A Liquid-to-Solid Gelling Polymer System for Cerebral Aneurysm Embolization:

Formulation, Characterization, and Testing

by

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ABSTRACT

Treatment of cerebral aneurysms using non-invasive methods has existed for decades. Since the advent of modern endovascular techniques, advancements to embolic materials have largely focused on improving platinum coil technology. However, the recent development of Onyx®, a liquid-delivery precipitating polymer system, has opened the door for a new class of embolic materials—liquid-fill systems. These liquid-fill materials have the potential to provide better treatment outcomes than platinum coils. Initial clinical use of Onyx has proven promising, but not without substantial drawbacks, such as co-delivery of angiotoxic compounds and an extremely technical delivery procedure.

This work focuses on formulation, characterization and testing of a novel liquid-to-solid gelling polymer system, based on poly(propylene glycol) diacrylate (PPODA) and pentaerythritol tetrakis(3-mercaptopropionate) (QT). The PPODA-QT system bypasses difficulties associated with Onyx embolization, yet still maintains non-invasive liquid delivery—exhibiting the properties of an ideal embolic material for cerebral aneurysm embolization.

To allow for material visibility during clinical delivery, an embolic material must be radio-opaque. The PPODA-QT system was formulated with commercially available contrast agents and the gelling kinetics were studied, as a complete understanding of the gelling process is vital for clinical use. These PPODA-QT formulations underwent *in vitro* characterization of material properties including cytotoxicity, swelling, and degradation behaviors. Formulation and characterization tests led to an optimized PPODA-QT formulation that was used in subsequent *in vivo* testing.

PPODA-QT formulated with the liquid contrast agent Conray™ was used in the first *in vivo* studies. These studies employed a swine aneurysm model to assess initial biocompatibility and test different delivery strategies of PPODA-QT. Results showed good biocompatibility and a suitable delivery strategy, providing justification for further *in vivo* testing. PPODA-QT was then used in a small scale pilot study to gauge long-term effectiveness of the material in a clinically-relevant aneurysm model. Results from the pilot study showed that PPODA-QT has the capability to provide successful, long-term treatment of model aneurysms as well as facilitate aneurysm healing.

To my Grandpa Bernie

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Chapter 1: INTRODUCTION TO CEREBRAL ANEURYSM EMBOLIZATION

1.1 Cerebral Aneurysms

An aneurysm is a “ballooning out” of an artery wall that occurs where the artery has been damaged or weakened. Damage to vessels can be caused by high blood pressure, smoking, trauma, or even genetic factors (Khurana, Meissner, and Meyer 2004). The hemodynamic forces of blood on the weakened artery wall can lead to aneurysm growth and eventual rupture. Most aneurysms occur within the aorta, but can happen anywhere in the arterial vasculature. An aneurysm is commonly thought of as a saccular bulge with a defined neck, but not all aneurysms have this feature. Fusiform aneurysms, for example, are characterized by bulging of an entire axial section of the artery, with no defined neck. Although these types of aneurysms can cause physiological problems, rupture of fusiform aneurysms is relatively rare (Lohani 2004).

Of particular interest to endovascular neurosurgeons are intracranial aneurysms (cerebral aneurysms). Intracranial aneurysms are generally more challenging to treat than peripheral aneurysms because they can be located in deep or eloquent areas of the brain. Furthermore, the rupture of cerebral aneurysms is a devastating event, leading to subarachnoid hemorrhage and often resulting in death (Hop et al. 1997). It is estimated that one in every 15 Americans will develop a cerebral aneurysm during their lives, according to the American Society of Interventional and Therapeutic Neuroradiology.

1.2 Treatment Techniques

Currently, aneurysm treatment involves one of two treatment techniques. Surgical clipping is the more traditional method, clinically accepted by the 1960s, which involves placement of a metal clip across the aneurysm neck, as seen in Figure 1.1, in order to prevent blood from entering the aneurysm (McKissock, Richardson, and Walsh 1965). Because blood cannot enter the weakened region, the aneurysm is protected from rupture. Preventing rupture and subsequent blood leakage into the brain space are the main goals of all aneurysm treatment techniques. However, surgical clipping is a highly invasive procedure, and not useful for deep aneurysms that are difficult to access via craniotomy.

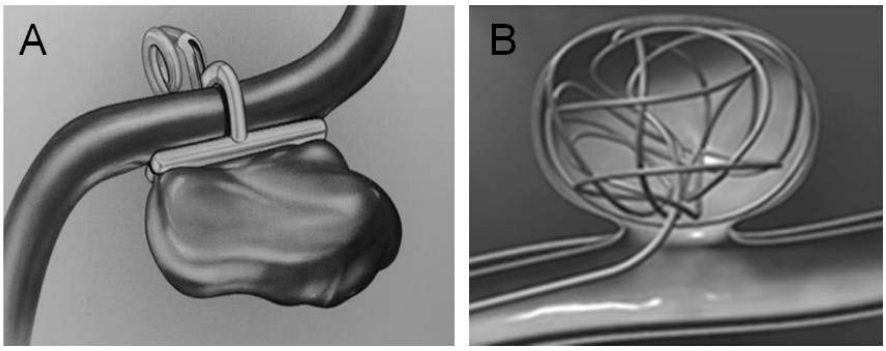


Figure 1.1 Techniques for cerebral aneurysm treatment. (A) Surgical clipping involves craniotomy and placement of a metal clip across the aneurysm neck. Image reproduced from: http://www.brain-surgery.net.au/recentops6.html. (B) Endovascular coiling is considered the “gold standard” of current treatments. Image reproduced from: http://www.brainaneurysm.com/aneurysm-treatment.html.

A less invasive treatment method is endovascular embolization. Embolization is as a process in which material is purposefully introduced into the circulation to occlude a vessel, abnormal structure, or an organ (Stedman 2000). In the case of aneurysm treatment, endovascular embolization involves internally guiding a microcatheter (via X-ray fluoroscopy) through a patient's vessels, then deploying a filler material into the aneurysm. This material occludes the aneurysm, preventing blood flow from entering the cavity. Due to its minimally invasive nature, this technique is attractive to both patients and clinicians (Prestigiacomo 2006). Endovascular methods were not routinely used before the early 1990s because of limitations in endovascular technology. With the advent of flow-directed microcatheters, balloon catheters, and detachable coils, the use of endovascular techniques for aneurysm treatment blossomed (Kanaan et al. 2005).

1.3 Clinically Available Endovascular Treatments

1.3.1 Coil Embolization

Currently, endovascular coiling is considered the "gold standard" in cerebral aneurysm treatment (Molyneux 2002), also shown in Figure 1.1. Flexible platinum coils are delivered sequentially into an aneurysm through a microcatheter, until no further coils can be placed. Guglielmi detachable coils were the first type of coil system on the market, introduced in 1991 (Linfante and Wakhloo 2007), but several modifications have been made to coil technology since then. Endovascular coils such as HydroCoil® (MicroVention, Tustin, CA), Matrix® detachable coils (Boston Scientific, Natick, MA), and Cerecyte® coils

(Micrus Endovascular, San Jose, CA) incorporate hydrogels to provide a specific function. HydroCoils contain a hydrogel coating that expands on contact with blood, designed to achieve better volumetric filling than non-coated platinum coils (Cloft and Kallmes 2004; Arthur et al. 2005; Fanning et al. 2007; Kang et al. 2007). An example of hydrogel-coated coils are shown in Figure 1.2.

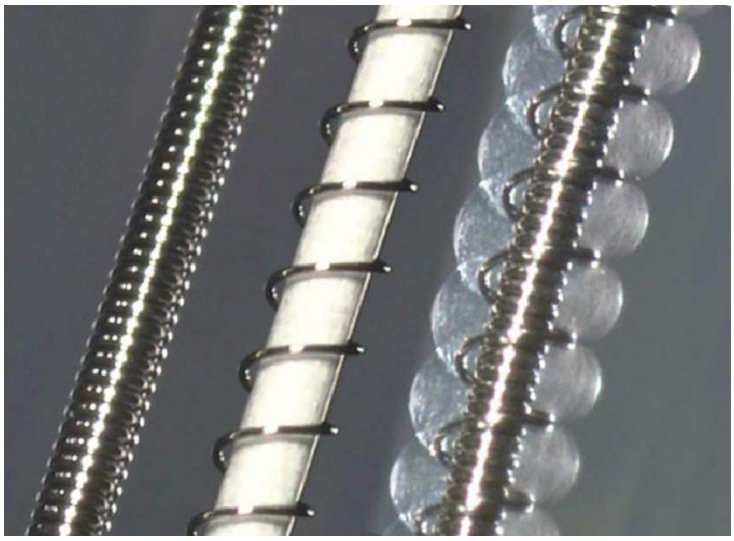


Figure 1.2 Hydrogel-coated coil. A bare platinum coil (*left*) compared to a hydrogel coated coil in its pre-hydrated state (*middle*) and post-hydrated state (*right*), showing the expanded translucent hydrogel. Reproduced with permission by Cloft and Kallmes (2004). Copyright (2004) American Society of Neuroradiology.

Matrix coils have a “bioactive” hydrogel coating which is designed to accelerate thrombus formation and enhance fibrous deposition, with the goal of improving aneurysm neck occlusion (Murayama, Tateshima, et al. 2003; Taschner et al. 2005; Fiorella et al. 2006). Cerecyte coils also use a bioactive polymer, but instead of a coating, polyglycolic acid is loaded on the inside of the

coil, giving the coil more structural support (Bendszus and Solymosi 2006; Bendszus, Bartsch, and Solymosi 2007).

The reason behind introducing these modifications is that aneurysms treated with coils are prone to recanalization (re-perfusion of blood flow into the aneurysm) due to compaction of coils into the aneurysm dome (Murayama, Nien, et al. 2003; van Rooij and Sluzewski 2007; Wakhloo et al. 2007). Overall, recanalization occurs in 15-35% of coil-treated aneurysms (Cognard et al. 1998; Cognard et al. 1999; Molyneux 2002; Murayama, Nien, et al. 2003; Raymond, Guilbert, et al. 2003; Henkes et al. 2004; Kurre and Berkefeld 2008; Ries and Groden 2009). However, recanalization rates are even worse after coil embolization of wide-necked aneurysms, with a 25-50% recanalization rate (Cognard et al. 1999; Hope, Byrne, and Molyneux 1999; Hayakawa et al. 2000), and large or giant aneurysms, with a 35-70% recanalization rate (Murayama, Nien, et al. 2003; Sluzewski , Menovsky, et al. 2003; van Rooij and Sluzewski 2007). The high recanalization rate is thought to be related to insufficient aneurysm filling by coils. In general, coil embolization can only fill about 30% of the aneurysm volume (Cloft and Kallmes 2004; Fiorella et al. 2006; Piotin et al. 2000; Slob, Sluzewski, and van Rooij 2005; Taha et al. 2006).The remainder of the aneurysm volume is occluded by blood that clots when it contacts the coils. However, it is the initial degree of occlusion that is directly related to the rate of recanalization, indicating that the body's clotting response will not protect from recanalization if an aneurysm is not initially filled with coils to a certain degree (Kawanabe et al. 2001; Lanzino et al. 2005).

While many modifications have been introduced to improve coils, these bioactive "advancements" have not been found to actually reduce recanalization

rates (Fiorella, Albuquerque, and McDougall 2006; Niimi et al. 2006; Cloft 2007; White and Raymond 2008). Recently, using stent-assisted coil embolization to treat large and wide-necked aneurysms has shown more success (Koebbe et al. 2006; Gao et al. 2010; Liang et al. 2010), but with significant additional costs and introducing the risk of in-stent stenosis (Simon, Reig, et al. 2010; Wells-Roth et al. 2005).

1.3.2 Onyx Embolization

Another class of embolic materials, liquid embolics, is aimed at improving the degree of aneurysm filling during embolization. Liquid can fill aneurysms more completely than coils, making them an attractive alternative (Murayama et al. 2000; Mawad et al. 2002; Molyneux et al. 2004). Currently, there is only one such liquid embolic device approved for use in the United States. Onyx ® (eV3 Neurovascular; Irvine, CA) is a liquid embolic system in which ethylene-co-vinyl alcohol is dissolved in an organic solvent. When delivered to an aneurysm, the co-polymers precipitate out of solution on contact with blood, forming a spongy solid cast, shown in Figure 1.3 (Murayama et al. 1998). The cast increases in size as more material is delivered, allowing greater aneurysm filling than achievable with coils. Initial studies have shown that Onyx® is more effective than coils in the treatment of large and giant aneurysms, with reported recanalization rates from 5-15% (Molyneux et al. 2004; Piske et al. 2009).

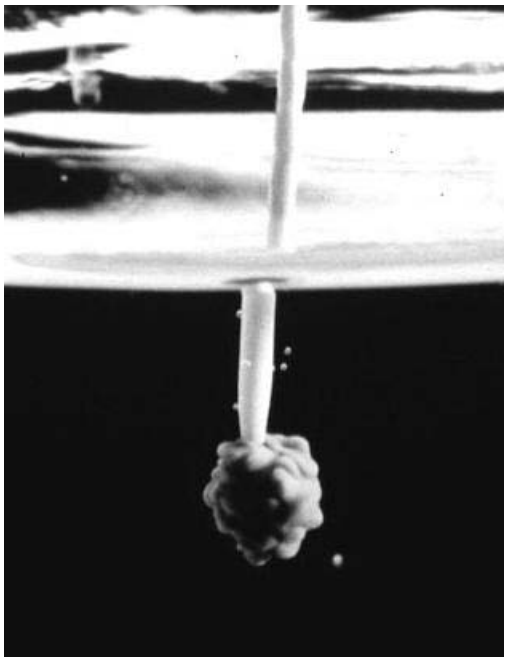


Figure 1.3 Precipitation of ethylene-co-vinyl alcohol (EVOH). EVOH co-polymers precipitate out of DMSO solvent when in contact with saline. Image reproduced with permission by Murayama et al. (1998). Copyright (1998) Lippincott, Williams & Wilkins.

However, there are significant drawbacks associated with Onyx® embolization. First, in order to get ethylene-co-vinyl alcohol into solution, it must be dissolved in an organic solvent, dimethyl sulfoxide (DMSO). DMSO diffuses away from the solid polymer cast during delivery and is released into the bloodstream. DMSO is known to cause angiotoxicity and vasospasm when injected too quickly (Murayama et al. 1998; Raftopoulos et al. 2000; Pamuk et al. 2005). As a result, Onyx must be delivered slowly. The delivery procedure requires multiple cycles of balloon inflation and deflation during injection, such that the organic solvent can diffuse away safely. The result is long procedure times (Molyneux et al. 2004; de Gast et al. 2008) as well as increased risk of

damaging the vessel wall during balloon cycling (Mawad et al. 2002). Recent studies have also reported that Onyx tends to migrate after embolization, increasing the chances of inadvertently occluding the parent artery (Struffert et al. 2008; Piske et al. 2009; Simon, Eskioglu, et al. 2010).

1.3.3 Flow Diverting Devices

Low porosity flow diverting stents, placed endoluminally across an aneurysm, have recently emerged as a new technique aimed at treating large, giant, and wide-necked saccular aneurysms, as well as fusiform aneurysms (Walcott et al. 2011). These devices are deployed endovascularly, and work to alter hemodynamics within an aneurysm by directing blood flow through the device, creating flow reduction and stagnation within the aneurysm itself. Flow stagnation results in thrombus formation and occlusion of the aneurysm, while endothelialization of the flow diverting device may occur over time.

While this new technology is in its infancy, initial reports suggest these devices may be able to successfully treat recanalization-prone aneurysms (Lylyk et al. 2009). However, aneurysm treatment with flow diverting devices may have some drawbacks. Initial investigations have suggested that in-stent thrombosis and stenosis are risks, possibly requiring patients to undergo long-term antiplatelet therapy (Klisch et al. 2011). Furthermore, these devices have resulted in delayed aneurysm rupture in some cases (Kulcsár et al. 2011), as well as ischemic stroke after unintentionally blocking perforating arteries with the flow diverting stent (van Rooij et al. 2010; Walcott et al. 2011).

1.4 Developmental Materials

Endovascular treatment options for cerebral aneurysms have multiplied in the past decade, yet most advancements have been geared towards improving existing coil technology. Onyx is the first and only clinically available liquid embolic system, but with many drawbacks. The field of volume filling embolics is ripe for development. The availability of technologically advanced endovascular tools makes it more enticing than ever to develop this new class of embolic materials. A few investigative embolic materials have been developed recently, such as calcium alginate and shape memory polymers.

1.4.1 Calcium Alginate

Calcium alginate was first examined for endovascular delivery by Becker and Kipke (2001). Alginate is a naturally occurring copolymer with mannuronic and guluronic acids blocks. When active guluronic acid sites associate with a divalent cation, such as calcium, polymer chains cross-link to form a gel matrix (Becker et al. 2005). In order to deliver this material to a lesion site, a double-lumen microcatheter has been used to bring both sodium alginate and the calcium chloride ($CaCl_2$) initiator to the desired site without reacting. Once the materials mix in the lesion site, the Ca^{2+} replaces a Na^{+} ion on alginate, resulting in rapid cross-linking. The byproduct of this system is NaCl, and the formed gel is nonadhesive as well as stable and biocompatible (Becker et al. 2007). Figure 1.4 shows calcium alginate in a bisected aneurysm model, showing the tissue-like nature of the solidified material (Soga et al. 2004).

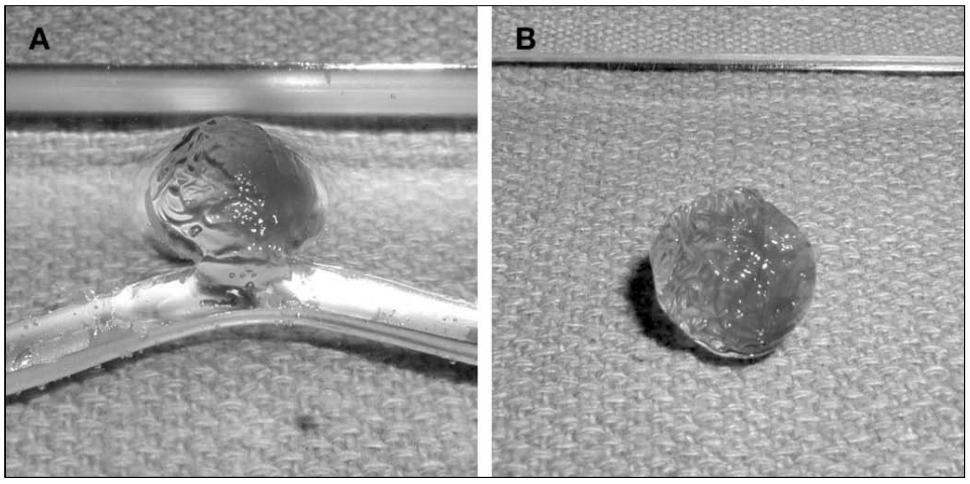


Figure 1.4 Calcium alginate as an embolic agent. An *in vitro a*neurysm model filled with calcium alginate “strings”, both within (A) and removed from the aneurysm model (B). Image reproduced with permission by Soga et al. (2004). Copyright (2004) Lippincott, Williams & Wilkins.

The advantages of this system include the absence of organic solvents, low toxicity, and the formation of a nonadhesive tissue-like gel. A downside to using calcium alginate for aneurysm embolization is that this material is not capable of a true liquid delivery because the components are extruded into the aneurysm in a string-like form, leaving unoccluded aneurysmal space after delivery. Calcium alginate may therefore behave like coils, where blood clots around the calcium alginate “strings” to occlude the aneurysm volume. If so, this system would also be prone to aneurysm recanalization, as seen with coils, especially since alginate is softer and thus potentially more amenable to compaction (Raymond, Metcalfe, et al. 2003). However, Becker et al. (2007) showed successful embolization after 3 months using a lateral wall aneurysm model in swine. Aneurysms were embolized with alginate under balloon

protection, resulting in initial complete occlusion and sustained occlusion at 3 months, with moderate thrombus formation within the aneurysm.

1.4.2 Shape Memory Polymers

Shape memory polymers (SMPs) are another class injectable embolic materials. While not liquid embolics, these materials have many similar features: they can be delivered through a microcatheter and are able to fill the entire aneurysm volume. Shape memory polymers are chemically structured so that they are able to reversibly take on a different physical shape in response to some stimuli (Small et al. 2007). Usually these different shapes include a compact form and an expanded form of the polymer. In the case of endovascular embolization, the expanded polymer can be pre-formed to fit specific contours of an individual aneurysm (Ortega et al. 2007). Upon interacting with a stimulus, such as heat or cold, the material is compacted into a shape that can be delivered through a microcatheter. The process of using shape memory polymers to embolize an aneurysm is shown in Figure 1.5, along with samples of expanded SMPs (Ortega et al. 2007).

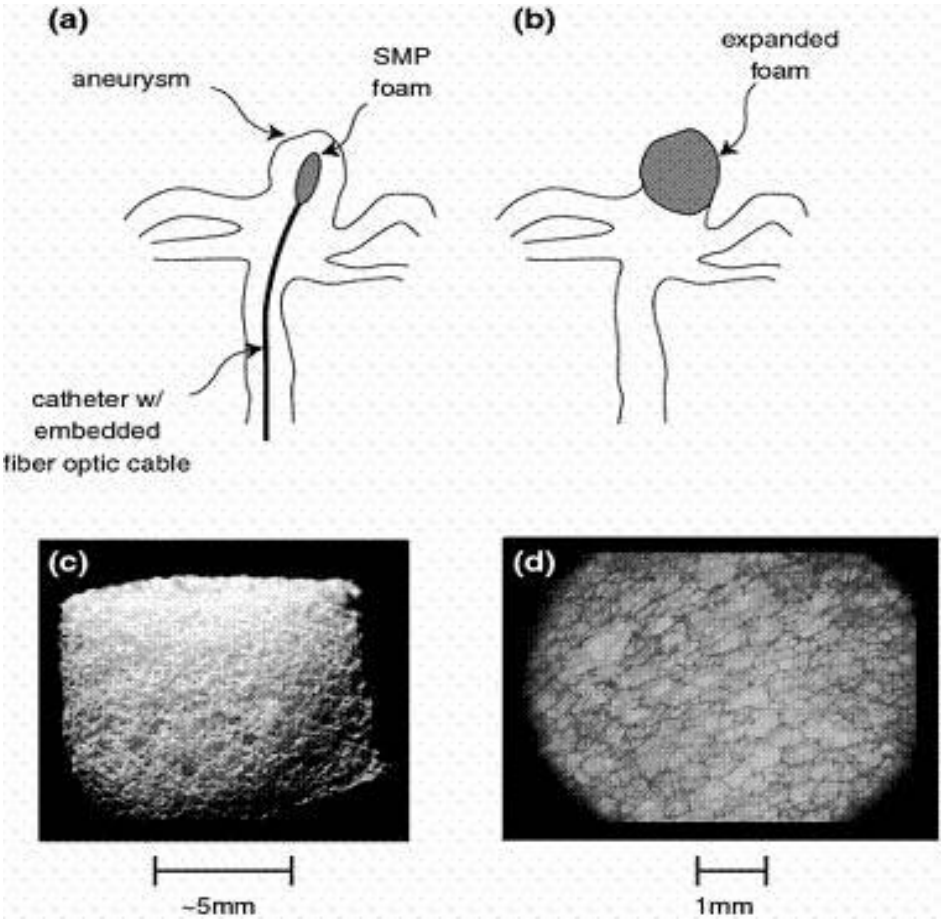


Figure 1.5 SMP foam for aneurysm embolization. (A) Compressed SMP foam is being delivered through a microcatheter. (B) After exposure to a stimulus, the foam expands. The porous structure of an SMP foam is shown in a primary, expanded shape (C) and close-up (D). Image reproduced with permission by Ortega et al. (2007). Copyright (2007) Springer.

