>et al.</i> have developed alginate microspheres with radiopaque barium sulfate nanoparticlesSeveral labs are investigating controlled release of bioactive agents	<ul style="list-style-type: none">[102–107][110, 116, 120]
<ul style="list-style-type: none">HepaSphere™ /Qu adraSphere® (Merit Medical)DC Bead (Biocompatibles)Oncozene™ (CellNova Biosciences, inc.)	<ul style="list-style-type: none">• Treatment of hypervascular tumors via TACE	<ul style="list-style-type: none">• Controlled, localized release of chemotherapeutics over period of days to weeks• Multiple size ranges available to suit target vessel size• Higher degree of control over location of injection for both embolization and drug delivery	<ul style="list-style-type: none">• Permanent, impeding multiple treatments often required in treating an aggressive tumor• Require preparation with contrast dye prior to injection• Require preparation with chemotherapeutic at least 1hr prior to injection	<ul style="list-style-type: none">• A combination of degradable, imageable, and drug capable microspheres for superior microsphere to treat malignant hypervascular tumors• Weng et al. recently developed a set of drug capable biodegradable microspheres	

microspheres

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Commercial Product	Clinical Applications	Advantages	Disadvantages	Next Generation in Development	Ref.
			<ul style="list-style-type: none">	<div>Lower penetration into tumor tissue compared to liquid embolics</div> <ul style="list-style-type: none"> <div>composed of modified chitosan</div> <div>The PEGMA degradable MS are also drug capable</div>	