These materials have an obvious application to fusiform aneurysms, which are difficult to treat using coils or liquid embolics due to migration into the parent vessel. Shape memory polymers can potentially remove this limitation since devices are pre-formed to the aneurysm shape. Metcalfe et al. (2003) investigated a porous polyurethane shape memory polymer as an embolic device

in an animal model. In this study, neointima formation was found after a 3-week period, but aneurysm obliteration was inconsistent. Of the 16 aneurysms embolized, only 1 showed complete obliteration and 8 showed residual necks or residual aneurysms after 3 weeks. Furthermore, none of these *in vivo* aneurysms were embolized using an endovascular delivery, calling into question the feasibility of delivering SMPs non-invasively. Since this 2003 study, *in vivo* investigation of SMPs for aneurysm embolization has not been reported.

While shape memory polymers may provide distinct benefits for aneurysm embolization, there are also potential limitations. For example, in order for the SMP to conform to the aneurysm's shape, it must be tailored to a specific aneurysm. This would involve patient-specific aneurysm dimensions to create the material, followed by precise delivery such that the SMP and aneurysm are oriented correctly when the material is expanded. Not only does this mean that additional material processing equipment must be available in the operating room, but it also requires an impeccably skilled neurointerventionalist to perform the embolization. With a true liquid embolic, the same material can be used for any shaped aneurysm and still provide an exact fit, without requiring additional material processing or a rigorous procedure.

1.5 Ideal Embolic Agent for Cerebral Aneurysm Embolization

All of the embolic agents discussed thus far have clear limitations for use in aneurysm embolization. An ideal endovascular embolic material would have a true liquid delivery in order to occlude the entire aneurysm volume in order to provide robust protection from aneurysm recanalization. This would be a major step up from coils, which are prone to recanalization. It would also provide an

advantage over Onyx and developmental materials discussed here (calcium alginate and shape memory polymers) because all of these materials do not encompass true liquid delivery and therefore cannot exactly fit an aneurysm's shape. Furthermore, the ideal embolic agent must be biocompatible and non-toxic, such that it either allows or encourages neointimal tissue growth *in vivo*.

Ease of delivery is also an important consideration. An ideal embolic agent should be delivered endovascularly in a one-time, straightforward procedure, in contrast to the drawn-out, technically challenging procedure associated with Onyx embolization. Furthermore, the less specialized equipment and additional processing procedures required, the easier it will be for neurointerventionalists to use this material in the clinic.

The work done here aims to showcase the development, optimization, *in vitro* testing, and initial *in vivo* studies of a novel polymer system for cerebral aneurysm embolization. The polymer system studied in this work has significant advantages for aneurysm embolization, making it closer to the "ideal" embolic agent than previously investigated materials. This material uses a time-dependent polymerization technique to cross-link into an elastic solid, rather than achieve solidification through polymer deposition. This type of liquid-to-solid transition allows the material to conform to the contours of the aneurysm, providing an exact fit and preventing blood re-entry. The time-dependent nature of solidification for straightforward delivery while in liquid form. Because of the true liquid delivery, balloon protection will be used to contain the material within the aneurysm until it cross-links to produce a mechanically sound embolic material. Furthermore, the material does not require the use of organic solvents

in formulation, because the reactive monomer components are already in liquid form prior to mixing.

1.6 PPODA-QT Cross-Linking Polymer System

The polymer system investigated here undergoes liquid-to-solid transformation through cross-linking of reactive chemical groups. The system is composed of two low-molecular weight monomers shown in Figure 1.6, poly(propylene glycol) diacrylate (PPODA, Mw~900), and pentaerythritol tetrakis (3-mercaptopropionate) (QT), and a basic water phase. When mixed in appropriate proportions, the organic monomer precursors (75% wt.) and pH-adjusted water phase (25% wt.) create a reverse-emulsion system (Vernon et al. 2003). The continuous phase consists of the organic monomer mixture, while the pH-adjusted aqueous phase is dispersed into droplets. Michael-type addition is initiated through diffusion of ^{-}OH groups from the high pH dispersed phase into the organic phase. Hydroxide ions deprotonate free thiol groups, which then nucleophilically attack and “add” onto acrylate-containing monomers.

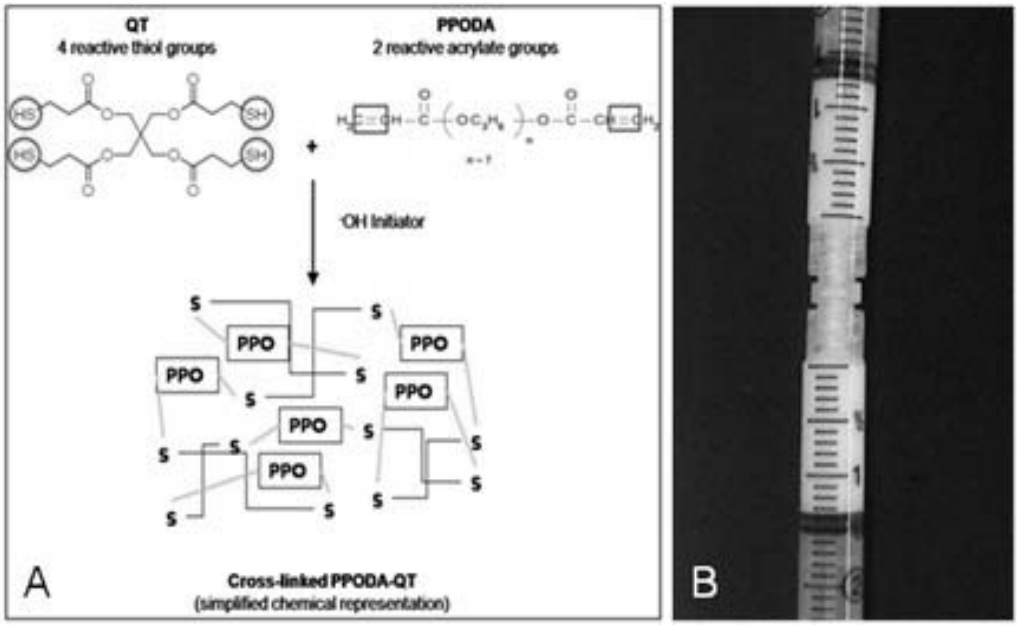


Figure 1.6 PPODA-QT gelling system. (A) Material precursors react to form a cross-linked network through (B) syringe-mixing of components.

Previous work has shown that the speed of cross-linking can be controlled in a number of ways. McLemore, Preul, and Vernon (2006) showed that the pH of the aqueous phase, buffer strength of the aqueous phase, and mixing duration all affected the speed of gel formation. In general, higher buffer strength, higher pH, and longer mixing are all associated with faster reaction times. This finding is critical to the material's suitability as an embolic agent, indicating that the material gel time is tailorable.

Initial investigations by Vernon et al. (2003) into phase-segregated Michael-type addition systems were valuable for gaining insight into how these types of materials behave. Up until then, very little work had been done in studying these systems, where hydrophobic monomers were mixed with a water-based initiator to form a cross-linked network. Vernon et al. (2003) initiated

investigation of a suitable system for hard tissue repair, and eventually for endovascular aneurysm embolization. Further investigation showed that the PPODA-QT system had repeatable reaction kinetics and suitable viscoelastic properties, such as a low viscosity “deliverable” region as well as an elastic modulus similar to that of an artery wall, between 10kPa – 10MPa (McLemore, Preul, and Vernon 2006; Nemir and West 2010). The work presented here builds on these past studies and describes the process of making the PPODA-QT system more applicable to cerebral aneurysm embolization.

1.6.1 Incorporation of Radio-Opacity

Embolic agents for cerebral aneurysm embolization must contain radiographic properties. These materials must be radio-opaque because the endovascular procedure is visualized via X-ray fluoroscopy. Seeing the material as it is delivered is crucial for successful aneurysm embolization. Platinum coils are innately radio-opaque, but polymer systems are not. Therefore, a radiographically dense material must be added to a polymer system to confer radio-opacity.

In this work, radio-opacity is added to the PPODA-QT system by incorporating a commercially available liquid contrast agent. Instead of using a high pH aqueous buffer as the initiating solution, a commercially available liquid contrast agent was substituted. Increasing the pH to an appropriate level allows the contrast agent to act as the initiating solution for Michael-type addition of PPODA and QT. While this technique was briefly discussed by McLemore, Preul, and Vernon (2006), there was no further investigation into the role of the contrast agent in the PPODA-QT system.

As this work will show, incorporation of a liquid contrast agent into the PPODA-QT gelling system is not a trivial matter. The choice of contrast agent is critical for a final polymer system formulation that has desired properties of an embolic material. In order to optimize the PPODA-QT system for cerebral aneurysm embolization, materials formulated with two different types of contrast agents were systematically compared. Once convinced of the suitability of a formulation, *in vivo* studies were performed using the optimized PPODA-QT system.

1.6.2 Experimentation and Testing

The two contrast agents initially investigated in this work are Conray™ and Omnipaque™ 300. Conray (Mallinckrodt, St. Louis, MO) is a high osmolar, an ionic contrast agent made up of (by 60% wt.) the radio-opaque salt iothalamate meglumine. Omnipaque, however, is a different class of contrast agent. Omnipaque is considered a low osmolar, nonionic contrast agent, composed primarily (48% wt.) of the radio-opaque molecule iohexol. The main difference between Conray and Omnipaque is these radio-opaque molecules. In Conray, iothalamate meglumine is dissociated, while iohexol does not dissociate in Omnipaque.

In normal clinical application, these contrast agents are used for angiography. Ionic contrast agents, such as Conray, result in hypertonic solutions since the number of particles doubles when the ionic salt dissociates. Ionic contrast agents are associated with more adverse physiological effects, such as pain, during clinical angiographic use (Wolf, Arenson, and Cross 1989). Nonionic contrast agents have lower osmolality because they do not dissociate, and are

associated with fewer instances of physiologic discomfort as well as nephrotoxicity (Rudnick et al. 1995). For incorporation into PPODA-QT polymer system, however, it is not immediately clear how Conray and Omnipaque would affect the resulting gels.

The work reported here is aimed at formulating, characterizing, and testing the PPODA-QT system, geared towards clinical aneurysm embolization. Chapter 2 investigates how formulations with Conray and Omnipaque affect the gelling process of the PPODA-QT system, while Chapter 3 compares *in vitro* characteristics of Conray- and Omnipaque- formulated gels in order to determine an optimal PPODA-QT formulation for cerebral aneurysm embolization. The last two chapters focus material testing *in vivo*. Chapter 4 is assesses initial biocompatibility and delivery strategies of the optimized PPODA-QT formulation, while Chapter 5 reports initial *in vivo* efficacy in a challenging animal model.

Chapter 2: GELLING PROCESS DIFFRERENCES IN REVERSE EMULSION, IN SITU GELLING POLYMERIC MATERIALS FOR INTRACRANIAL ANEUYSM EMBOLIZATION, FORMULATED WITH INJECTABLE CONTRAST AGENTS

2.1 Introduction

Endovascular embolization has been routinely used to treat intracranial aneurysms since the mid-1990s. This technique has gained popularity due to the non-invasiveness of the procedure compared to surgical intervention (Brilstra et al. 1999; Byrne, Molyneux, and Brennan 1995). The advent of microcatheters, flexible stents, and balloon occlusion has triggered widespread use of embolization for conditions that could not previously benefit from the technique (Higashida, Hieshima, and Halbach 1991; Linfante and Wakhloo 2007). With the ability to perform embolization routinely, many new types of embolic materials are being introduced. A variety of endovascular coils exist for treating intracranial aneurysms. Some include hydrogel coatings or fillings in order to trigger biologic activity or increase the coil packing density within an aneurysm (Murayama, Nien, et al. 2003; Cloft and Kallmes 2004; Arthur et al. 2005; Taschner et al. 2005; Bendszus and Solymosi 2006; Fiorella, Albuquerque, and McDougall 2006; Bendszus, Bartsch, and Solymosi 2007; Fanning et al. 2007; Kang et al. 2007). Precipitation-based copolymer systems such as Onyx® (eV3, Irvine, CA), in which polymers precipitate out of solution and form a solid mass immediately on contact with blood, are also being optimized for use in both intracranial aneurysms and arteriovenous malformations (AVMs) (Murayama et al. 1998; Becker and Kipke 2001; Jahan et al. 2001; Soga et al. 2004; Song et al. 2004; Weber et al. 2007; Velat et al. 2008).

While there are now many embolic materials available for cerebral aneurysm occlusion, there are drawbacks and limitations to all techniques, providing need for the development of more desirable materials. Currently, coil embolization is the most common and considered an effective endovascular method for treating intracranial aneurysms. The International Subarachnoid Aneurysm Trial in 2002 showed that intracranial aneurysm treatment with coil embolization resulted in significantly less patient morbidity rates when compared to microsurgical clipping, given that the study compared aneurysms that were suitable for treatment by either method (Molyneux 2002). However, coil embolization problematically results in a higher rate of aneurysm recanalization, which is thought, in part, to be related to the relatively low packing density achievable during coil placement (Piotin 2000; Kawanabe 2001; Molyneux 2002; Cloft 2004, Sluzewski 2004; Lanzino 2005; Slob 2005; Fiorella 2006).

Precipitating copolymer systems, such as Onyx ® Liquid Embolic System, can achieve greater aneurysm volume filling (Mawad 2002 and Molyneux 2004). However, the Onyx system has drawbacks as well, some of which may impose serious risk. Onyx (ethylene-vinyl alcohol) requires dimethyl sulfoxide (DMSO) to dissolve the copolymers, leading to injection of the organic solvent into the body during embolization. Injection of DMSO has been shown to induce vessel necrosis and vasospasm when injected too quickly (Murayama et al. 1998; Chaloupka et al. 1999; Raftopoulos et al. 2000; and Pamuk et al. 2005). In addition, there have been reports of electrocautery-induced ignition of Onyx (Schirmer, Zerris, and Malek 2006).

In order to overcome the drawbacks of commercially available embolic materials, we have developed a liquid, *in situ* gelling polymeric material that can

potentially be used as an embolic agent for intracranial aneurysms. This polymer system is advantageous because its liquid-to-solid gelling characteristics will allow non-invasive endovascular delivery of the material into the aneurysm. Upon delivery, this material will be able to significantly increase the volume of the aneurysm filled with material and conform to the shape of the aneurysm, without simultaneously injecting organic solvents into the body. Initial *in vivo* injections of this material have shown ease of delivery and indicated no instances of "gluing" the catheter tip to tissue, which is another consideration when using liquid-to-solid gelling materials (Debrun et al. 1997; Duffner et al. 2002; McLemore et al. 2005; McLemore, Preul, and Vernon 2006).

This Michael-type addition polymer system has been described in detail previously (McLemore, Preul, and Vernon 2006; Vernon et al. 2003). Briefly, two multi-functional hydrophobic monomers are combined through syringe mixing to create a homogeneous organic phase. These monomers are poly(propylene glycol) diacrylate (PPODA) and pentaerythritol tetrakis(3-mercaptopropionate) (QT), shown in Figure 2.1. After mixing the organic monomers, a basic initiating solution is introduced through syringe mixing, resulting in an unstable reverse-emulsion. The organic monomers (PPODA and QT) make up the continuous phase and the initiating solution is dispersed. Flux of ions across phase boundaries initiates the reaction through deprotonation of thiol groups located on QT, which then react with the acrylate groups on PPODA. The Michael-type addition reaction scheme is shown in Figure 2.1C. Once the initiator is introduced, the organic phase cross-links and eventually undergoes network formation in a time-dependent manner.

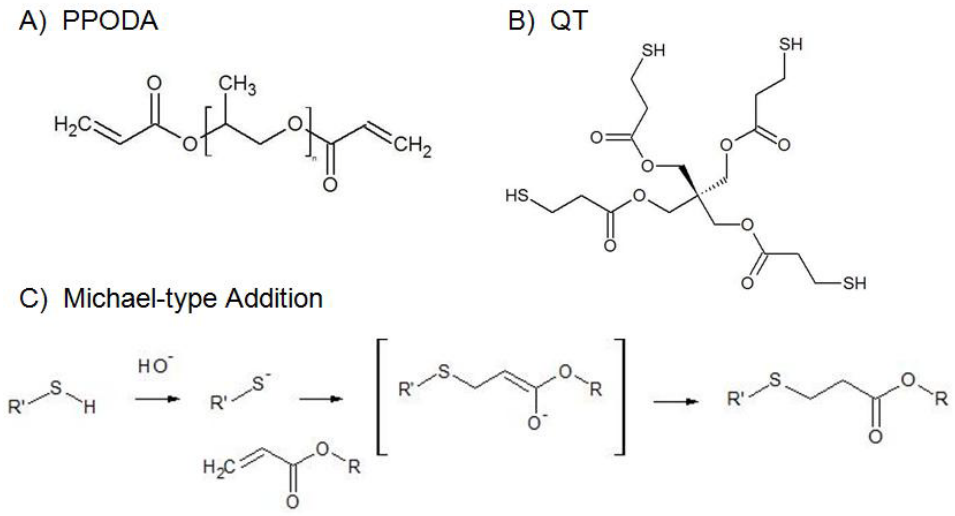


Figure 2.1 Components and reaction scheme. (A) Poly(propylene glycol) diacrylate, also called PPODA, M_w≈900; (B) Pentaerythritol tetrakis(3-mercaptopropionate), also called QT, M_w≈488; (C) Michael-type addition reaction scheme. Deprotonated thiol group of QT performs nucleophilic attack on acrylate group of PPODA.

The rate of reaction kinetics can be adjusted by changing certain parameters, such as increasing the pH of the initiating solution and increasing the duration of mixing the initiating solution and organic components, both of which increase the reaction rate (McLemore, Preul, and Vernon 2006).

Since the PPODA-QT polymer system encompasses a true liquid delivery of the embolic material, chemical reaction kinetics become very important. The speed of “solidification” has implications for how this material may be used clinically. For example, treating an AVM via endovascular embolization requires extreme care to prevent the embolic material from reaching the draining vein (Chun-Ho Yu and Kin-Ming Cheng 2004; Alexander and Tolbert 2006). A material that takes minutes to solidify has a much greater chance of entering the

draining vein than a material that solidifies in seconds (Lieber et al. 2005). As such, embolic materials that solidify almost instantly upon delivery are preferred, leading to almost exclusive use of NBCA and Onyx in endovascular AVM treatment. For aneurysm embolization, however, there can be more flexibility with respect to a material's "gel time" due to the common practice of using an endovascular balloon to occlude the parent artery during delivery of the embolic agent (Moret, Pierot, and Boulin 1994; Moret et al. 1997; Murayama et al. 2000; Mawad et al. 2002, Molyneux et al. 2004; Gallas et al. 2005, Piske et al. 2009). The balloon is able to keep the embolic material contained within the aneurysm until it is fully filled—or in the case of the PPODA-QT system, able to cross-link. Therefore, using a material that undergoes true liquid delivery is much more feasible for treating an aneurysm (or vascularized tumor lesion, potentially) than for treating an AVM, as long as parent artery occlusion is performed across the aneurysm neck during the procedure. While it is still critically important to monitor and control the gel time of the PPODA-QT material, a system that requires minutes to gel is a viable alternative to currently available materials for aneurysm embolization.

In this work, the high pH initiating solution used to start the reaction between PPODA and QT consists of an injectable radio-opaque contrast agent, which will allow the polymer to be seen during clinical injection by digital subtraction angiography (DSA). Two different injectable contrast agents were analyzed—one is a high osmolar, ionic contrast agent, while the other is a low osmolar, nonionic agent. Rheological and scanning electron microscopy (SEM) analyses were performed on each formulation to identify differences in gel formation attributed to using different contrast agents. Once differences were

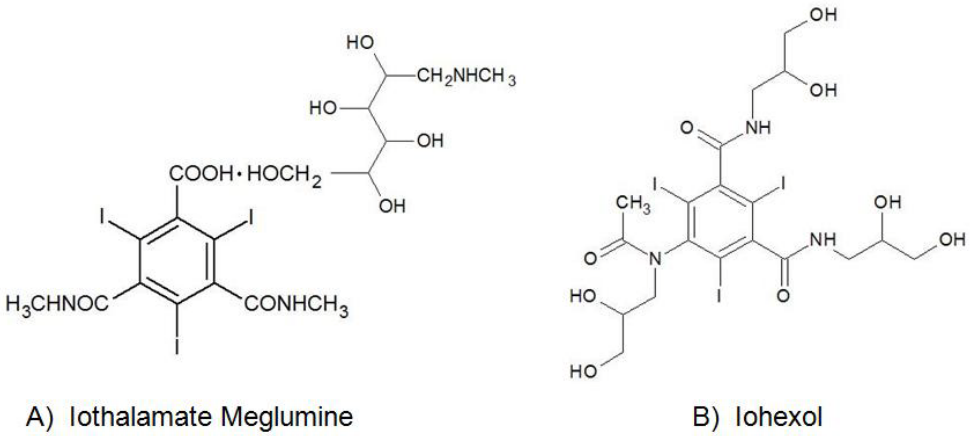
identified, analysis of the results helped determine why these differences are seen.

2.2 Materials and Methods

2.2.1 Polymer System

The polymer system consists of a liquid mixture of two organic monomers that cross-link to form a solid network through Michael-type addition chemistry. Poly(propylene glycol) diacrylate, ~Mw 900 (PPODA) and pentaerythritol tetrakis(3-mercaptopropionate), abbreviated QT (both purchased from Sigma, St. Louis MO), were combined such that an equal number of functional groups were available to react with each other.

The injectable contrast agents used in this work are Conray™ (Mallinckrodt, St. Louis, MO) and Omnipaque™ 300 (GE Healthcare, Princeton, NJ), composed of radio-opaque molecules shown in Figure 2.2. Conray™ is a high osmolar, ionic contrast agent, the main component of which is iothalamate meglumine, at a concentration of 600 mg/mL (742 mM). Iothalamate meglumine provides radio-opaque contrast and makes up 60% wt. of Conray. When dissolved in water, this molecule dissociates to form the conjugate base of iothalamic acid and meglumine. Omnipaque™ 300 (GE Healthcare, Princeton, NJ) is considered a low osmolar, nonionic contrast agent, composed primarily (48% by wt.) of the radio-opaque molecule iohexol. Iohexol is present in a concentration of 647 mg/mL (788 mM). Iohexol does not dissociate when dissolved in water.



A) Iothalamate Meglumine B) Iohexol

Figure 2.2 Radio-opaque molecules of Conray and Omnipaque. (A) Iothalamate meglumine (Mw≈809) is the radio-opaque molecule used in Conray. When dissolved in Conray, iothalamate meglumine dissociates into iothalamate anions and meglumine cations; (B) Iohexol (M_w≈821) provides radio-opacity in Omnipaque. When dissolved in Omnipaque, iohexol does not dissociate.

These injectable contrast agents were pH-adjusted with 5N NaOH and incorporated into the polymer system as the aqueous initiating component. Conray™ was adjusted to pH 10.8 and Omnipaque™ 300 was adjusted to pH 12.2. Due to the fact that these contrast agents have very different compositions (iothalamate meglumine vs. iohexol), it is necessary to adjust each contrast agent to a different pH in order to achieve gel times that are on the same scale. Further explanation of why each particular pH was chosen can be found in the discussion section. The addition of NaOH to each contrast agent did not negatively affect the radio-opacity of these solutions, as the solutions (as well as the resulting gels) are visible under X-ray even after pH adjustment (McLemore, Preul, and Vernon 2006). The contrast-providing molecules in both Conray and Omnipaque do provide a weak buffering capacity for each solutions, so it is not

likely that the addition of NaOH will compromise their structural or functional integrity.

To begin the reaction, each component was first weighed and aliquoted. Components were measured such that the final gel concentration was 75% (wt.) organic components and 25% (wt.) aqueous contrast agent. The organic components, PPODA and QT, were weighed into 3cc syringes so that they had an equal number of reactive groups. In these experiments, 0.488g of QT was syringe-mixed with 1.80g of PPODA using a luer-loc syringe coupler to attach component syringes. After 30 seconds of mixing the organic components, 0.763g of pH-adjusted contrast agent (either Conray or Omnipaque) was introduced. Mix times of 0.5 and 1.5 minutes were used to make gels.

2.2.2 Rheological Testing

Rheology was performed in replicates of three for each formulation (Conray pH 10.8 or Omnipaque pH 12.2) and mix time (0.5 or 1.5 minutes). Oscillatory time sweeps were performed at 22^{o}C over the course of the reaction, with a constant frequency of 1 Hz and constant stress of 10 Pa. The gel time for each treatment group was determined by rheology, measured at the point where the phase angle (δ) equals 45^{o}, indicating the sample has equal proportions of solid-like and liquid-like characteristics.

Using rheology data, changes were also observed in the material viscosity over time on a semi-log scale. Viscosity can also be used to monitor the progression of the reaction, by which the material's strength can be evaluated over time to identify when the reaction is complete. Examining both the viscosity

and phase angle profiles provided a complete picture of how each of these formulations progress through gelling.

2.2.3 Scanning Electron Microscopy

The pore structure of gelled polymer samples was examined using scanning electron microscopy (FEI XL30 EFSEM). Polymer samples were prepared as previously described, then cut with a razor blade in thin sections through the radial plane of the cross-linked gel. Samples were left to air dry, followed by gold sputter-coating. Imaging was done at a working distance of approximately 10 mm, and captured at 2000X and 5000X magnification.
In order to compare pore structures present on Conray- and Omnipaque-formulated materials, image analysis was done on 5000X magnification images of 0.5 and 1.5 minute mixed gels (n=6 images per group). A spline masking analysis programmed in MATLAB (The MathWorks, Inc.) was used to quantify pores.

2.2.4 Statistical Analysis

Statistical analysis was accomplished through multiple-comparison ANOVA for the gel time responses. Pore size distributions obtained from SEM images underwent a logarithmic transformation to obtain normalcy of the data prior to statistical analysis. A 2-tailed student's t-test was used to compare gel times and droplet size distributions. The statistical significance threshold was chosen to be $p<0.05$. When specific data are reported, they are given as the average value ± standard deviation.

2.3 Results

2.3.1 Rheological Testing

Phase angle profiles for one replicate of Conray- and Omnipaque-formulated gels (1.5 minute mix) are shown in Figure 2.3, highlighting representative curves for each formulation. The average gel time is marked on each curve where δ=45^{o}, for n=3 replicates. An ANOVA analysis indicated that both radio-opaque agent and mix time had a significant effect on the gel time (p<0.0001).

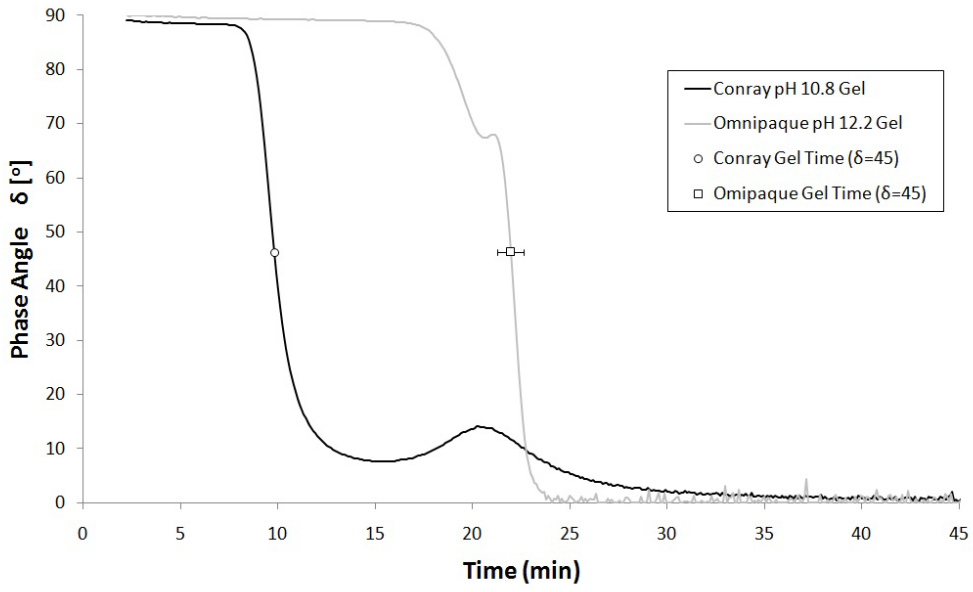


Figure 2.3 Phase angle profiles of Conray and Omnipaque gels. Gels were mixed for 1.5 minutes. Conray samples (black line) mixed for 1.5 minutes had an average gel time of 9.8 ± 0.1 minutes (○), while 1.5 minute mixed Omnipaque samples (grey line) reached δ=45^{o} in 22.2 ± 0.68 minutes (□). For each condition, n=3.

Comparing between radio-opaque agents, Figure 2.4 shows that Conray-formulated samples reached the gel point significantly faster than Omnipaque-formulated samples, with $p<0.05$ for each mix time pair. When comparing the 0.5-minute and 1.5-minute mixed samples within a formulation group, both Omnipaque- and Conray-formulated samples show statistically reduced gel times for the 1.5-minute mixed samples (p=0.007 and p=0.003, respectively, using 2-tailed t-tests). Overall, this data indicates that materials made with Conray at pH 10.8 reach the gel point faster than gels formulated with Omnipaque at pH 12.2, and longer mixing also results in faster gelling.

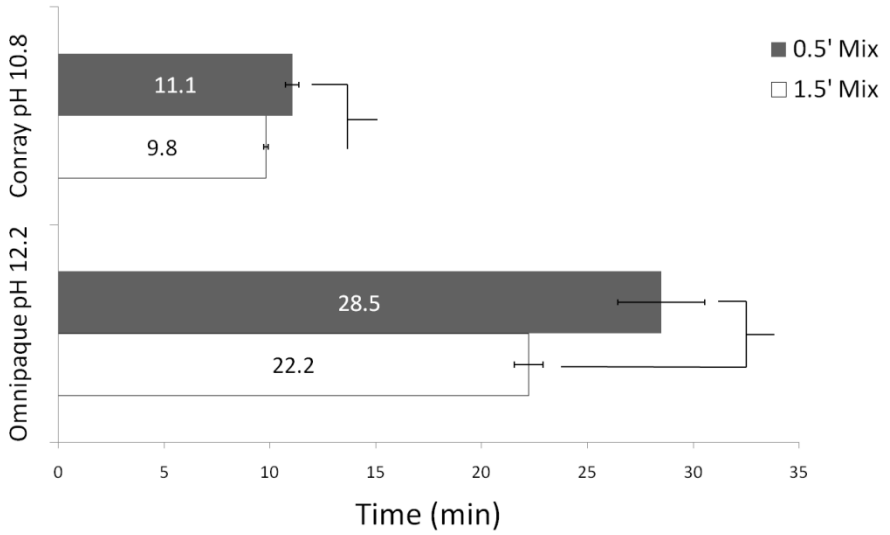


Figure 2.4 Average gel times. Gel times (min) ± standard deviation are shown for Conray- and Omnipque-formulated gels mixed for 0.5 minutes (dark grey) and 1.5 minutes (white). For each condition, n=3.

A semi-log plot of complex viscosity vs. time is shown in Figure 2.5. Curves for each formulation indicate that there are distinct regions of viscosity change corresponding to the reaction kinetics for each formulation. Figure 2.5 also highlights these separate regions for each 0.5 minute mix formulation, with clear boxes marking regions of the Conray gel viscosity profile, and grey boxes highlighting regions of viscosity change in Omnipaque gels.

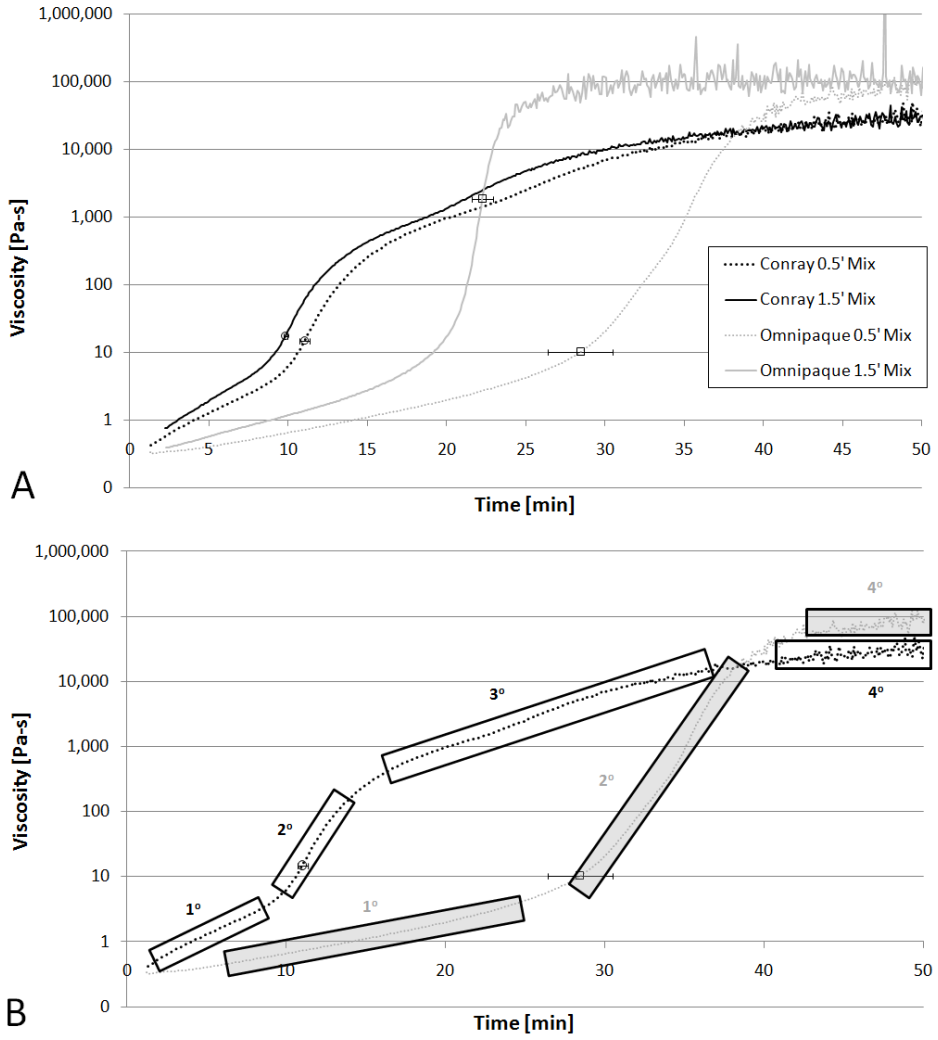


Figure 2.5 Viscosity profiles during the reaction process. (A) Conray gel (black lines) and Omnipaque gels (grey lines) mixed for 0.5 (dashed) and 1.5 (solid)

minutes. Average gel time ± standard deviation is shown on each profile. Average gel time for Conray samples are shown as circles, while average gel times for Omnipaque samples are squares. (B) The 0.5-minute mix profile is shown for both radio-opaque formulations. Regions of the viscosity profile are identified for Conray gels (white boxed regions) and Omnipaque gels (grey boxed regions).

2.3.2 SEM Analysis

SEM imaging showed that gels formulated with both Conray and Omnipaque contain a distribution of isolated, non-interconnected, pores due to dispersion of the aqueous phase (contrast agent). The droplet structures within gels were compared in terms of droplet size number of droplets per image. Figure 2.6 shows the surfaces of Conray-formulated and Omnipaque-formulated gels at 2000X magnification for each mix time. Qualitatively, these images show that increasing the material mix time results in smaller pores.

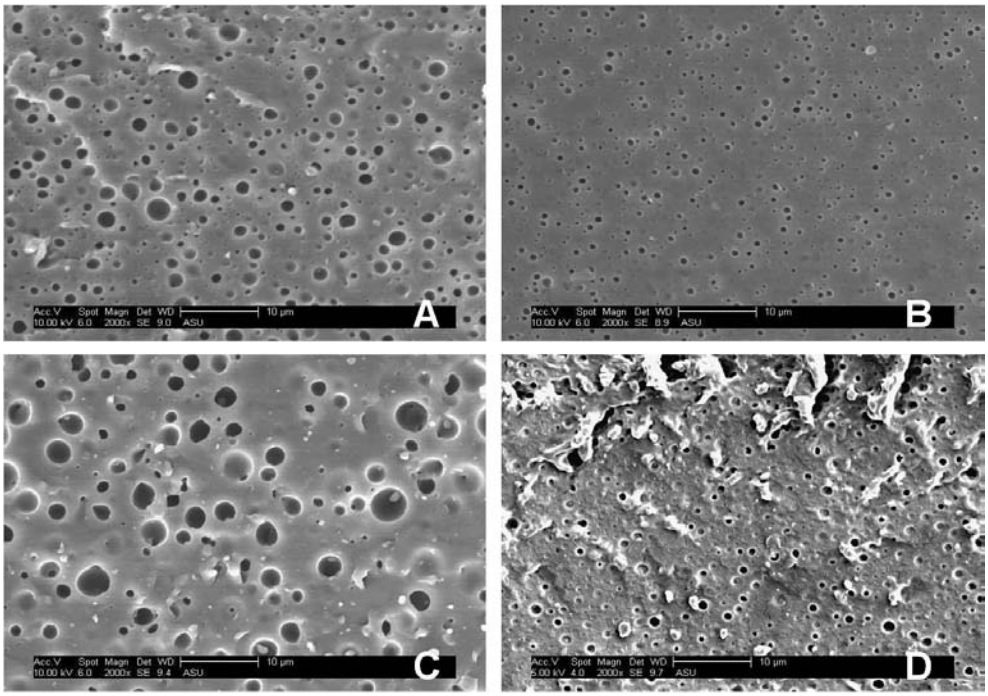


Figure 2.6 SEM images of Conray and Omnipaque gels. Conray-formulated (A, B) and Omnipaque-formulated (C, D) gels at mix times of 0.5 minutes (A, C) and 1.5 minutes (B, D). Magnification 2000X. Uneven gel surface is due to slicing with a razor blade when preparing samples.

Quantitative droplet analysis was done on 5000X magnification SEM images for 0.5 and 1.5-minute mixed gels (n=6 images per formulation). Figure 2.7 shows visual output of the spline masking program, in which the SEM image of a 1.5-minute mixed Conray gel is shown at 5000X before and after masking.

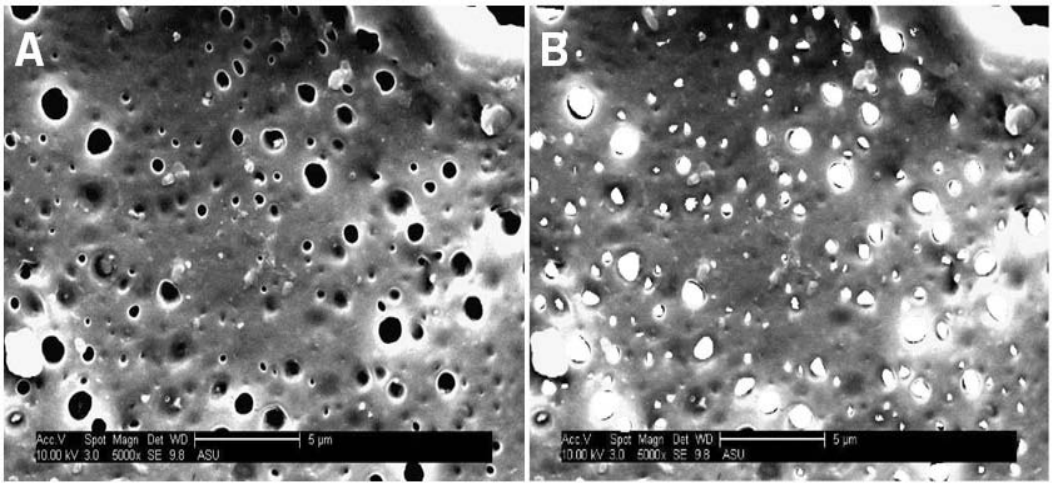


Figure 2.7 SEM masking analysis. Conray-formulated gel mixed for 1.5 minutes, 5000X magnification. (A) Image before masking analysis; (B) Image after masking analysis.

From image quantification, Conray gels were found to have an average of 42.7 ± 5.7 droplets per image when mixed for 0.5 minutes, and 147.8 ± 10.6 droplets when mixed for 1.5 minutes (p=0.005). Omnipaque gels have an average of 25.3 ± 9 and 87.0 ± 31.7 droplets per image with mix times of 0.5 and 1.5, respectively (p=0.005). Conray-formulated gels were found to have statistically more droplets than Omnipaque-formulated samples when mixed for the same amount of time (0.5 minute mix, p=0.0049; 1.5 minute mix, p=0.0082). For both gel formulations, more droplets are seen when the material is mixed for a longer amount of time.

The average size distribution of droplets in each treatment group is shown in Figure 2.8. A log transformation to achieve normalcy was first applied to droplet size data (nm^2), which were then represented as a histogram. In Figure 2.8, the number of droplets per image is seen to increase for both contrast agent

formulations when longer mixing is applied to the system. Also, Omnipaque-formulated gels display a shift towards larger sized droplets in both the 0.5 and 1.5 minute-mixed treatments. Student's t-tests performed on the transformed droplet size data show statistical differences between populations of relevant treatment combinations (Con0.5 vs. Con1.5 $p<0.001$; Omn0.5 vs. Omn1.5 $p<0.001$; Con0.5 vs. Omn0.5 $p=0.008$; Con1.5 vs. Omn1.5 $p<0.001$).

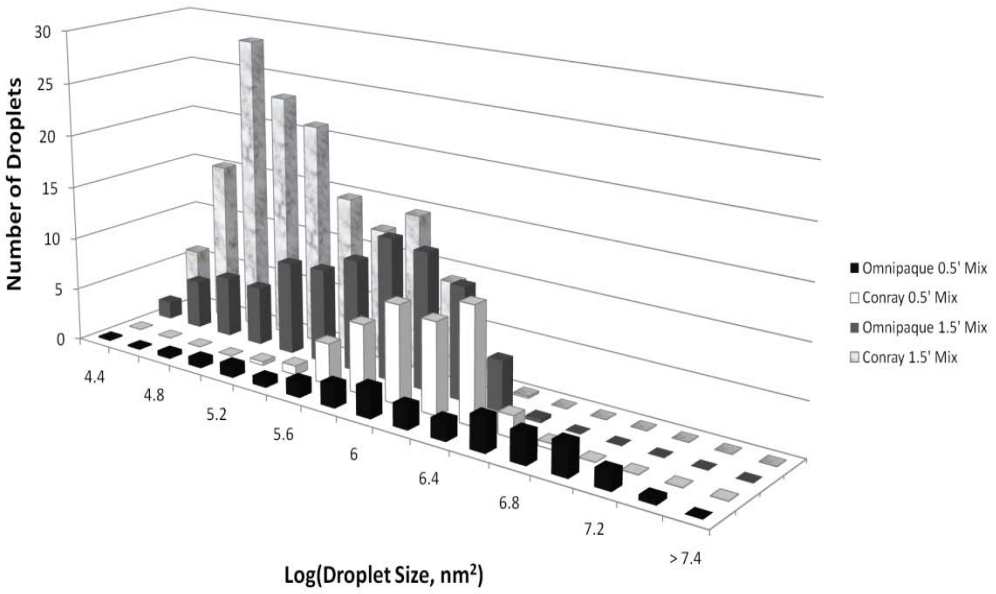


Figure 2.8 Droplet distribution from SEM analysis. Histogram showing the average number of droplets per treatment group (n=6) distributed into equally spaced categories of droplet area (nm^2), after performing a log transformation of the droplet area. Data is shown for Omnipaque 0.5-minute mixed samples (black bars), Conray 0.5-minute mixed samples (white bars), Omnipaque 1.5-minute mixed samples (grey bars), and Conray 1.5-minute mixed samples (marble bars).

2.4 Discussion

2.4.1 PPODA-QT Reverse Emulsion System

Properties of PPODA-QT gelling polymer systems have been investigated previously and reported in a variety of publications (Vernon et al. 2003; Vernon et al. 2004; McLemore, Preul, and Vernon 2006; McLemore, Lee and Vernon 2009). When a PPODA-QT mixture, making up 75% (wt.) of the total formulation, is further mixed with an aqueous solution making up the remaining 25% (wt.), the resulting solution is an unstable reverse emulsion. The continuous phase is made up of organic monomers (PPODA and QT), while the aqueous solution is dispersed. If the aqueous solution is basic, diffusion of ions across the phase boundary will initiate deprotonation of thiol groups on QT, which then react with PPODA. However, if the aqueous and organic phases separate before network formation is able to take place, the material will not form a fully cross-linked gel.

There are a number of factors that have been found to increase the rate of reaction so that cross-linking occurs faster than phase separation (McLemore, Preul, and Vernon 2006). For example, increasing the pH of the dispersed phase introduces more $^{-}$OH groups to the system. This heightens the capacity for reaction initiation by creating more ion flux during mixing, as well as providing a higher concentration of ions in the post-mixed droplets.

Another method for achieving faster reaction kinetics is enhancing ion diffusion by increasing the mixing time. Increasing mix time extends the duration of convective ion transfer between phases, which further increases the number of reaction initiation sites in the organic phase. Longer mixing also works to forcefully maintain the dispersed phase in small droplets. Maintaining small

droplet sizes increases the surface area-to-volume ratio of droplets, thereby enhancing diffusion during mixing. Once mixing is finished, droplets are no longer maintained at small sizes and can merge with neighboring droplets, given that the viscosity of the continuous phase is low enough to allow droplet mobility.

Reaction kinetics can also be altered through surfactant action, which stabilizes droplets within the emulsion by inhibiting droplet coalescence. McLemore, Lee, and Vernon (2009) examined how certain surfactants affect the gelling process of PPODA-QT systems. The authors found that large, non-ionic surfactants at low concentrations were able to increase reaction kinetics when compared to initiating solutions that did not contain surfactants. The authors concluded that gel times were faster due to the ability of surfactants to stabilize droplet sizes, resulting in enhanced ion flux across phase boundaries.

2.4.2 Incorporating Different Initiating Solutions

In previous studies, increasing the reaction kinetics of PPODA-QT materials has been accomplished through techniques mentioned above: increasing pH, increasing mix time, and adding surfactant (McLemore, Preul, and Vernon 2006; McLemore, Lee, and Vernon 2009). In almost all previous work, 100-150 mM phosphate-buffered saline (PBS), pH-adjusted to basic conditions, was used as the aqueous initiating solution. Overall, there has been little discussion regarding how different initiating solutions affect the gelling process. Due to the clinical necessity of fluoroscopic visibility, two aqueous contrast agents were incorporated into PPODA-QT gels.

In this study, NaOH was added to Conray and Omnipaque to achieve pH 10.8 and 12.2, respectively. The rationale for adjusting these contrast agents to

different pH values relates to differences in their composition. As previously mentioned, Conray is composed mostly of the iothalamate meglumine, an ionizable salt. Iothalamic acid has a pKa of about 3.5, meaning that at a pH above 3.5, iothalamate molecules have been deprotonated and are negatively charged, in the form of COO^- instead of COOH (Busetti et al. 2008). Therefore, at a pH of 10.8, there are many free ^-OH groups in solution. Iohexol, on the other hand, has a pKa of 11.35. At pH 10.8, there are not many free ^-OH groups available to react, resulting in slow reaction kinetics. At pH 12.2, where pH>pKa, there are more free ^-OH groups and the reaction can occur faster than it would at pH 10.8.

While it is difficult to compare these contrast agents given their different "buffering" molecules, pH values that produced similar gel times (<30 min) for both formulations were examined. Since the actual gel times of each formulation can be adjusted by changing the pH, the primary goal of this work is to identify differences in how each formulation progresses through gelling. As discovered during this study, the amount of free ^-OH ions available to initiate Michael-type addition reactions is not the only factor in determining reaction kinetics. The observed differences in the gelling process, dependent on which contrast agent is used, may have implications for clinical applicability of the material, and thus are worth investigating.

Both rheological and SEM analysis identified differences in the gelling process when using different radio-opaque agents. For example, while all Conray-formulated gels reached $\delta=45^o$ faster than their Omnipaque-formulated counterparts (Figure 2.4), Omnipaque gels reached their plateau viscosity sooner than Conray-formulated gels (Figure 2.5). Furthermore, SEM analysis showed

that Conray-formulated gels have a larger number of droplets, and these droplets are smaller in size than corresponding Omnipaque gels (Figure 2.8). The observed differences in rheology profiles and droplet characteristics indicate that these materials have unique gelling processes.

2.4.3 Interaction of Contrast Agents with Organic Phase

The explanation for why there are differences in the gelling process relates to the interaction of each contrast agent with the PPODA-QT organic phase. While both formulations employ the same chemical reaction that leads to network formation, the contrast agents interact differently with the organic phase. Therefore, the actual molecular composition of each contrast agent plays a major role in the gelling process.

Surfactants have been mentioned as a way to increase the stability of aqueous droplets in an emulsion. This would give rise to less droplet coalescence, increased ion flux across phase boundaries, as well as fewer observable differences in gel time and droplet size when the material is mixed for different amounts of time. The gel time of Conray-formulated gels shows much less dependence on mix time (although still statistically significantly different) than when compared to Omnipaque-formulated gels. Therefore, it is possible that iothalamate meglumine is acting as a surfactant and stabilizing small droplets, resulting in faster reaction kinetics.

The “Conray-as-a-surfactant” theory also implies that droplet size dictates gel time, because (1) droplet sizes during mixing are identical, so all ion flux that occurs during mixing is identical, and (2) after mixing, smaller stabilized droplets have greater ion flux than unstabilized, coalescing droplets, due to their greater

surface area-to-volume ratio. All differences in gel time are assumed to arise from the rate of droplet coalescence after mixing is complete. In general, Conray- and Omnipaque-formulated gels follow this rule. For gels made with the same mix time, Conray materials gel faster, have smaller pores, and have more pores than Omnipaque materials.

Table 2.1 shows average gel times and droplet sizes for Conray and Omnipaque materials, mixed for 0.5 and 1.5 minutes. While all Conray materials gel faster, the average droplet size in Conray gels mixed for 0.5 minutes is 1.28 μm^2, compared to 0.60 μm^2 for 1.5-minute mixed Omnipaque samples. This comparison shows that while the Conray-formulated materials have a larger average droplet size, they still gel faster than Omnipaque-formulated samples (11.1 ± 0.32 min vs. 22.2 ± 0.68 min). This finding conflicts with the Conray-as-a-surfactant theory, which implies that gel time is dependent only on droplet size.

Table 2.1 Gel Time and Droplet Size Comparison

Formulation	0.5 Minute Mix		1.5 Minute Mix	
	Gel Time (min)	Size (μm^2)	Gel Time (min)	Size (μm^2)
Conray pH 10.8	11.1 ± 0.32	1.27	9.8 ± 0.11	0.21
Omnipaque pH 12.2	28.5 ± 2.0	3.47	22.2 ± 0.68	0.54

Another mechanism to explain kinetic differences due to formulation involves the solubility of each initiating solution in the organic phase. A more soluble initiating solution would bring ions into the organic phase through dissolution, rather than from ion flux via droplets alone. Essentially, this works to scatter many initiation sites throughout the organic phase, and speed up the

“global” cross-linking event, in which molecules are essentially immobilized within the material.

While neither radio-opaque molecule—iothalamate meglumine or iohexol—seem to be acting as a surfactant, they do both contain hydrophilic and hydrophobic regions and therefore may be somewhat soluble in the PPODA-QT organic phase. Examining each molecule individually, it is readily apparent that dissociated iothalamate and meglumine ions are much smaller than iohexol. This size discrepancy of the radio-opaque molecules may contribute to differences in the miscibility of each contrast agent within the organic phase. Since Conray contains smaller, more easily solvated molecules, it is likely that the Conray solution is more miscible with the PPODA-QT phase than iohexol-containing Omnipaque. Conray’s heightened miscibility means that there is a greater capacity for widespread ion transfer, resulting in numerous reaction initiation sites and a more rapid “global” cross-linking event than is possible for a less miscible aqueous solution.

Furthermore, if Conray is better integrated with the PPODA-QT organic phase, it is more likely that Conray-formulated gels will retain their *in vivo* radio-opacity better than Omnipaque-formulated gels. Since iohexol is less able to integrate with the organic phase, it is present only within the dispersed aqueous phase droplets. Over time, the contents of droplets will “wash out” into the bloodstream as blood contacts the polymer. If the radio-opaque molecule is only present within aqueous droplets, such as iohexol, then the material will lose radio-opacity to some degree. The integration of contrast-providing molecules within the organic phase in Conray-formulated gels should protect against this loss of radio-opacity.

2.4.4 Effect of Solubility on the Gelling Process

During the course of mechanical mixing, it is assumed that aqueous droplets are reduced to equal sizes, regardless of material formulation. This assumption reflects identical mixing conditions. Given the small droplet sizes and convective transfer, ion flux between droplets and the organic phase is assumed to be identical, unless there are differences in the number of available ^{-}OH ions. However, solubility differences will also alter the amount of ions that reach the organic phase. A greater number of ions will contact the organic phase through dissolution of the more soluble contrast agent, which will significantly increase the number of reaction initiation sites in the organic phase.

In Omnipaque-formulated gels, there are much fewer initiation sites in the organic phase due to the low miscibility of Omnipaque in PPODA-QT. Therefore, increasing the mixing time affects gelling kinetics by creating more initiation sites through convective ion transfer. Even with longer mixing, the number of reaction initiation sites is suspected to be much less than in Conray-formulated gels.

Since fewer reaction sites are created during mixing for Omnipaque-formulated materials, ion flux from post-mixed droplets dominates the reaction kinetics. These post-mixed droplets are allowed to coalesce for a longer period of time because of the long 1^{o} region of slow material viscosity increase (Figure 2.5). During this time, the organic phase viscosity is not high enough to completely inhibit droplet coalescence, resulting in the presence of large droplets. Large coalesced droplets, paired with fewer reaction initiation sites in the organic phase, could lead to a reverse emulsion system where coalesced droplets provide “local” reaction initiation sites at phase boundaries. This

supports a slow, but prolonged initial viscosity increase, because local reaction sites may not cause the entire material viscosity to change rapidly at first.

However, as the reaction progresses, local reaction sites will eventually "link up" with other local reaction sites, resulting in rapid material viscosity increase caused by network formation (2^o region). After network formation is complete, the material viscosity reaches a maximum and the gelling process is finished (4^o region).

Comparatively, a more soluble aqueous phase shows different viscosity profile behavior. As expected, the initial viscosity increase is gradual, where monomers are reacting and droplets are able to coalesce without being hindered by high continuous phase viscosities. Due to Conray's enhanced miscibility, there are a greater number of reaction initiation sites in the bulk, which cause network formation to happen much sooner. Following network formation, there is a region of gradually increasing viscosity (3^o region), which is missing from Omnipaque gels. This gradual viscosity increase may happen as a result of the rapidness of the "global" cross-linking event. When global cross-linking occurs, all of the end-groups on PPODA and QT may not have yet reacted, but are immobilized in the cross-linked gel. After immobilization, many of the end-groups eventually react, but the speed of this process would be inhibited due to lack of mobility. The observable phenomenon here is that the rate of viscosity increase slows, resulting in the gradual 3^o region seen in Figure 2.5.

The lack of mobility of end groups after global network formation in Conray-formulated gels may also account for the fact that Conray-formulated gels do not reach as high of a final viscosity as Omnipaque-formulated gels. It is

unlikely that the same degree of cross-linking is achievable for gels that have immobilized unreacted end-groups, even if many of them do eventually react.

While this theory explains the gelling behavior of PPODA-QT gels made with Conray at pH 10.8 and Omnipaque at pH 12.2, there still may be a discrepancy regarding the number of available $^{-}$OH ions, which could potentially alter the gelling process. For example, it is possible that if the pH of Omnipaque is increased, then resulting PPODA-QT gels may also undergo a rapid “global” cross-linking event and show the same viscosity profile seen for gels made with Conray at pH 10.8. In order to verify that the viscosity profiles are distinct for gels made with each contrast agent regardless of pH, rheological analysis was performed on PPODA-QT gels made with Omnipaque at pH 13.0 mixed for 0.5 minutes. The resulting viscosity profile, displayed with curves of gels made with Conray pH 10.8 and Omnipaque pH 12.2 (both 0.5 minute mix) are shown in Figure 2.9. From this figure, it is apparent that both of the Omnipaque-formulated gels have a similar viscosity profiles even though gels made with Omnipaque at pH 13.0 gel much faster (4.3 ± 0.59 min vs. 22.2 ± 0.68 min). Consequently, neither Omnipaque curve shows a 3^{o} region of gradual viscosity increase, which is characteristic of Conray-formulated gels.

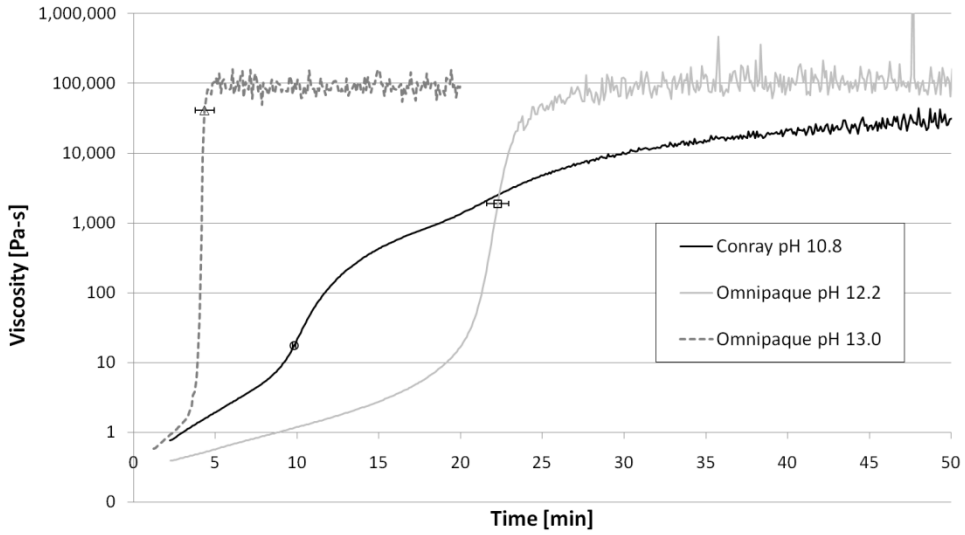


Figure 2.9 Viscosity profile of faster gelling Omnipaque formulation. PPODA-QT gels are made with Conray at pH 10.8 (black line), Omnipaque at pH 12.2 (light grey line), and Omnipaque at pH 13.0 (grey dashed line). Average gel time ± standard deviation is shown on each curve, with n=3 replicates per group.

This analysis demonstrates that the manner in which gelling progresses in PPODA-QT gels is dominated by the composition of the initiating solution rather than the amount of free $^{-}$OH ions present, even though the gel time itself is affected by the $^{-}$OH concentration.

2.5 Conclusion

These results suggest that Conray is more soluble in the PPODA-QT organic phase, allowing for widespread reaction initiation. For this formulation, reaction kinetics are dominated by processes that occur as a result of enhanced solubility of the aqueous initiating solution in the organic phase. Due to a higher number of initiation sites, the “global” cross-linking event happens sooner than

when using Omnipaque at pH 12.2. After network formation, the reaction is slowed due to immobility of the reactive end groups on each polymer. At the end of the reaction, however, the material does reach a final viscosity no more groups are able to react.

Omnipaque seems to be less soluble in the organic phase. This results in a “localized” reaction effect where viscosity increase is gradual until locally reacted sites “link up” to form a completely cross-linked gel. The “localized” reaction sites are dominated by ion flux from post-mixed, coalescing droplets. While network formation takes longer to occur in Omnipaque-formulated gels made with pH 12.2, the rate of reaction is much faster once cross-linking begins, since global cross-linking occurs after local sites have fully reacted. As a result, there are likely fewer unreacted end groups in Omnipaque-formulated gels, giving rise to their observed higher final viscosity values.

Chapter 3: IN VITRO DELIVERY, CYTOTOXICITY, SWELLING, AND DEGRADATION BEHAVIOR OF A LIQUID-TO-SOLID GELLING POLYMER SYSTEM FOR CEREBRAL ANEURYSM EMBOLIZATION

3.1 Introduction

Embolic materials are commonly used to occlude cerebral aneurysms by endovascular delivery through a microcatheter. Endovascular procedures are less invasive than open surgical aneurysm treatment and are therefore routinely applied to many types of cerebral aneurysms (Byrne, Molyneux, and Brennan 1995; Brilstra et al. 1999; Friedman et al. 2003; Henkes et al. 2004; Cekirge et al. 2006). There are a variety of embolic materials on the market including standard platinum coils, “bioactive” coils which generally contain a biocompatible polymer filling or coating (Murayama, Tateshima, et al. 2003; Cloft and Kallmes 2004; Bendszus and Solymosi 2006; Kang et al. 2007) and the precipitation-based copolymer material, Onyx® (eV3, Irvine, CA) (Murayama et al. 1998; Weber et al. 2005; Velat et al. 2008).

Platinum coil embolization is considered to be the gold standard of endovascular cerebral aneurysm treatment. However, coils have a tendency to compact in the aneurysm dome in 15-35% of treated cerebral aneurysms (Cognard et al. 1998; Cognard et al. 1999; Molyneux 2002; Murayama, Nien, et al. 2003; Raymond, Guilbert, et al. 2003; Henkes et al. 2004; Kurre and Berkefeld 2008; Ries and Groden 2009), which can lead to reperfusion of blood flow into the aneurysm and recanalization with the potential for eventual aneurysm rupture. Recanalization is especially common after treating wide-necked and giant aneurysms, with recanalization rates of 25-50% (Cognard et al.

1999; Hayakawa et al. 2000; Hope, Byrne, and Molyneux 1999) and 35-70%, respectively (Murayama, Nien, et al. 2003; Sluzewski, Menovsky, et al. 2003; van Rooij and Sluzewski 2007). Recanalization is likely related to the relatively low packing density achievable during coil placement, generally ~30% of the aneurysm volume (Piotin et al. 2000; Cloft and Kallmes 2004; Fiorella, Albuquerque, and McDougall 2006). However, packing densities are even lower for wide-necked and giant aneurysms (Kawanabe et al. 2001; Murayama, Nien, et al. 2003; Sluzewski, Menovsky, et al. 2003; Slob 2005).

The Onyx ® Liquid Embolic System can achieve higher filling percentages, but this system has other drawbacks, such as co-delivery of an angiotoxic organic solvent (dimethyl sulfoxide, DMSO) that is used to dissolve the copolymer prior to injection. DMSO has been has been associated with vessel necrosis and vasospasm when injected too quickly (Murayama et al. 1998; Chaloupka et al. 1999; Pamuk et al. 2005). As a result, Onyx must be delivered slowly. The delivery procedure requires multiple cycles of endovascular balloon inflation during Onyx injection, then balloon deflation and re-perfusion such that the organic solvent can diffuse away safely. The result is an even longer procedure time than coiling (Molyneux et al. 2004; de Gast et al. 2008) as well as increased risk of damaging the vessel wall during balloon cycling (Mawad et al. 2002). Recent studies have also reported that Onyx tends to migrate after embolization, increasing the chances of inadvertently occluding the parent artery (Molyneux et al. 2004; Piske et al. 2009; Struffert et al. 2008; Simon, Eskioglu, et al. 2010).

Calcium alginate has also been investigated as an endovascular material for cerebral aneurysm embolization (Raymond, Metcalfe, et al. 2003; Soga et al.

2004; Becker et al. 2007). Like Onyx, this material is not capable of a true liquid delivery because the components react at the end of the delivery catheter and are extruded in a strand-like form or in globular form into the aneurysm sac. It has been suggested that calcium alginate embolization therefore may work similarly to coils, where a blood clot forms around the calcium alginate “strings” to occlude the aneurysm volume. This system would also likely be prone to recanalization as is seen with coils, especially since alginate is softer and thus potentially more amenable to compaction than coils (Raymond, Metcalfe, et al. 2003). However, Becker et al. (2007) showed successful embolization after 3 months using a swine lateral wall aneurysm model. Aneurysms were embolized with alginate under balloon protection, resulting in initial complete occlusion and sustained occlusion at 3 months, with moderate thrombus formation within the aneurysm. In this study, two of the 8 aneurysms required multiple delivery attempts to achieve a complete initial fill. In these two cases, the main drawback observed with calcium alginate was that the liquid-to-solid gel transition was very rapid, and the additional amount of injected calcium alginate did not coalesce into the existing alginate mass, but produced material separation between the sequential injections, allowing blood to seep into the space (Becker et al. 2007).

An ideal embolic material for cerebral aneurysm embolization would be able to fill the entire aneurysm volume through a true liquid delivery, without co-delivery of organic solvents. Furthermore, the material should allow a straightforward, controlled, one-time delivery in which complete or near complete filling is achieved.

To address such aims, we have developed a water-based, *in situ* cross-linking polymer system for cerebral aneurysm embolization (Vernon et al. 2003;

McLemore, Preul, and Vernon 2006; Riley et al. 2011). The polymer system is composed of liquid monomers poly(propylene glycol) diacrylate and pentaerythritol tetrakis(3-mercaptopropionate), which undergo a cross-linking reaction when mixed with a basic aqueous solution. Before monomers form a cross-linked network, there is a window in which the viscosity is low enough to deliver the polymer using a non-invasive method. This material can be delivered as a true liquid, whereby the gelling reaction is completed once inside the aneurysm. This advantage allows for complete aneurysm volume filling and conformation to the aneurysm shape without the use of organic solvents.

In this work, two different formulations were examined, in which the initiating solution is a commercially available liquid contrast agent, either Conray™ or Omnipaque™ 300. Crucial for successful delivery, embolic materials must be radio-opaque for visualization during clinical delivery. Incorporating contrast agents into the PPODA-QT system fulfills this requirement. Previous work has shown that incorporating Conray™ or Omnipaque™ 300 into the PPODA-QT system produces different gelling kinetics, which has implications for deliverability (Riley et al. 2011). This study aims to identify a number of *in vitro* characteristics of gels made with different contrast agents, which will help identify an optimal formulation for cerebral aneurysm embolization. The material properties investigated here include mock delivery into an aneurysm model to assess delivery feasibility, a cytotoxicity analysis, as well as characterization of material swelling and degradation.

3.2 Materials and Methods

3.2.1 Polymer System Formulation

The polymer system consists of a liquid mixture of two organic monomers that cross-link to form a solid network through Michael-type addition chemistry, described in detail previously (Vernon et al. 2003; McLemore, Preul, and Vernon 2006; Riley et al. 2011). Poly(propylene glycol) diacrylate, ~Mw 900 (PPODA) and pentaerythritol tetrakis(3-mercaptopropionate), abbreviated QT (both purchased from Sigma, St. Louis MO), were combined with an equal number of functional groups.. The monomer components and reaction scheme are shown in Figure 3.1. Once syringe-mixed for 30 seconds to create a homogeneous phase, the liquid organic monomers are syringe-mixed with a basic initiating solution to start the reaction. In this work, commercially available injectable contrast agents were pH-adjusted with 5N sodium hydroxide and used as the initiating solution.

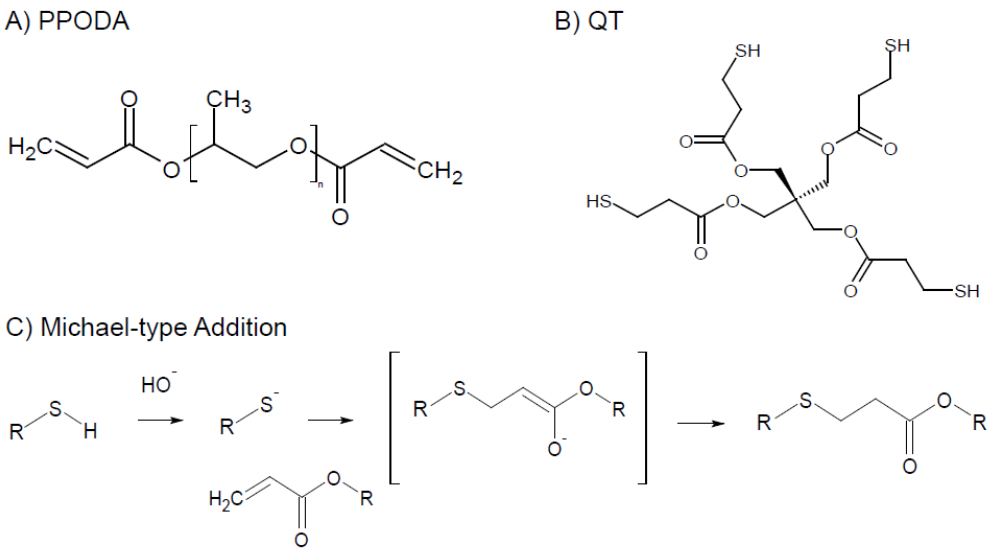


Figure 3.1 Components and reaction scheme. (A) Poly(propylene glycol) diacrylate, (PPODA) M_w≈900; (B) Pentaerythritol tetrakis(3-mercaptopropionate),

(QT) M_w≈488; (C) Michael-type addition reaction. Deprotonated thiol nucleophilically attacks acrylate group.

Conray™ (Mallinckrodt, St. Louis, MO) and Omnipaque™ 300 (GE Healthcare, Princeton, NJ) were the injectable contrast agents used in this work. Conray™ is a high osmolar, ionic contrast agent, while Omnipaque™ 300 (GE Healthcare, Princeton, NJ) is considered a low osmolar, nonionic contrast agent. Based on previous work (Riley et al. 2011), Conray™ was adjusted to pH 11.0 and Omnipaque™ 300 was adjusted to pH 12.6 in order to achieve gelling in the range of 5-20 minutes, measured by rheology. These gel times are tailorable, where increasing the pH of the contrast agent speeds the gel time. In order to obtain information about how mixing duration (mixing time) affects *in vitro* properties, a wider range of gel times (5-20 minutes) was evaluated since shorter mixing duration results in slower gel times (McLemore, Preul, and Vernon 2006; Riley et al. 2011). In a clinical setting, a faster gelling formulation (~5-10 minutes) would be more appropriate.

Sample preparation was done by first weighing and aliquoting each component. The final gel concentration was 75% (wt.) organic components and 25% (wt.) aqueous contrast agent. For the mock delivery model, components were aliquoted into 1cc syringes: PPODA=1.08 g; QT=0.293 g; contrast=0.458 g. For cytotoxicity, swelling, and degradation experiments, components were aliquoted into 3cc syringes as follows: PPODA=1.80g; QT=0.488g; Contrast=0.763g. Prior to cytotoxicity experiments, all precursors were sterilized with 0.2 µm syringe filtration, then weighed, aliquoted, and mixed in a sterile environment.

Once aliquoted, air was purged from all component syringes. The PPODA- and QT-containing syringes were mixed for 30 seconds (pre-mix) using a luer-loc syringe coupler to attach syringes. After the pre-mix, the organic mixture was pushed into one syringe, and the empty syringe was replaced with the contrast-containing syringe (either Conray or Omnipaque). Mixing in the contrast agent marked the beginning of the "mix time", chosen to be either 0.5 or 1.5 minutes. All mixing was performed at ~3 syringe strokes/second.

3.2.2 Rheological Testing

Previous studies investigating rheology of PPODA-QT formulations with Conray and Omnipaque showed low sample-to-sample variability with respect to sample gel time, with standard deviations ranging from 0.1-0.3 minutes for Conray gels, and 0.7-2 minutes for Omnipaque formulations (Riley et al. 2011). In this study, only one gel time experiment was done per formulation. Rheology was performed by an oscillatory time sweep at 22^{o}C with a constant frequency of 1 Hz and constant stress of 10 Pa. Gel time was determined to be the time when the phase angle (δ) equals 45^{o}, indicating that the sample has an equal proportion of solid-like and liquid-like properties (Vernon et al. 2000; Blakely et al. 2010; Riley et al. 2011)

3.2.3 Delivery Feasibility

In vitro delivery of the polymer system into a glass aneurysm model was performed as a "proof of concept" experiment to assess feasibility of delivery. The experimental setup shown in Figure 3.2 was designed to mimic embolization

in vivo, with the added benefit of being able to visualize the material as it filled the glass aneurysm.

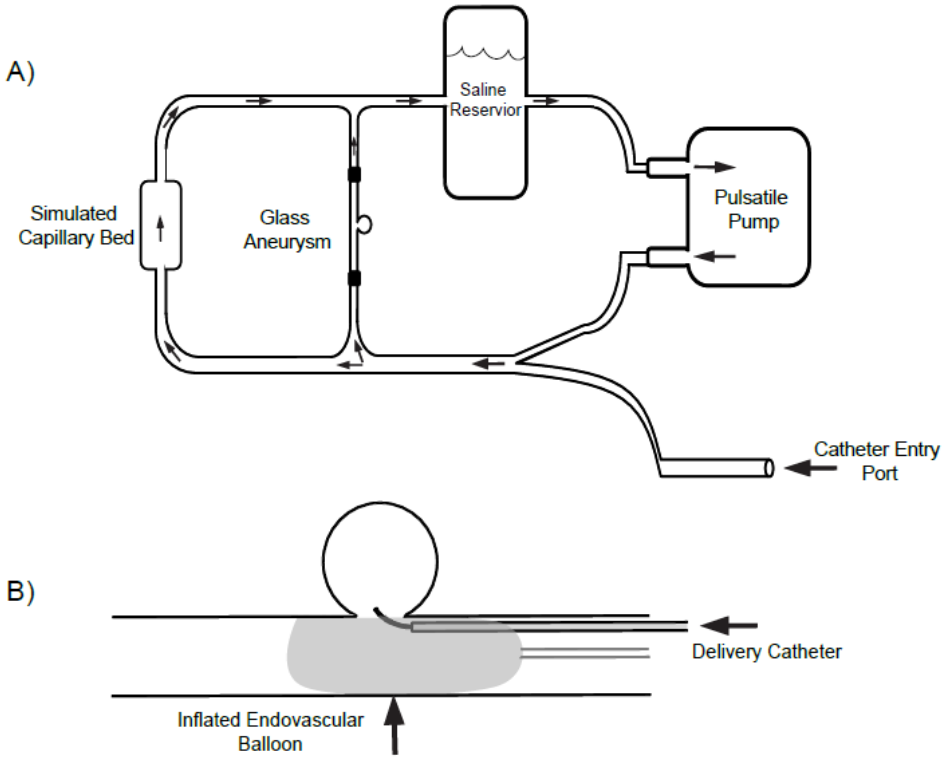


Figure 3.2 Mock delivery into a glass aneurysm model. (A) System set-up. Catheters were introduced to the system through the catheter entry port and positioned in the 10 mm glass aneurysm. (B) Prior to polymer delivery, the endovascular balloon was inflated, securing the delivery catheter tip within the aneurysm. During delivery, the balloon prevented the polymer from flowing into the glass “parent artery”, containing it within the aneurysm volume.

The glass aneurysm model consists of a sidewall aneurysm with a 10 mm diameter neck opening and a 10 mm parent vessel diameter. Before occluding the glass aneurysm, a balloon catheter (HyperGlide^{TM},Micro Therapeutics) was

inflated across the aneurysm neck to contain the polymer system prior to cross-linking, displayed in Figure 3.2B. As shown here, the tip of the delivery catheter (Renegade^{TM}, Boston Scientific) was positioned inside the aneurysm before balloon inflation. After the balloon was properly inflated, the polymer system was delivered. For this experiment, the polymer system was formulated with Conray at pH 11.0 as described above, and delivered to the glass aneurysm immediately after completing a 1.5-minute mix.

3.2.4 Cytotoxicity

The cytotoxic effects of pH-adjusted Conray and Omnipaque, both alone and within the PPODA-QT polymer system, were examined through direct and indirect cytotoxicity testing using a cell proliferation assay. 3T3 fibroblast cells were seeded in 24-well plates at 5000 cells/well for 1 day prior to exposure to experimental materials. Cells were cultured in Dulbecco's Modified Essential Medium (DMEM), supplemented with 5% calf serum, 1% L-glutamine, and 1% penicillin/streptomycin. Cells were maintained in a 37^{o}C incubator with 5% CO_2. Material precursors (PPODA, QT, and contrast agents) were sterilized in a biosafety cabinet by passing each precursor through a sterile 2-µm syringe filter before use.

A direct cytotoxicity experiment was performed to determine the effect of each pH-adjusted contrast agent on cell proliferation. For each experimental group (n=4), 50 µL of contrast was added to 1 mL of cell culture media. Treatment groups included non-pH adjusted Conray and Omnipaque (at pH 7.4), as well as Conray at pH 11.0 and Omnipaque at pH 12.6, which are the pH levels used to make PPODA-QT gels. After 3 days in direct contact, cells were assayed

using the Promega CellTiter 96® Cell Proliferation Assay performed according to manufacturer instructions, followed by reading the absorbance at 490 nm using a FLOstar Omega microplate reader (BMG Labtech; Offenburg, Germany). Positive control wells (n=4) contained cells and culture media only, while negative control wells (n=4) contained only media (no cells). The amount of 50 µL was chosen such that the amount of contrast directly contacting cells would be slightly greater than would be available from indirect gel exposure, as described in the subsequent section. Therefore, the direct contrast exposure experiment represents a “worst-case-scenario” challenge for cells when exposed to contrast agents.

In order to assess the effect of gel eluates on growing cells, an indirect assay was performed. Gels were made aseptically with either Conray or Omnipaque and mixed for either 0.5 or 1.5 minutes, as described previously. Once mixed, 0.5 mL of the mixed material was injected into 8-µm Transwell® inserts and allowed to react for 10 minutes (Conray gels) or 30 minutes (Omnipaque gels). For each experimental group (n=4) gel-containing inserts were then placed in the cell-seeded 24-well plate, such that the cell culture media contacted the inserts’ 8-µm pores. Positive and negative control wells (n=4 each) were included as previously mentioned. After 3 days of incubation with gel-containing inserts, the proliferation assay was performed.

For both the direct and indirect cytotoxicity experiment, a standard curve was created to determine number of cells in each well after the proliferation assay. Cell proliferation was represented as percent of cell viability resulting after the treatment, with all values scaled to the 100% viability of positive control wells.

3.2.5 Swelling

The swelling ratio was determined for Conray- and Omnipaque-formulated gels mixed for 0.5 and 1.5 minutes. The swelling ratio (u), shown below, is an adaptation of the swelling ratio (q) described by Vernon et al. (2004). In this work, the swelling ratio (u) represents the percent of the sample's weight increase over time when compared to the sample's original weight after formulation.

$$u = \left(\frac{W_s - W_i}{W_i}\right) \times 100\%$$

Gels were prepared with Conray and Omnipaque and allowed to solidify in the syringe for ~60 minutes before removal. All gels had a diameter of 8 mm (inner diameter of 3cc syringe). Samples were cut into lengths of ~7.5 mm and randomly divided into n=3 samples per group. Samples were placed in a 15 mL vials filled with 5 mL of 150 mM phosphate buffered saline (PBS), replaced every 2 weeks.

Time points of 1 day, 1 month, 4 months, 8 months, and 10 months were examined. Each time point contained two sets of samples, one placed at 37^{o}C to simulate body temperature and the other at 50^{o}C to represent accelerated swelling conditions. At each time point, a sample's weight was measured gravimetrically. A total of 40 sets of n=3 samples were prepared:

$$2\ Contrast\ Agents \times \frac{2\ Mix\ Times}{Contrast\ Agent} \times \frac{5\ Time\ Pts.}{Mix\ Time} \times \frac{2\ Temps.}{Time\ Pts.} = 40\ Sets$$

Each sample was weighed before placement in PBS to determine its initial weight, W_i at time t=0. At each subsequent time point, the sample weight, W_s, was measured and recorded.

3.2.6 Degradation

The same samples used for swelling determination were also used for examining degradation characteristics. Young's modulus has been shown to be directly correlated with cross-link density in the PPODA-QT system (Birdno and Vernon 2004). Calculating the Young's modulus at different times allowed us to track the progression of material hydrolysis. After measuring the weight at each designated time point, samples underwent compression testing using a Sintech 1/S load frame with a speed of 20 mm/min. Results from 50^{o}C samples represented accelerated degradation conditions.

The Young's modulus for each sample was calculated from compression data as the slope within the linear region of the resulting stress vs. strain curve. Strain and stress were calculated as follows:

$$Strain = \frac{h_0 - h}{h_0}$$

$$Stress\ (MPa) = \frac{F \times h}{A_0 \times h_0}$$

Where h_0 was the initial sample height (mm) and h (mm) was the compressed height at a given applied load. In the strain equation, F was the applied load (N) and A_0 (mm^2) was the sample's compressed surface area.

3.2.7 Statistical Analysis

A one-way analysis of variance (ANOVA) was used in cytotoxicity, swelling, and degradation experiments. One-way ANOVA was chosen over a two-way ANOVA because our previous research has shown that gels formulated with Omnipaque and Conray have different modes of reaction kinetics (Riley et al. 2011). As a result, each contrast agent and mix time combination acts more like an independent formulation as opposed to a consistent formulation where the level of a variable is changed. A significance level of $\alpha = 0.05$ was used.

For direct the cytotoxicity experiment, one-way ANOVA was used to determine if pH level of each contrast agent affected resulting cell viability. In the indirect experiment, a one-way ANOVA was used to determine cell viability differences between each gel formulation (contrast agent at prescribed mix time). A post-hoc Tukey's multiple comparison test was performed to identify individual differences, using a 95% confidence interval to determine significant differences. For the swelling experiment, swelling ratios of each formulation were compared at different time points using one-way ANOVA. This statistical analysis implies that a gel made with a specific contrast agent at a given mix time held at a prescribed temperature can be considered a unique formulation. Similarly, Young's moduli results from the degradation study were compared for different formulations at a time point using the one-way ANOVA.

For comparison of one treatment group over time (for both swelling and degradation), we also used one-way ANOVA. Had the same samples been measured at each time point, repeated-measures ANOVA would have been appropriate. However, because different samples were measured at different

time points within a unique treatment group (samples destroyed by compression testing at each time point), one-way ANOVA was used.

3.3 Results

3.3.1 Rheological Testing

Results from rheology showed that at pH 11, Conray gels have a gel time ($\delta=45^{o}$) of 6.6 minutes with a 0.5 minute mix time, and 6.0 minutes with a 1.5 minute mix time. Onmipaque gels have gelling times of 18.8 minutes when mixed for 0.5 minutes, and 13.6 minutes when mixed for 1.5 minutes.

3.3.2 Delivery Feasibility

This “proof-of-concept” experiment was done using the polymer system formulated with Conray at pH 11.0 and mixed for 1.5 minutes. This formulation represents the most challenging delivery case because it has the fastest gel time of all formulations, and thus was more likely to present delivery issues. The material was delivered to the aneurysm immediately after completing the 1.5-minute mix. The panel of images in Figure 3.3 shows delivery of the material over time. The procedure was free of complications and allowed for a one-time, continuous delivery resulting in complete filling of the model aneurysm in ~1 minute.

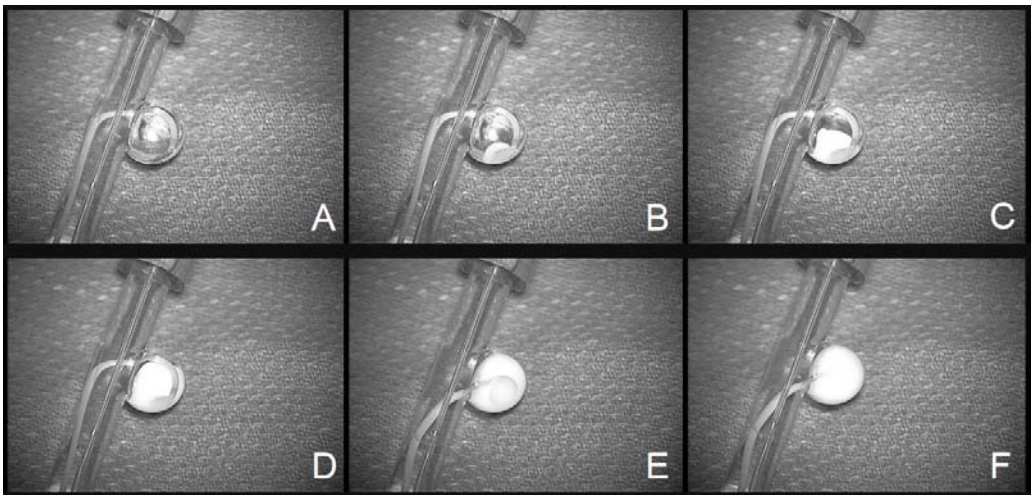


Figure 3.3 Delivery feasibility experiment. PPODA-QT gel formulated Conray at pH 11.0, and mixed for 1.5 minutes was delivered to the model aneurysm. Filling progresses in time from panels A-F, where no filling has occurred in A, and F is completely filled.

3.3.3 Cytotoxicity

Figure 3.4A shows the percent cell viability for each treatment group when 50 µL of contrast agent was added to the experimental wells. Results are reported as a percentage of the positive control cells, which were taken to represent 100% viability. Along with percent viability, representative images of each treatment group are shown at 20X magnification.

Both Conray formulations significantly hindered cell proliferation compared to the positive control, resulting in only 5.1% ± 2.1% viability when Conray at pH 7.4 was added, while the pH 11.0 Conray solution resulted in 2.2% ± 1.8% viability. Omnipaque at pH 12.6 also hindered cell growth, showing 1.1% ± 2.1% viability. When Omnipaque at pH 7.4 was added, the result was 73% ± 23% viability, statistically lower than the positive control yet significantly greater

than all other treatment groups, by Tukey's comparison with a 95% confidence interval. The corresponding 20X image shows that these cells have retained normal morphology, while cells exposed to other treatments are not growing normally.

Percent viability data for indirect gel exposure is displayed in Figure 3.4B, along with 20X magnification images of cells from each treatment group. This figure shows that while they still have significantly less proliferation than the control group, gel formulations made with Conray using a 1.5 minute mix (72% ± 14% viability) and a 0.5 minute mix (69% ± 6.0% viability) resulted in significantly greater cell viability than gels formulated with Omnipaque. Omnipaque gels mixed for 1.5 minutes produced 19% ± 15% cell viability, and the 0.5 minute mix formulation resulted in 1.1% ± 1.4% viability. Both Conray gel formulations show resulting cell morphology similar to that of the positive control cells, while Omnipaque gel formulations are rounded and have few attachments.

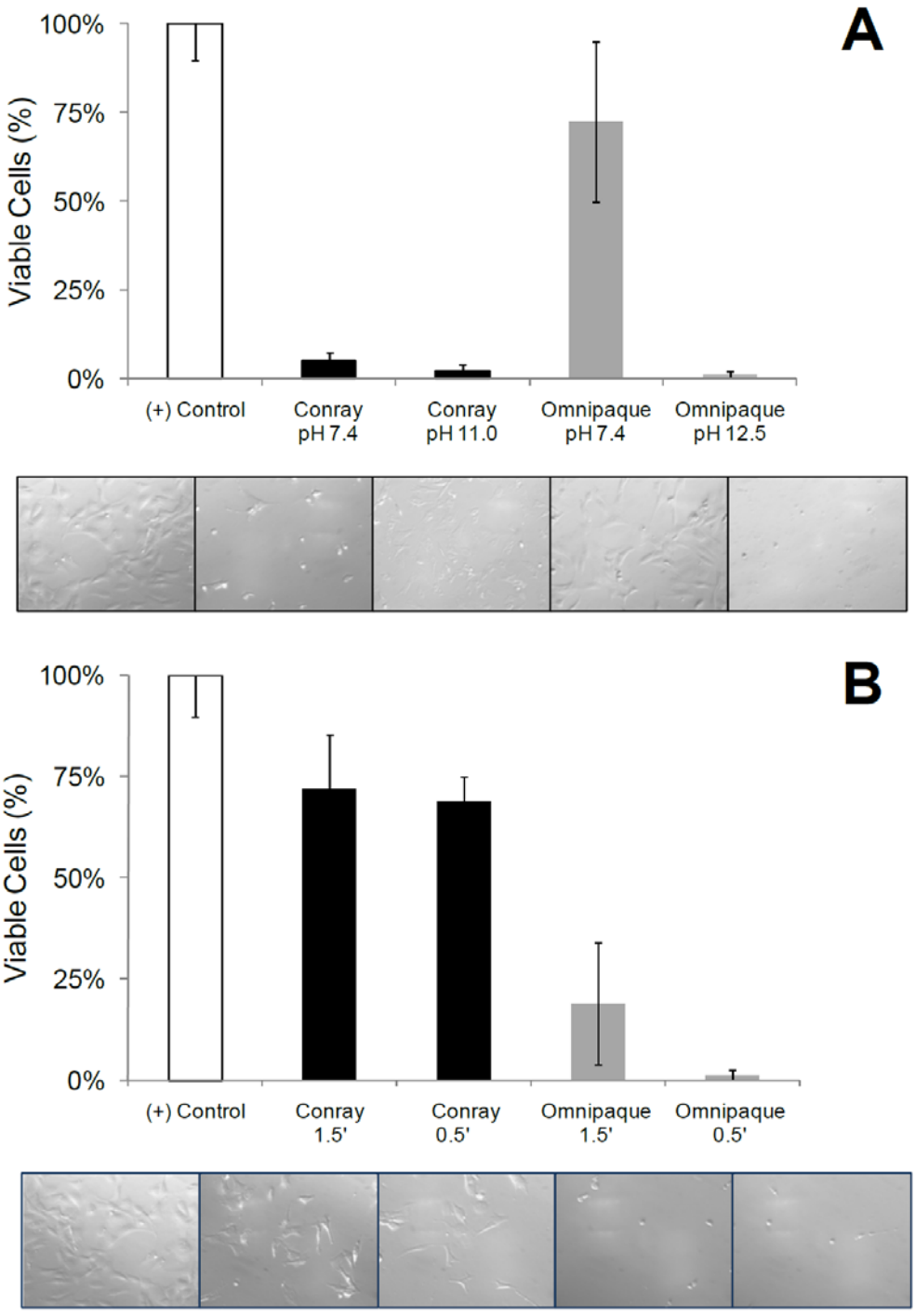


Figure 3.4 Cytotoxicity assays. Cytotoxicity assays were performed using a proliferation assay on 3T3 fibroblast cells after 3-day incubation with the treatment. The positive control group corresponds with 100% cell viability.

Assays included: (A) Direct exposure to 50 µL of a contrast agent, at physiologic pH (7.4) or adjusted to the pH level used in making PPODA-QT gels; and (B) indirect exposure to PPODA-QT gels made with high-pH contrast agents. Components were mixed, then injected into cell culture inserts with 8 µm pores. The mixture was allowed to solidify and then placed in contact with the media of growing 3T3 cells. Corresponding 20X images taken after the 3-day incubation are shown below each treatment group.

3.3.4 Swelling

The swelling ratio was calculated for gels made with Conray and Omnipaque, mixed for 0.5 minutes or 1.5 minutes. Swelling ratios were examined at two different temperatures, 37^{o}C representing physiologic conditions, and 50^{o}C representing accelerated swelling conditions. Swelling ratio was calculated as the percent increase in the polymer's weight over time, when exposed to 150 mM PBS. This polymer system takes up water over time, resulting in weight gain and swelling of the sample. Results of the swelling experiment are shown in Figure 3.5.

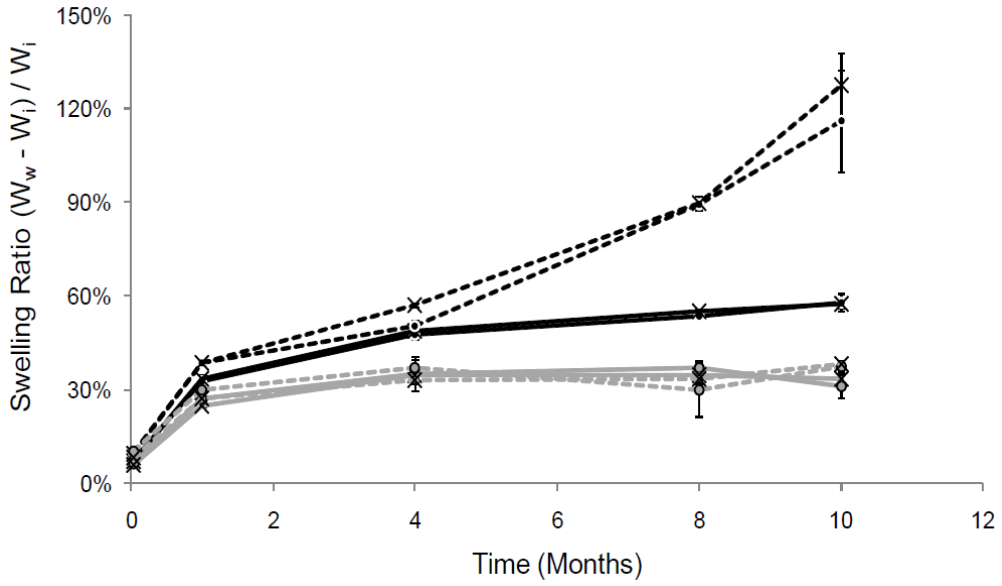


Figure 3.5 Swelling results. Swelling ratio represents the percent increase in weight of a polymer sample as a result of water uptake over time in Conray-formulated gels (black lines) and Omnipaque-formulated gels (grey lines). Solid lines represent samples maintained under 37°C conditions, while dotted lines represent samples kept at 50°C. Formulations with different mix times are distinguished by data point markers, where (o) represents a mix time of 0.5 minutes, and (x) represents a mix time of 1.5 minutes.

At one month, all polymer samples at both temperatures show similar levels of swelling, with Conray formulations clustered around ~35% weight increase, and Omnipaque formulations near ~25% weight increase. There are statistical differences between some treatment groups, but in general, Conray gels have a statistically higher swelling ratio than Omnipaque gels at 1 month.

At 4 months, the differentiation between groups is more striking. All Conray-formulated gels have statistically higher swelling ratios (~50%) than all Omnipaque-formulated gels (~35%), regardless of mix time and temperature.

Furthermore, there were no statistical differences between formulations within the Omnipaque group, indicating that temperature and mix time do not affect the swelling ratio of Omnipaque gels at 4 months. Gels formulated with Conray, however, showed statistically higher swelling ratios for formulations at 50^{o}C. The 1.5 minute mix formulation at 50^{o}C had statistically different swelling ratios than all other Conray formulations except the 0.5 minute mix formulation at 50^{o}C.

The 8-month swelling data highlights greater differences within the Conray-formulated gels, which show statistical differences in swelling between temperatures, but not mix times. Conray gels (both 0.5 and 1.5 minute mixes) kept at 50^{o}C show statistically greater weight increases (~90%) than their 37^{o}C counterparts (~54%). Similar to the 4-month data, Omnipaque gels at 8 months do not show statistical differences in swelling between formulations, maintaining a weight increase of ~35%. These statistical trends were maintained throughout the remainder of the experiment, showing that swelling of Conray gels is highly dependent on temperature, while different temperatures did not affect the swelling of Omnipaque gels.

3.3.5 Degradation

Degradation was monitored by testing the compressive strength of gel samples over time, when exposed to 150 mM PBS, both at 37^{o}C and 50^{o}C. At each time point, the Young's modulus was calculated from compression data. Young's modulus has been shown to be related to cross-link density of these gels, so tracking the Young's modulus over time serves as a proxy for monitoring hydrolytic degradation of the PPODA-QT polymer system.

Degradation data is shown in Figure 3.6. Results from samples held at 37^{o}C are shown in plot A, while 50^{o}C results are displayed in plot B. At 37^{o}C, the Young's moduli of Omnipaque gels are not statistically different from each other at all time points, at ~2.4 MPa. Conray gels at 37^{o}C show a greater difference in Young's modulus with mix time, where gels made with a 0.5 minute mix gels maintain an average Young's modulus of 2.3 MPa over time, showing no statistically significant differences over the 10-month span. The 37^{o}C 1.5-minute mixed Conray gels shown no statistical differences in Young's modulus before 10 months, with an average value of 2.0 MPa, which significantly drops to 1.4 MPa at 10 months. All samples at 37^{o}C show no statistical differences in Young's modulus at each time point until 8 and 10 months, when the Young's modulus of the 1.5-minute mixed Conray gels becomes significantly lower than that of the Omnipaque gels.

Degradation of the 50^{o}C samples is shown in Figure 3.6B. Between treatment groups, No statistical differences in Young's modulus were observed at each time point until 8 months, when the Conray-formulated gels showed a significant drop in Young's modulus (to ~0.50 MPa) compared to Omnipaque gels (~1.9 MPa), which was sustained at 10 months. This figure shows that under accelerated conditions, Conray-formulated gels tend to degrade faster than Omnipaque-formulated gels. Furthermore, mixing time does not appear to play a large part in degradation kinetics within each contrast-agent formulation.

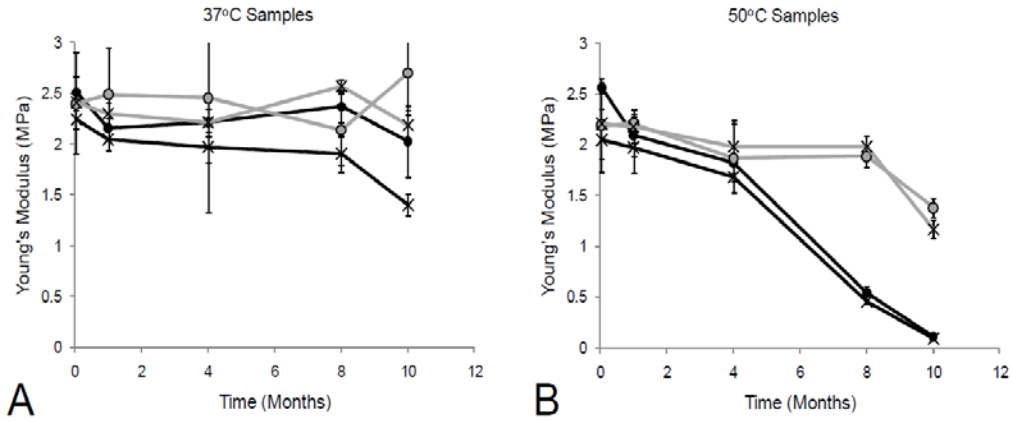


Figure 3.6 Degradation results. The Young's modulus for each formulation is shown over time, when samples were kept at (A) 37°C, and (B) 50°C. Conray-formulated gels are shown in black and Omnipaque-formulated gels are shown in grey. Formulations with different mix times are distinguished by data point markers, where (o) represents a mix time of 0.5 minutes, and (x) represents a mix time of 1.5 minutes.

3.4 Discussion

Due to the highly hydrophobic nature of the organic phase, the PPODA-QT gelling system forms a reverse emulsion when an aqueous initiating phase is introduced. At 75% of the material composition, the hydrophobic PPODA and QT monomers make up the continuous phase, while the high-pH aqueous solution is dispersed into droplets (Vernon et al. 2003; McLemore, Preul, and Vernon 2006; Riley et al. 2011). Diffusion of ^{-}OH groups from the aqueous droplets into the organic phase initiates the addition reaction between PPODA and QT, eventually leading to network formation and resulting in solidification of the material. When different contrast agents are incorporated, it has been shown that the gelling process is dependent on the contrast agent used. The underlying chemical

reactions do not change, but composition of the contrast agent results in differences in the large-scale gelling kinetics between formulations.

It was previously found that Conray-formulated gels exhibit rapid, wide-spread global cross-linking, in which network formation allows the entire material to take on solid characteristics rapidly, even though chemical cross-linking between PPODA and QT is not fully complete. Unreacted monomers are essentially immobilized in the network and eventually react with other nearby monomers, evidenced by a long period of slow viscosity increase after the network formation event (Riley et al. 2011).

In contrast, Omnipaque-formulated gels seem to first cross-link in local satellite regions, followed by a network formation event only when these regions grow large enough to connect with other locally cross-linked regions. This results in a long period of very low viscosity, in which local regions become highly cross-linked, followed by a sharp increase to the material's final viscosity when the local regions "link-up" (Riley et al. 2011).

Differences in large-scale gelling kinetics indicate that PPODA-QT gels made with either Conray or Omnipaque may also show differences in *in vitro* behavior. This study was focused on identifying certain *in vitro* characteristics of each gel formulation that have practical implications treating cerebral aneurysms. Specifically, investigation of delivery feasibility, cytotoxicity, swelling, and degradation characteristics of PPODA-QT gels formulated with either Conray or Omnipaque were performed.

3.4.1 Delivery Feasibility

Endovascular delivery is the cornerstone of this polymer system's applicability in cerebral aneurysm treatment. The material must be deliverable through a microcatheter in order to be useful. Therefore, a mock embolization experiment was performed using a glass aneurysm model. The delivery catheter was typical of those used in embolization procedures, employing a balloon catheter for parent vessel protection as would be done *in vivo* when using a liquid embolic material. From rheology, it was found that the formulation made with Conray (pH 11) mixed for 1.5-minutes would be the most challenging case because it reached the gel time faster than all other formulations, potentially being too viscous to deliver through a small catheter. During the experiment, the gel was delivered by hand (no syringe pump) immediately after the 1.5-minute mix, resulting in smooth delivery through the microcatheter, even with the 6-minute gel time. The experimental aneurysm was filled in ~1 minute.

Since the Conray formulation was easily delivered to the model aneurysm, the Omnipaque formulation would also be assumed to be easily deliverable. Subsequent mock deliveries using the Omnipaque gel formulation have proven smooth delivery as well.

3.4.2 Cytotoxicity: Implications for *In Vivo* Use

The effect of the PPODA-QT gelling system on locally growing cells is an important consideration when determining a more optimal formulation to use *in vivo*. Cytotoxicity experiments performed in this study represent more challenging conditions than would be present *in vivo*, due to incubating cells with the contrast agent or gel formulation for 3 days without replacing the cell culture media. However, this study did highlight important differences between formulations.

The direct exposure study involved placing Conray and Omnipaque, either pH 7.4 or adjusted to the level used in gels, directly into contact with growing 3T3 cells. Conray at pH 7.4 and 11.0 was shown to be highly detrimental to cell proliferation. Omnipaque, however, was only severely toxic at pH 12.6, while the pH 7.4 case showed relatively low cell cytotoxicity. These results do not mean that commercially available Conray and Omnipaque are unsafe. These results simply set a baseline of how 3T3 cells respond to Conray and Omnipaque for the purposes of this specific “worst-case-scenario” experiment.

Given the cytotoxic nature of Conray alone, one might expect that in the gel exposure study, the Conay-formulated gel would be at least as toxic as the Omnipaque-formulated gel. However, this was not observed—Conray gel formulations showed significantly less cytotoxicity than Omnipaque gels. This outcome can be attributed to the differences in large-scale gelling kinetics between the Conray- and Omnipaque-formulated gels. The rapid global network formation occurring in Conray gels works to immobilize unreacted monomers as well as the aqueous Conray phase. Therefore, most potentially toxic constitutions are initially trapped in the network and cause only limited cytotoxicity over the 3-day exposure period. Omnipaque-formulated gels do not undergo rapid, global network formation, so potentially toxic components are not immediately trapped within the gel. While local cross-linking should be occurring uniformly, it is possible that some regions, including regions in contact with the inserts, may not have completely cross-linked. These regions would not necessarily be immobilized when network formation occurs, leading to an influx of high-pH Omnipaque into cell culture media during the 3-day exposure period and resulting in extensive cytotoxicity.

The initial cytotoxicity results obtained from these experiments are a worst-case estimate of a potential *in vivo* response. However, it is possible that limiting the initial cytotoxicity is key to producing an effective aneurysm treatment. The goal of all aneurysm embolization is not only to fully occlude the aneurysm volume, but also to facilitate neointimal tissue growth over the embolic material. This physiologic "barrier" will better protect the aneurysm from blood reperfusion and thus further reduce the aneurysm's risk of future rupture compared to cases in which neointimal tissue growth is absent (Abrahams et al. 2001; Metcalfe et al. 2003; Murayama, Tateshima, et al. 2003). Therefore, a PPODA-QT gel formulation that shows better initial biocompatibility may also be better-equipped to facilitate desired neointimal tissue growth.

3.4.3 Swelling: Implications for *In Vivo* Use

Conray gels show greater swelling than Omnipaque formulations, and greater sensitivity to temperature. At 37^{o}C, the swelling of Conray gels increased by ~58% after 10 months, but increased by ~120% under 50^{o}C conditions. *In vivo*, of course, the temperature will be consistent at 37^{o}C, but the 50^{o}C case shows that Conray gels have the capability of swelling more than Omnipaque gels, which did not show much difference in swelling at either temperature, swelling by only 30%-40% after 10 months.

The differences seen here can be explained by the gelling kinetics as well. The rapid global network formation event associated with Conray-formulated gels results in immobilization of components before completely cross-linked. While many monomers do cross-link after network formation occurs, there are some that do not. Omnipaque-formulated gels undergo extensive local cross-

linking initially, so when the local sites link up to form the network, there are fewer unreacted functional groups. The degree of cross-linking in Omnipaque gels is higher than in Conray gels, which, as expected, leads to greater swelling and more temperature dependence in Conray-formulated gels.

In general, swelling is an undesirable characteristic of an embolic material for aneurysm treatment. A saccular aneurysm results from the “ballooning out” a weakened arterial region. Placement of a material into that weakened region, followed by uncontrollable or unexpected swelling, will cause a pressure increase on the aneurysm wall and may lead to aneurysm rupture. Therefore, characterizing the swelling properties of embolic materials is necessary. It is not known how much swelling is considered too much for an aneurysm, because it is likely to vary aneurysm-to-aneurysm.

Swelling was calculated as the percent increase in polymer weight over time. Polymer samples in 150 mM PBS took up water over time, increasing their mass. This weight gain can be directly equated to an increase in volume using the density of water. The increase in polymer volume over time can be related to a change in polymer dimension as well. Assuming that the polymer system is delivered to a perfectly spherical aneurysm, thus taking on a perfectly spherical shape, the expected volume increase can be used to estimate the diameter change in the swelled state. For Conray-formulated gels at 37^{o}C, the final swelling increased by ~58%. A 58% increase in spherical volume results in a 17% increase in the polymer radius. For a commonly sized 7 mm-diameter aneurysm, the new polymer diameter would be 8.15 mm in the swelled state occurring over 10 months. Omnipaque-formulated gels increased in volume by ~35% at 37^{o}C, which would result in a 10.5% increase in sphere radius. For a 7

mm aneurysm, this volume increase results in a new polymer diameter of 7.74 mm. The difference in new diameter between a swelled Conray gel (8.15 mm) and a swelled Omnipaque gel (7.74 mm) in a 7-mm aneurysm is only 0.41 mm. While this comparison does not address the issue of how much swelling is too much or quantify the osmotic pressure in the gel leading to the swelling, it does provide a way to compare the potential effects of swelling between these PPODA-QT formulations. However, the opposite effect of gel shrinkage would be detrimental for aneurysm embolization because a shrinking embolic material would facilitate aneurysm recanalization.

In this experiment, *in vitro* conditions provided a “worst-case-scenario” for testing swelling properties. Gel samples were submerged in 150 mM PBS such that water penetration occurred over the entire surface area of the samples. *In vivo*, only the portion of gel exposed at the aneurysm neck would be in contact with blood in the parent vessel—a much smaller surface area for aqueous penetration. It is conceivable that while swelling will occur to some degree *in vivo*, it may require a longer time period to achieve the swelling ratios found in this study.

3.4.4 Degradation: Implications for *In Vivo* Use

The degradation experiment performed in this work is correlated to the swelling experiment, in that at each time point the same samples were first measured to collect swelling data, then compressed for mechanical strength analysis. Therefore, one would expect to see similar trends between the swelling and degradation results for each gel formulation. At 37^{o}C, the degradation results are difficult to distinguish, with Conray and Omnipaque formulations showing

similar Young's moduli. At 10 months the gap widens somewhat, where the Conry 1.5-minute mix formulation shows lower mechanical strength. Eventually, because Conray gels have a lower starting cross-link density than Omnipaque gels, they would be expected to degrade faster. The 50^{o}C condition provides proof of this hypothesis, since this state represents accelerated degradation conditions. In this case, faster degradation of the Conray-formulated samples is observed, clearly distinguishable by 8 months.

Embolic agents are designed to be non-degradable in order to provide long term structural support within the aneurysm. Problems arise when a void is left in a treated aneurysm volume, as commonly happens when coils compact. The PPODA-QT formulations discussed here can be considered non-degradable, in the sense that they do not undergo rapid degradation in the manner of hydrogels designed specifically for fast degradation. Degradation occurring within the PPODA-QT system at 37^{o}C is slow, on the order of many months or even years. Even in the 50^{o}C case, when the Conray-formulated gels show a drop in Young's modulus from ~2.25 MPa to ~0.1 MPa over the 10-month study period, the gel itself remains intact in its original shape, albeit visibly swelled.

The degradation analysis performed here provides sound evidence that long periods of time are required to weaken PPODA-QT gels through hydrolysis. Even when significantly weakened over the course of the experiment, gels maintained their shape. Furthermore, as previously mentioned, these *in vitro* experiments provide "worst-case-scenario" conditions that lead to more pronounced and more rapid mechanical degradation than would actually be seen *in vivo*.

3.5 Conclusion

In this study, pertinent *in vitro* behavior of PPODA-QT gels formulated with Conray and Omnipaque was examined, as related to their potential use in cerebral aneurysm treatment. Delivery feasibility, cytotoxicity, swelling, and degradation behavior of different gel formulations were examined. Conray-formulated gels showed better initial biocompatibility, while also responding with greater long-term swelling and more rapid degradation when compared to Omnipaque gel formulations. Initially, biocompatibility may play a vital role in successful in *vivo* treatment, where a less cytotoxic formulation may be more capable of facilitating neointimal cell growth over the material. Swelling and degradation are longer-term considerations, since these responses are significant after months rather than days.

In general, these cytotoxicity, swelling, and degradation experiments can be considered “challenge” situations because the observed responses were more pronounced that they would likely be *in vivo*. Overall, the benefits and drawbacks of both formulations must be weighed in order to determine an optimal formulation. Initial cytotoxicity data may be more important than the longer-term swelling and degradation behavior, since those responses will be much less pronounced *in vivo,* especially if neointimal tissue growth occurs. The new tissue layer would prevent direct fluid penetration, further delaying the effects of both polymer swelling and degradation. For these reasons, Conray-formulated gels may be better-equipped to encourage *in vivo* neointimal tissue growth, which would help minimize potential concerns of excessive swelling and degradation of the polymer system.

Chapter 4: IN VIVO ANEURYSM EMBOLIZATON IN A SWINE LATERAL WALL ANEURYSM MODEL: ONE MONTH BIOCOMPATIBILITY AND DELIVERY STRATEGY ANALYSIS

4.1 Introduction

Treatment of cerebral aneurysms was revolutionized in the early 1990s, when improvements in embolic devices, namely endovascular coils, allowed endovascular embolization to become mainstream. Less invasive than surgical intervention, endovascular treatment is routine and preferred for many types of cerebral aneurysms (Byrne, Molyneux, and Brennan 1995; Brilstra et al. 1999; Friedman et al. 2003; Henkes et al. 2004; Cekierge et al. 2006). Many types of embolic materials are now on the market, including a variety of endovascular coils (Murayama, Tateshima, et al. 2003; Cloft and Kallmes 2004; Soga et al. 2004; Bendszus, Bartsch, and Solymosi 2007; Kang et al. 2007). There is only one liquid delivery system approved for aneurysms, Onyx® (eV3, Irvine, CA), which is a precipitation-based system employing ethylene vinyl alcohol copolymer (EVOH) and dimethyl sulfoxide (DMSO).

However, these materials are not ideal. Coil embolization is considered the most effective endovascular aneurysm treatment, yet coils tend to compact over time and have a significant rate of recanalization, especially after treating giant aneurysms (35-70% recanalization rate) and wide-necked aneurysms (25-50% recanalization rate) (Cognard et al. 1999; Hope, Byrne, and Molyneux 1999; Hayakawa et al. 2000; Murayama, Nien, et al. 2003; Sluzewski, Menovsky, et al. 2003; van Rooij and Slizewski 2007; Youn et al. 2010). This phenomenon is likely related to the low packing density achievable during coil placement (Piotin

et al. 2000; Kawanabe et al. 2001; Sluzewski et al. 2004; Slob, Sluzewski, and van Rooij 2005). Advancements to traditional coil technology have included a hydrogel filling or coating, designed to swell and therefore occlude more of the aneurysm space than bare coils. However, coated coils have not actually translated into consistent improvements, over bare coils (Niimi et al. 2006; Cloft 2007; White and Raymond 2008).

In principle, greater aneurysm volume filling can be achieved by liquid systems. Initial clinical experience with Onyx has shown an improvement in recanalization rates over coils for large and giant aneurysms (Molyneux et al. 2004; Piske et al. 2009). However, Onyx has significant drawbacks that make it less appealing. Onyx involves co-delivery of DMSO, which is used to dissolve the EVOH copolymer. When delivered too quickly, DMSO has been shown to induce vessel necrosis and cause vasospasm (Murayama et al. 1998; Chaloupka et al. 1999; Pamuk et al. 2005). As a result of co-formulation with DMSO, EVOH liquid must be delivered very slowly and in stages, making for a challenging delivery technique (Molyneux et al. 2004; Struffert et al. 2008).

An ideal embolic material for endovascular delivery would be able to achieve complete or near-complete aneurysm volume filling initially, which cannot be done with coils. Furthermore, an ideal embolic agent would not necessitate formulation with organic solvents, given their potential angiotoxicity. Another critical aspect of an ideal embolic agent is that it can be administered in a straightforward manner, requiring only a short, one-time delivery technique. To date, there are no such materials on the market for cerebral aneurysm embolization.

In the quest for a better embolic material, we have developed a water-based, *in situ* gelling polymer system based on poly(propylene glycol) diacrylate (PPODA) and pentaerythritol tetrakis(3-mercaptopropionate) (QT). The PPODA-QT material possesses advantages in liquid-to-solid gelling characteristics that allow for endovascular delivery with a similar degree of aneurysm volume filling as EVOH, but without using a partner system such as an organic solvent. As a result, delivery technique associated with PPODA-QT is more straightforward, without requiring the material to be delivered over prolonged stages.

The terms “delivery technique” and “delivery strategy” have very specific definitions in the context of this work. Delivery technique refers to the manner in which a liquid embolic is delivered through a catheter to the aneurysm, relating to its inherent material properties or characteristics. Delivery strategy refers to a method of aneurysm embolization, regardless of embolic agent used.

This work reports the first *in vivo* investigation of PPODA-QT in experimental aneurysm models. The aims of this study were to (1) determine the initial biocompatibility behavior of PPODA-QT in a large (i.e. human gauge) animal aneurysm model, and (2) identify a delivery strategy suitable for PPODA-QT in order to maximize the potential benefits of the material. Delivery strategies examined were: complete (100%) aneurysm filling with PPODA-QT, sub-complete (80-90%) aneurysm filling with PPODA-QT, and a combination treatment involving placement of a framing coil followed by PPODA-QT embolization.

4.2 Materials and Methods

4.2.1 PPODA-QT Formulation

Formulation of PPODA-QT involves two multi-functional hydrophobic monomers poly(propylene glycol) diacrylate (PPODA) and pentaerythritol tetrakis(3-mercaptopropionate) (QT), shown in Figure 4.1. These monomers are mixed in equimolar ratios of reactive groups. A basic initiating solution is introduced to begin Michael-type addition between PPODA and QT (Figure 4.1C). As described by Riley et al. (2011), radio-opacity can be incorporated into the polymer system through the use of a liquid contrast agent, which can replace the basic initiating solution by simply increasing the pH of the contrast agent. The optimized formulation of this polymer system was used in the experiments reported here.

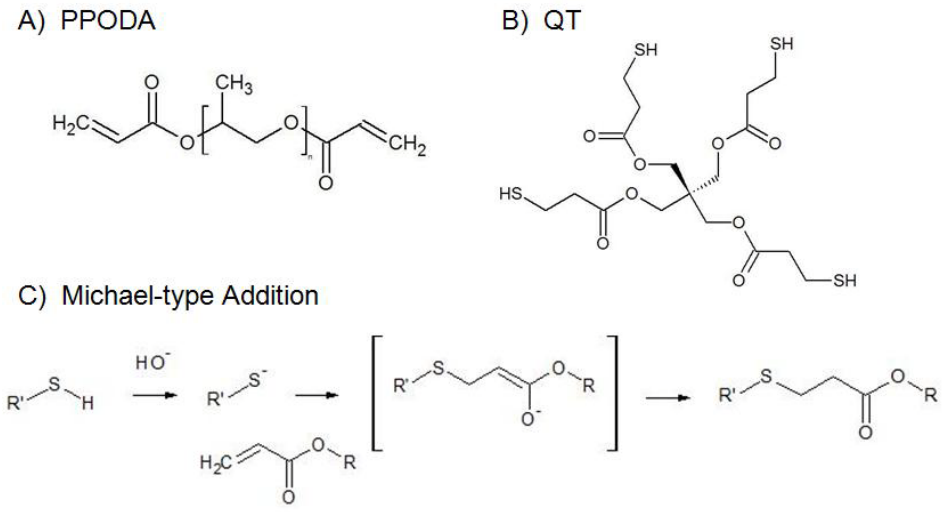


Figure 4.1 Components and reaction scheme. (A) Poly(propylene glycol) diacrylate, also called PPODA; (B) Pentaerythritol tetrakis(3-mercaptopropionate), also called QT; (C) Michael-type addition reaction. Deprotonated thiol group of QT nucleophilically attacks acrylate group of PPODA.

Poly(propylene glycol) diacrylate, Mw ~900 (PPODA), and pentaerythritol tetrakis(3-mercaptopropionate) (QT) were purchased from Sigma (St. Louis MO). The liquid contrast agent used in this work was iothalamate meglumine (Conray^{TM}, Mallinckrodt, St. Louis, MO), a high osmolar, ionic contrast agent. Conray was adjusted to pH ~11.0 with 5N sodium hydroxide (NaOH), based on parameters investigated in previous work (Riley et al. 2011). The addition of NaOH did not negatively affect radio-opacity of the solution or the resulting gels.

To begin the reaction, each component was weighed and aliquoted in a sterile environment after filtration through 0.2 µm syringe filters. Components were measured and aliquoted into 1cc syringes such that final concentrations were 75% (wt.) organic components and 25% (wt.) aqueous contrast agent. For these experiments, 0.293g of QT was syringe-mixed with 1.08g of PPODA using a luer-loc syringe coupler to attach component syringes. After 30 seconds of mixing, 0.458g of Conray at pH 11 was introduced. Conray was mixed in with the organic components for 2 minutes.

The gel time of PPODA-QT was determined using parallel plate rheology on a Physica MCR 101 rheometer (Anton Paar, Graz, Austria). A total of n=3 samples were mixed as described above, and then placed on the rheometer. An oscillation time sweep was performed at 25^{o}C with a constant stress of 10 Pa and constant frequency of 1 Hz. The gel time was taken to be the time at which the phase angle, δ, reached 45^{o}. A phase angle of 90^{o} represents a purely viscous liquid, while 0^{o} is considered an elastic solid. Therefore, a phase angle of 45^{o} represents the point at which the polymer material has an equal proportion of

solid-like and liquid-like properties, and is commonly used to define the gel point. The average and standard deviation of gel time for n=3 samples was calculated.

4.2.2 Surgical and Endovascular Procedures

The animal studies reported in this work were approved by the Institutional Animal Care and Use Committee at Barrow Neurological Institute and are in line with guidelines as set forth by the USDA, NIH, and AAALAC. Aneurysms were surgically created in the right common carotid artery of adult Yorkshire swine (~130 pounds) as previously described (Becker et al. 2007). A lateral wall carotid artery aneurysm was created by making a 10 cm incision on the right side of the animal's neck to access the right common carotid artery and external jugular vein (EJV). A 2 cm section of the EJV was removed and sewn over a 5 mm opening made in the common carotid artery in order to form the aneurysm sac. Resulting aneurysms were oval shaped with neck diameters of 5-6 mm, heights of 5-8 mm, and largest width dimensions of 7-12 mm. Animals were intubated and anesthetized with and maintained on 2% isoflurane plus oxygen during the procedure. Animals were given 3000 IU IV bolus of heparin immediately after conclusion of aneurysm construction and prior to embolization, with heparin maintenance of 500 IU IV every 30 minutes during the embolization-angiographic procedure. Post-operative aspirin was given orally at a dose of 81.25 mg/day.

Experimental aneurysms were embolized immediately after creation via a standard endovascular access procedure through the right femoral artery. Catheters were housed in an 8-French guide catheter for introduction into the vasculature. Embolization procedures were performed by first directing the filling

catheter (Renegade™ Hi-Flo, Boston Scientific) to the aneurysm and placing the tip inside the aneurysm sac. Next, the 30-mm balloon catheter (HyperGlide™, eV3) was inflated across the aneurysm neck. Upon delivery of PPODA-QT, the inflated balloon prevented the polymer from flowing into the parent vessel until solidification occurred. Procedures involving a 3D coil (CASHMERE™ 14, Micrus Endovascular) were done by depositing the coil prior to inflating the endovascular balloon, followed by balloon inflation during delivery of PPODA-QT to fill the remaining aneurysm volume.

Control procedures, either coil embolization alone or no-treatment controls, were not performed due to published reports on outcomes of such treatments and our own experience. (Becker et al. 2007). Given the exploratory and small nature of this study, it was decided that these controls would not provide enough significant new information to justify the animal sacrifice. Furthermore, our study goals were not aimed at comparing effectiveness results to other treatments.

4.2.3 Study Outline

Three different PPODA-QT delivery strategies were investigated in this study, designed to have 3 surviving animals per treatment group, with a one-month end point. Group 1: complete aneurysm filling with PPODA-QT; Group 2: sub-complete—80% to 90%—aneurysm filling with PPODA-QT; and, Group 3: placement of a 3D platinum coil followed by filling the remaining aneurysm volume with PPODA-QT. In all groups, aneurysm filling was achieved through visualization of PPODA-QT under digital subtraction angiography (DSA). For Group 2 aneurysms, the road-mapped aneurysm image was physically traced on

the viewing screen. Filling was performed such that 80-90% of the outlined angiogram (2D-image) was filled with the polymer.

In all treatment groups, material leftover in the syringe (non-injected material) was monitored to gauge its degree of solidification. While PPODA-QT has been found to react more quickly at body temperature (McLemore, Preul, and Vernon 2006), monitoring was done at room temperature to ensure the polymer was suitably solidified upon deflation of the endovascular balloon. For each procedure, the balloon was inflated for ~15 minutes, during which time the polymer was mixed, delivered, and underwent solidification.

Due to the goals of this study being determination of biocompatibility and a proper delivery mode, the gel time of PPODA-QT was purposefully set at a longer time than would be allowed for clinical use. The gel time of PPODA-QT is tailorable, meaning it can be further optimized to achieve shorter gel times, which would be more suitable in a clinical setting. However, because this was the first *in vivo* study using PPODA-QT and the goals of this study required successful material introduction, a procedure time of 15 minutes allowed enough flexibility in the delivery window during this first-time-use investigation.

4.2.4 Analysis

Outcomes from this study include each group's animal survival rate, the degree of aneurysm occlusion immediately after the procedure and at one month post-embolization, as well as the thickness of neointimal tissue growth at the polymer-vessel interface. In each treatment group, experiments were performed such that 3 animals survived to the one month time point. Survival rate was

calculated as the number of animals surviving divided by the total number of animals that underwent treatment.

The degree of aneurysm occlusion was determined using the Raymond-Roy classification system (Roy, Milot, and Raymond 2001). Class 1 indicates total obliteration, including the aneurysm neck. Class 2 means a residual neck is present, but there is no opacification of the aneurysm sac. Class 3 indicates that a residual aneurysm is present due to opacification of the aneurysm sac. The Raymond-Roy scale was used to classify angiograms at 30-minutes post-embolization and at the end of the one month survival time. In Group 2, the degree of occlusion was further quantified to determine the percentage of 2D aneurysm space filled by the polymer. A spline area calculation program was developed in MATLAB to calculate percent aneurysm filling.

Explanted aneurysms were macroscopically observed for the presence of new tissue growth in the ostium of PPODA-QT filled aneurysms, followed by histological verification. Prior to histology, Group 1 and 2 samples were fixed in 10% formaldehyde and sent to the Medical College of Georgia (Augusta, GA) for paraffin embedding, sectioning, and staining. Samples were stained with hematoxilin and eosin (H&E) and Masson's trichrome. Group 3 samples were fixed in 70% ethanol and sent to TAACH Pathology (Phoenix, AZ) for plastic embedding, sectioning, and proprietary staining. Explanted Group 3 samples were processed differently from Group 1 and Group 2 due to presence of the platinum coils, which required a different sectioning protocol. The thickness of neointimal tissue over the aneurysm neck was measured from histology images at 10X magnification. Two slides per treatment group underwent neointimal

tissue thickness measurements, with n=5 measurements per slide. Statistical analysis (one-way ANOVA) was performed between treatment groups.

4.3 Results

4.3.1 PPODA-QT Formulation

Previous studies with PPODA-QT have shown that the gel time kinetics are reproducible and have low sample-to-sample variability (Riley et al. 2011). In the current study, rheological analysis for gels formulated with Conray at pH 11.0 and mixed for 2 minutes resulted in gel times of 10.2 ± 0.5 minutes.

4.3.2 Overall Study Results

Results from the one month studies are summarized in Table 4.1. The important outcomes of this study include the animal survival rate, degree of occlusion at 30 minutes and at one month post-embolization, as well as the presence of neointimal tissue in the ostium. From Table 4.1, the most obvious concerns arise from the Group 1 survival rate of 60%. This was directly related to the delivery strategy, where the target of 100% aneurysm filling resulted in 2/5 aneurysm model failures. Further explanation of this outcome can be found in the discussion.

Table 4.1 One Month Study Results

Group		Total # Animals	Animals Surviving to1 Month	Survival Rate	Raymond-Roy Classification		Cell Layer Present (Full/Partial)	Material in Vessel? (Y/N)
					30-min	1-Mo		
Group 1	Complete PPODA-QT Fill	5	3*	60%	1, 1, 1	1, 1, 1	F, F, F	N, N, N
Group 2	Sub-Complete PPODA-QT Fill	3‡	3	100%	2, 3, 3	1, 1, 1	P, F, F	N, N, N
Group 3	Coil + PPODA-QT Fill	3	3	100%	1, 1, 1	1, 2, 1	P, P, P	N, Y, Y

***** 2 animals died in Group 1 due to aneurysm model failure 5-7 days after the embolization procedure.

‡ Another animal was initially included in this group, but died due to excessive clotting complications the same day of embolization. This response was anomalous to the study and therefore the animal was removed from the study group.

Groups 2 and 3 resulted in 100% animal survival and complete angiographic occlusion at one month. Representative angiographic images from each treatment group are shown in Figure 4.2. These images show angiograms before embolization, immediately after the procedure, and at one month post-embolization. Images of aneurysm neck regions from explanted samples are shown for representative groups in Figure 4.3, confirming the presence of neointimal tissue growth in all groups. However, more pronounced tissue coverage is visible in Groups 1 and 2 than in Group 3. Material in the parent vessel was seen only in Group 3.

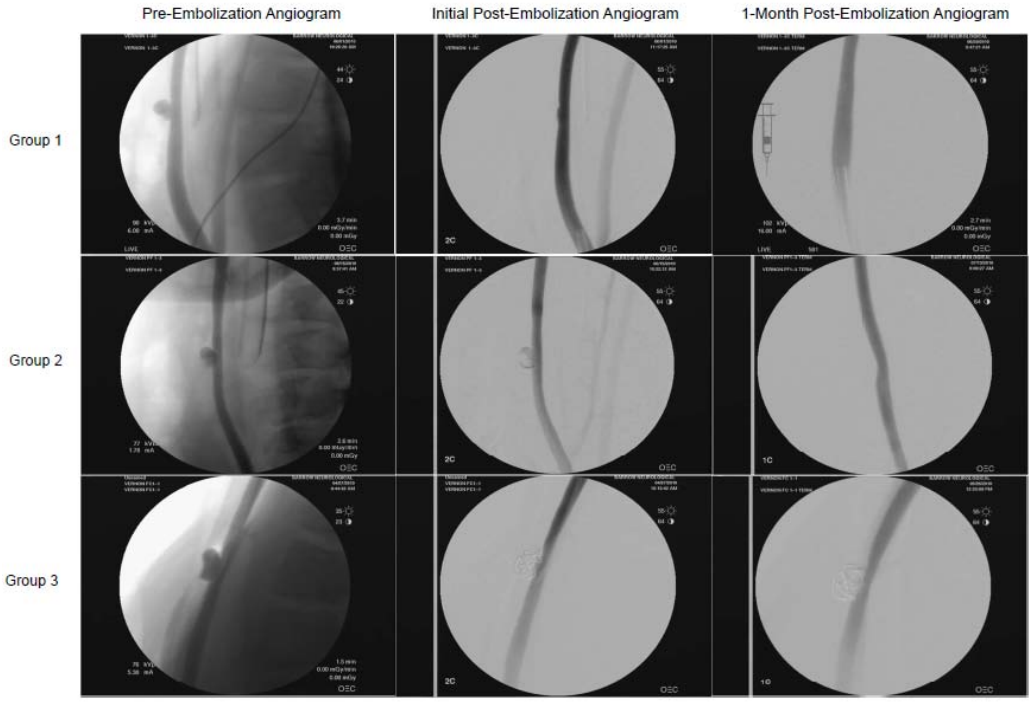


Figure 4.2 Angiographic images of experimental aneurysms. Images show aneurysms before embolization (*first column*), immediately after embolization (*second column*), and one month post-embolization (*third column*), for each treatment group.

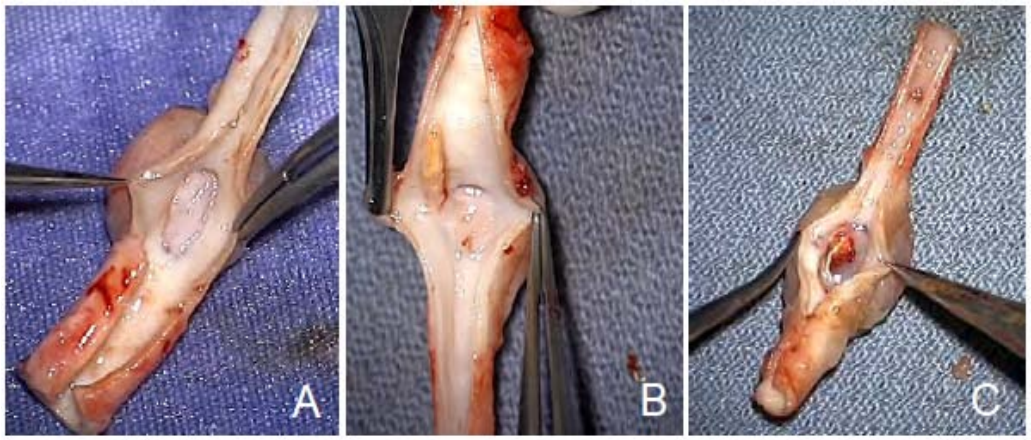


Figure 4.3 Explanted aneurysms from each treatment group. The luminal side of the aneurysm neck/vessel interface has been exposed by cutting axially along

the opposite side of the vessel wall. Neointimal tissue growth over the aneurysm neck orifice can be seen in each image. Group 1 (A) and Group 2 (B) samples showed more complete tissue over growth than observed in Group 3 aneurysms (C) by macroscopic observation.

Initial filling percentages for aneurysms in Group 2 are displayed in Table 4.2. Given the sub-complete (<100%) occlusion in Group 2 aneurysms, yet their complete angiographic occlusion at one month, PPODA-QT showed positive progressive occlusion behavior without resulting in parent artery occlusion in any Group 2 sample.

Table 4.2 Aneurysm Volume Filling Percent in Group 2 Animals

Group 2 Aneurysm	Filling % (Area)
1	87.1%
2	80.1%
3	82.0%

4.3.3 Neointimal Tissue Growth Analysis

Figures 4.4 and 4.5 show histological images from a representative Group 2 sample stained with Masson's Trichrome and H&E, respectively. These images highlight NI tissue growth in the aneurysm neck region.

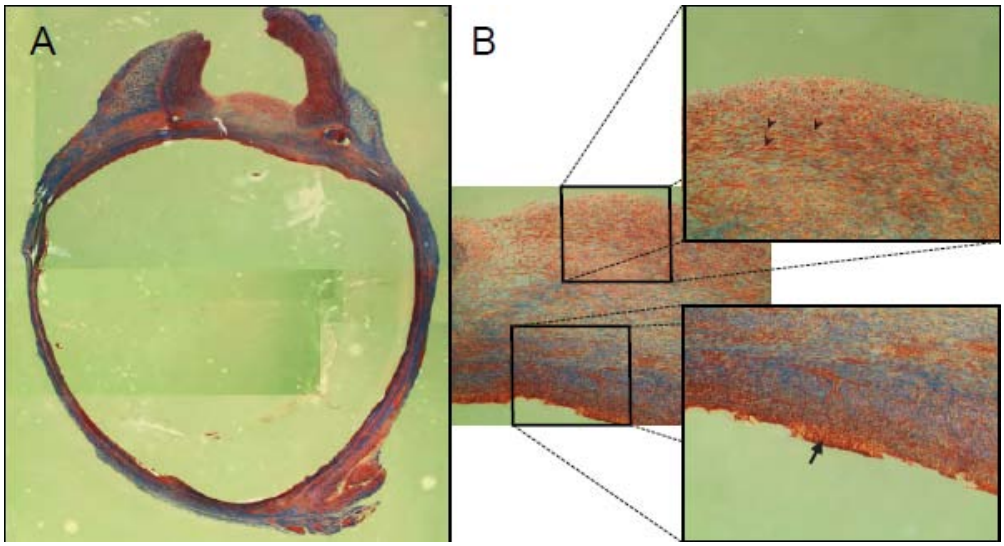


Figure 4.4 Histology image of a Group 2 aneurysm. Tissue was stained with Masson's Trichrome and viewed at 8X (A) and 50X (B) magnification. (A) Cross-sectional slice of the aneurysm has been reconstructed from a series of images at 8X magnification. The tissue "flaps" at the top of (A) make up the original parent vessel, bisected for macroscopic observation. (B) NI tissue growth in the ostium at 50X magnification. At the PPODA-QT interface (*bottom insert*), a thin layer of blood (arrow) transitions into a dense collagen layer. Tissue closer to the parent vessel interface (*top insert*) has much less collagen, but contains fibrous neointimal tissue (arrowheads).

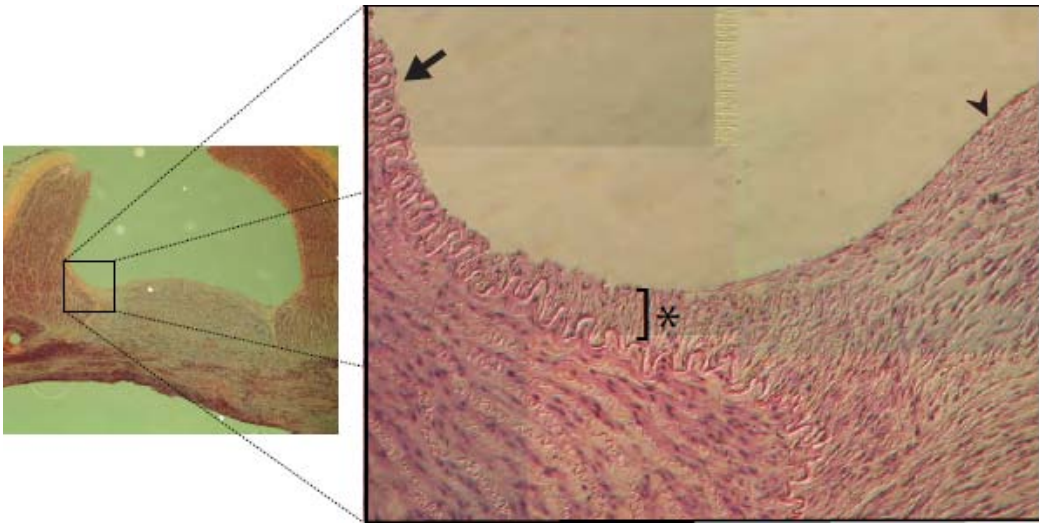


Figure 4.5 Neoendothelial layer over a Group 2 aneurysm. Sample is stained with H&E, at 20X and 200X magnification. The neoendothelial cell layer is present at the luminal interface (arrowhead). The internal elastic lamina of the parent vessel is also visible (arrow), as well as the region of initial NI tissue growth from the parent vessel over the neck region (*).

Neointimal tissue thickness measurements for each treatment group are shown in Figure 4.6. This graph shows similar thicknesses across all treatment groups, with Group 1 displaying the most variability in measurements, as a result of one replicate that showed significantly more tissue growth than others. Group average measurements are as follows: Group 1) 1.55 ± 0.88 mm; Group 2) 1.43 ± 0.51 mm; Group 3) 0.44 ± 0.38 mm.

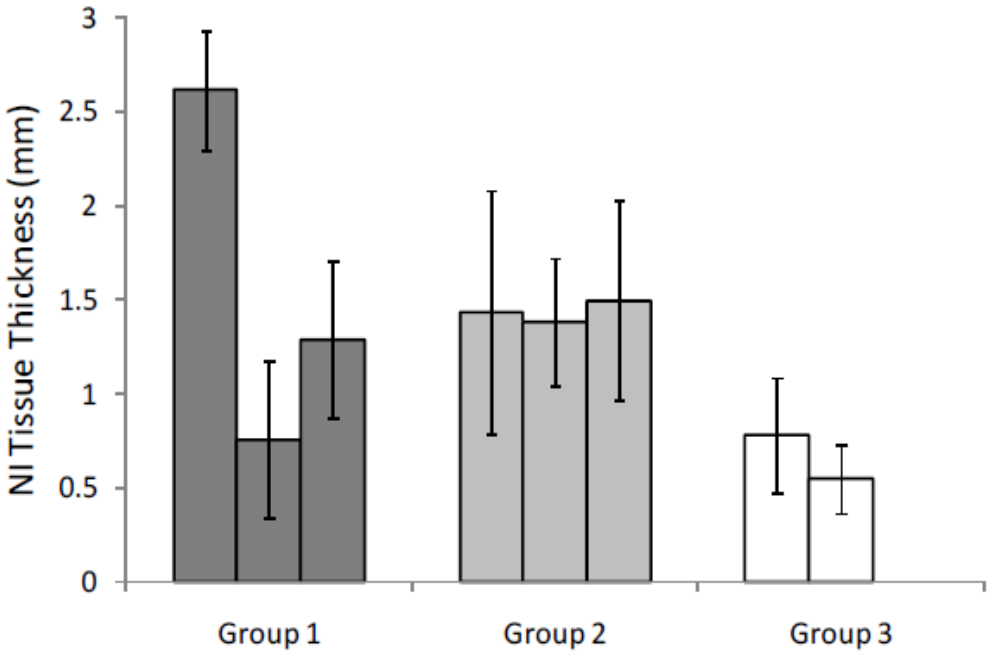


Figure 4.6 Neointimal tissue thickness measurements. A total of n=10 measurements were taken per aneurysm with 3 aneurysms per treatment group, except for Group 3. One aneurysm sample in Group 3 did not show any measurable NI tissue thickness. Average group measurements are: Group 1) 1.55 ± 0.88 mm; Group 2) 1.43 ± 0.51 mm; Group 3), 0.44 ± 0.39 mm. Statistical testing found that NI tissue thickness measurement in Groups 1 and 2 were not significantly different (p=0.53), but showed significantly thicker NI tissue growth than Group 3 (p<0.001).

Only two of the 3 explanted aneurysm replicate samples in Group 3 showed NI tissue growth over the ostium that could be measured. One sample showed little if any NI tissue over the ostium, which is shown in Figure 4.7. In fact, during tissue processing the coil ejected itself through the still patent aneurysmal ostium. This sample was recorded as not showing NI tissue

overgrowth. Statistical testing found that Group 1 and 2 were not significantly different from each other (p=0.53), but both groups showed significantly more NI tissue growth than Group 3 (p<0.001). A marked difference was noted in Group 3 in that there remained spaces and channels within the aneurysm with tenuous adhesions attached to the coil strands.

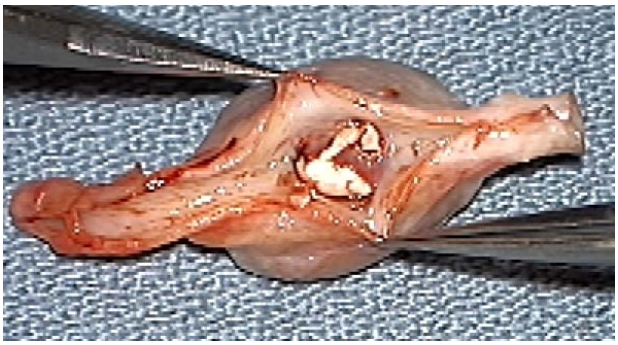


Figure 4.7 Explanted Group 3 aneurysm with no measurable NI tissue in the ostium. This sample also displays a considerable amount of PPODA-QT in the parent vessel, likely hindering NI tissue growth in the ostial region.

4.4 Discussion

4.4.1 PPODA-QT System for Aneurysm Embolization

PPODA-QT is a novel liquid-to-solid gelling material with ideal properties for cerebral aneurysm embolization. PPODA-QT transitions from a liquid into a solid by cross-linking of PPODA and QT monomers, catalyzed in the presence of free $^{-}$OH groups. In formulating the gel, sufficient $^{-}$OH levels can be incorporated by increasing the pH of an aqueous buffer. After mixing all components together, PPODA and QT begin to react, and developing first into growing polymer chains, and then into a solid network over time. The speed of reaction can be increased

or decreased by changing the pH of the aqueous solution, with higher pH leading to faster gel times. The aqueous solution used for PPODA-QT formulation was the contrast agent Conray. Once the pH of Conray was increased to an appropriate level, it was mixed in with PPODA and QT to form the gel. This Conray-formulated system provided fluoroscopic visibility of the gel *in vivo*.

PPODA-QT can be delivered through a single catheter once all components are mixed together. In-depth characterization of PPODA-QT has shown that there is a delivery window available in which PPODA-QT can be administered as a liquid. The duration of the delivery window is dependent on the material gel time, and is therefore also tailorable. As the chemical reaction progresses, the PPODA-QT gel becomes an elastic solid, showing substantial cross-linking and high viscoelastic strength (Vernon et al. 2003; McLemore, Preul, and Vernon 2006; Riley et al. 2011), capable of withstanding the forces of blood flow.

4.4.2 PPODA-QT Delivery Technique vs. Other Liquid Embolics

The PPODA-QT system is different than any other liquid embolic that has been used clinically. This study was not designed to take particular aim at Onyx, but because EVOH is the only available liquid embolic system currently available in the United States, it naturally assumes a role for comparative relevance. We also compare the delivery technique of PPODA-QT to delivery of the calcium alginate gelling system, which has been investigated previously for aneurysm embolization and used in a small number of patients (Becker and Kipke 2001; Raymond, Metcalfe, et al. 2003; Soga et al. 2004; Becker et al. 2007).

4.4.2.1 EVOH-DMSO Delivery Technique Comparison

In contrast to the EVOH-DMSO system, PPODA-QT is a self-contained gelling system. The solidification reaction used in PPODA-QT is “self-reactive” in that the catalyst (free $^{-}$OH groups) is formulated into the gelling system. With Onyx, EVOH solidifies only when DMSO is allowed to diffuse away. In this respect, Onyx uses polymer deposition to achieve formation of a solid material, while PPODA-QT chemically cross-links into a solid over time, once components are mixed.

Delivery of EVOH is performed very slowly in staged fashions, so that DMSO can diffuse away and Onyx can cure. EVOH-DMSO is injected for a period of time while the endovascular balloon is inflated, followed by periods of balloon deflation and reperfusion. This process is repeated until a sufficient amount of EVOH is delivered to occlude the aneurysm. While this technique does promote safe delivery of the copolymer, it results in a technically challenging procedure and prolonged procedure times, with an average of about 95 minutes, as reported in the CAMEO trial (Molyneux et al. 2004; Struffert et al. 2008).

4.4.2.2 Calcium Alginate Delivery Technique Comparison

Similarly to the EVOH-DMSO system, calcium alginate is a precipitation-based system, composed of a naturally occurring copolymer (alginate) that cross-links to form a gel matrix in the presence of divalent ions, such as calcium. This gelling system was first investigated for endovascular delivery by Becker and Kipke (2001). In order for the material to gel *in situ*, the liquid alginate precursor must be co-delivered with an ionic solution. Aqueous calcium chloride

($CaCl_2$) was employed for this purpose. Co-delivery of alginate and $CaCl_2$ was accomplished by using a double-lumen catheter so that components did not react prior to leaving the delivery catheter. Once mixed, however, Ca^{2+} integrated into the alginate, resulting in rapid cross-linking (Becker et al. 2005; Becker et al. 2007). While the advantages of this system include low material toxicity and formation of a “tissue-like” gel, it has inherent delivery challenges as well.

From our experience with calcium alginate, although biocompatibility aspects were favorable, the rapid cross-linking of this material is problematic for delivery purposes. Because cross-linking happens so rapidly, filling the aneurysm was the result of a “one-shot” injection. Although in most instances the experimental aneurysms were filled well, problems with incremental alginate delivery occurred upon the delivery of additional gel to an existing gel mass. The new alginate gel mass did not adhere to the already-delivered and cured gel. This outcome proved troublesome because blood was able to perfuse the spaces between alginate pieces, resulting in dislodgement of the alginate pieces (unpublished data).

The PPODA-QT system, as already discussed, does not require or even permit incremental delivery. Because the gelling process of PPODA-QT is self-contained, the aneurysm can be filled while the material is still in liquid form, which is impossible with calcium alginate and EVOH. As long as it is within the delivery window, PPODA-QT can be injected through a small catheter continuously, with the parent vessel under balloon protection as the liquid fills the aneurysm volume.

In this study, PPODA-QT delivery was completed in 1-2 minutes in all animals. Because PPODA-QT has proven to have reproducible gelling kinetics

(Riley et al 2011), it solidifies in a predictable time frame. Furthermore, this time frame can be tailored to the needs of the procedure by adjusting the pH of the aqueous component. The uncomplicated delivery technique associated with PPODA-QT, as well as the absence of organic solvent delivery, may enhance clinical acceptance of this liquid embolic material.

4.4.3 Study Goals and Controls

With these properties indicating an improvement in delivery technique over currently available liquid embolics, we aimed to evaluate initial biocompatibility as well as direct the future delivery strategy of PPODA-QT through this small scale study. This study was designed to assess two specific outcomes. The first study question was: does PPODA-QT show good initial biocompatibility in an *in vivo* aneurysm model? The second study question was: given its straightforward delivery technique, are there specific strategies for PPODA-QT delivery that stand out as potentially better or worse for future clinical use?

One important note regarding study goals is that this first-time *in vivo* study was not meant to evaluate efficacy of the PPODA-QT compared to other treatment methods. There are not enough study subjects to make statistically valid comparisons to other studies, and we believe the swine animal model itself is not preferable for testing long term efficacy, although it is acceptable for short term survival studies in which material handling characteristics are evaluated at human gauge. We do report outcomes, such as occlusion scores and NI tissue thickness measurements between treatment groups, but these are tools for comparing between delivery strategies within this particular study.

Along these same lines, control groups were not deemed necessary. Previous studies, including our experience, have shown outcomes from untreated experimental lateral wall aneurysms, as well as coiled experimental aneurysms in swine, providing the necessary control information without having to use additional animals. These previously published reports and our experience indicate that experimental lateral wall aneurysms in swine are unstable when left untreated, being prone to either spontaneous thrombosis or rupture (Guglielmi et al. 1994; Byrne et al. 1997; Becker et al. 2007). Conversely, coiled aneurysms in the swine model have previously shown variable to moderate neointimal tissue coverage over time frames similar to our one-month study (Byrne et al. 1997; Murayama, Tateshima, et al. 2003).

4.4.4 PPODA-QT Containment within Model Aneurysms

One of the main concerns neurointerventionalists have with using any liquid embolic material is the possibility of material escaping out of the aneurysm and blocking arteries downstream, without the ability to retrieve the material once it escapes. This is a valid concern due to the serious and potentially fatal consequences of such an event. However, the Onyx Liquid Embolic System has been used clinically since the early 2000s. Reported rates from large-scale clinical studies indicate that Onyx has comparable complication rates to endovascular coiling when comparing similar aneurysm populations (Molyneux et al. 2004).

In our study, we report no instances of liquid PPODA-QT escaping past balloon protection before material solidification. The lateral wall aneurysm model used in this study is not representative of the tortuous vessel geometry that can

be associated with pathological aneurysms, but successful containment of PPODA-QT shown in this study may at least satisfy initial safety concerns.

4.4.5 Initial *In Vivo* Biocompatibility

Biocompatibility of PPODA-QT gels could have been analyzed using any *in vivo* model, but due to the parallel interest in assessing different delivery strategies with near human gauge, the lateral wall carotid artery aneurysm model in swine was used. Previous studies with PPODA-QT in a swine AVM model have shown good biocompatibility (McLemore, Preul, and Vernon 2006), so this study served to reconfirm positive biocompatibility outcomes in a swine aneurysm model.

One month after embolization, macroscopic observation and histology analysis showed that PPODA-QT had no untoward effects on local tissue in the aneurysm region (Figure 4.4 and Figure 4.5). The swine model also allowed assessment of biocompatibility in terms of neointimal tissue response. Because swine are known for their aggressive clotting cascade and adept aneurysm healing after almost any embolic treatment, we were able to estimate potential toxicity of the PPODA-QT system by its affect on NI tissue growth. Groups 1 and 2, in which PPODA-QT alone was delivered to aneurysms, showed a similar level of NI tissue thickness (Figure 4.6) as achieved with other embolic agents in the swine aneurysm model (>1000 μm) (Bouzeghrane et al. 2010). These results indicate that PPODA-QT does not hinder NI tissue growth in the neck region and therefore displays good biocompatibility at one month post-embolization.

4.4.6 Delivery Strategy Analysis

4.4.6.1 Group 1: Complete Fill

Complete obliteration of an aneurysm is the overarching goal in embolization treatment. One of the benchmarks used to assess an embolic material is its ability to completely prevent blood flow into the aneurysm immediately after delivery. This benchmark is monitored for all embolic agents, especially emerging therapies (Gallas et al. 2005; Cloft 2006; Bendszus, Bartsch, and Solymosi 2007; Geyik et al. 2007; Piske et al. 2009). Therefore, one of the delivery strategies used for PPODA-QT in this study was 100% aneurysm volume filling by the polymer.

Group 1 (n=5) consisted of experimental aneurysms that were completely (100%) filled with PPODA-QT. The delivery technique involved continuous injection (1-2 min.) with an endovascular balloon inflated across the aneurysm neck until the aneurysm was completely filled. Once PPODA-QT was considered gelled, the balloon was deflated. Initial angiography in all Group 1 aneurysms showed total occlusion. While histology results of surviving animals showed excellent biocompatibility and considerable NI tissue growth, the main concern with this group was the low survival rate.

In order to compare outcomes and histology results between all treatment groups in this study, 3 animals in each group were required to survive to the one month time point. However, during the study, two animals in Group 1 died prematurely, within 5-7 days of the embolization procedure. Two additional animals were placed in this group, which survived to one month, making the total number of animals in this group 5 instead of 3.

Autopsy of the two non-surviving animals showed that the aneurysm model had failed, but PPODA-QT was still in place within the aneurysm. This means that the material did not dislodge from the aneurysm and flow upstream and therefore permit blood flow to rupture the unfilled aneurysm. Instead, there is evidence that these two aneurysms failed as a result of overfilling aneurysms with PPODA-QT, rather than blood re-perfusion. If blood re-perfusion caused experimental aneurysms to rupture, the initially sub-completely filled aneurysms of Group 2 would have also showed rupture problems, but this was not the case.

Overfilling experimental aneurysms is a likely cause of the observed aneurysm model failure in two of 5 Group 1 animals. Simply overstretching the surgically created aneurysm during PPODA-QT filling could have caused failure. During delivery, the endovascular balloon is inflated across the aneurysm neck to prevent PPODA-QT from escaping before it solidifies. Blood inside the aneurysm can escape around the balloon, but the more viscous polymer cannot. When attempting to fill the aneurysm to 100% capacity, there is a likelihood of stretching the surgically created aneurysm sac, which is a portion of the compliant external jugular vein, such that the stretched aneurysm volume is greater than the volume originally meant to be occluded. Because the endovascular balloon is physically keeping PPODA-QT within the aneurysm, the material is allowed to solidify even though the aneurysm walls are in an over-stretched state. The perpetually stretched state of the surgically created aneurysm walls may result in tearing of the tissue. This would result in what looks like a ruptured aneurysm, because the “aneurysm” walls did in fact tear.

If this sequence of events caused animal death in these two cases, the overstretching problem may be limited to experimentally created aneurysms. The

external jugular vein is more compliant than normal carotid arteries, and would likely be much more compliant than a pathological aneurysm. A pathological aneurysm is less likely to stretch, due to disruption of its internal elastic lamina (Meng et al. 2007), so complete angiographic occlusion would be seen before any stretching is allowed to occur.

However, one concern with pathological aneurysms is that they may be very weak. It has been suggested that the intra-aneurysmal pressure (IAP) during delivery may be high enough to actually rupture the aneurysm. This is a concern when delivering any embolic device under balloon occlusion. Murayama et al. (2000) analyzed IAP during Onyx delivery in a swine model with balloon occlusion over the aneurysm neck and found a spike in IAP when 100% filling was attempted. However, they did indicate that sub-total occlusion of the aneurysm volume (80-90% filling) resulted in alleviation of high IAP during filling, even with the balloon in place.

Another potential concern is the swelling behavior associated with PPODA-QT gels. In previous studies (described in Chapter 3) we have reported that PPODA-QT shows moderate *in vitro* swelling behavior at 37^{o}C. Over the first 7 days in a physiologically simulated environment (150 mM phosphate-buffered saline, PBS), PPODA-QT gels tend to swell by ~15% in volume. For a theoretical 7-mm aneurysm that is perfectly spherical, this 15% volume increase actually results in a spherical diameter increase of 0.33 mm (increasing from 7.0 to7.33 mm). It is unlikely that a 15% increase in PPODA-QT volume alone is enough to rupture an experimental aneurysm, especially one made of the compliant external jugular vein. Furthermore, this volume increase occurring over 7 days was measured under benchtop conditions, which are likely overestimates of

swelling that would occur *in vivo*. However, if the aneurysm is initially completely filled such that the aneurysm is stretched, subsequent gel swelling of 15% could cause rupture. Results from this experimental group highlight the potential safety concerns of filling aneurysms to 100% capacity with any material that may swell or expand.

4.4.6.2 Group 2: Sub-complete Fill

Previous studies with Onyx have shown that initially occluding 100% of the aneurysm volume may not be necessary to achieve complete aneurysm healing. A number of clinical reports have indicated that progressive volume occlusion of an aneurysm can be achieved with Onyx over time (Molyneux et al. 2004; Piske et al. 2009). In our experience with calcium alginate in swine an canine models, sub-100% filling of the aneurysms resulted in excellent aneurysmal ostium healing. Taking this into consideration, this study also assessed the effect of sub-complete aneurysm volume filling with PPODA-QT, in order to determine if progressive aneurysm occlusion was possible. Of course, the swine model is known for its aggressive clotting cascade and robust healing after experimental aneurysm embolization (Dai, Ding, et al., “Histopathologic,” 2005; Kadirvel et al. 2007; Raymond et al. 2007), but this model afforded proof-of-concept information regarding whether progressive occlusion was possible or not.

Group 2 aneurysms were filled with PPODA-QT such that 80-90% of the aneurysm volume was filled when visualized by DSA. All aneurysms in this group were completely obliterated by one month, and showed complete neointimal tissue growth over the aneurysm ostial regions. Results from this group indicate

that progressive occlusion of an aneurysm is achievable with PPODA-QT, even when some residual contrast filling of the aneurysm was seen immediately post-embolization. Along with NI tissue thickness measurements, these aneurysms showed successful healing.

Histology indicated that initially, blood does clot in response to contact with PPODA-QT (also seen in surviving Group 1 aneurysms), but this clot is mild and replaced by a fibrous capsule at the polymer interface by one month. Closer to the parent vessel, neointimal tissue becomes more organized and presents a neoendothelial cell layer at the parent vessel interface. While a mild clotting response is observed in response to the polymer, the clot was not problematic, did not propagate in the vessel, and tissue reorganization apparently worked to seal off the aneurysm from the vascular system resulting in a favorable healing surface at one month.

Overall, the lack of undesirable outcomes resulting from Group 2 aneurysms indicates that sub-complete occlusion with PPODA-QT may be a suitable delivery strategy for cerebral aneurysms. Given the size and nature of this study, no definite conclusions can be drawn, but results reported here at least suggest that initial sub-complete occlusion is worth exploring for future delivery of PPODA-QT.

4.4.6.3 Group 3: Coil and Polymer Fill

The final treatment group studied was a combination treatment including a single 3-dimensional platinum coil, followed by filling the remaining aneurysm volume with PPODA-QT. Initial studies with Onyx and coil combination treatments have indicated generally positive occlusion results, but not

necessarily reduction in the occurrence of Onyx migration (Murayama et al. 2000; Cekirge et al. 2006). Other investigational liquid embolics have employed this delivery scheme because the material itself is not adhesive or lacks flow-resistance (viscoelastic strength)—such agents may perform better with a coil scaffold to anchor the material so it does not wash out of the aneurysm (Becker et al. 2007; Takao et al. 2009). While PPODA-QT gels show suitable viscoelastic strength to be used in aneurysm embolization, we wanted to examine the framing coil plus PPODA-QT combination treatment in order to determine if this delivery strategy is advantageous.

Group 3 consisted of first placing a 3D framing coil into an experimental aneurysm, followed by attempting to completely fill the remaining space with PPODA-QT. Estimation of volume of PPODA-QT added to the aneurysm was difficult because of the radio-opacity differences between the coil and gel. Platinum coils are more radiographically dense than the PPODA-QT, which made PPODA-QT visibility difficult during delivery, a phenomena commonly when using a combination of coils and liquid embolics (Murauama et al. 2000; Takao et al. 2009). Furthermore, addition of PPODA-QT after coil placement may have resulted in adherence to the coil and less PPODA-QT penetration into the remaining aneurysm space, reducing the occurrence of aneurysm stretching. As a result, we believe the volume of PPODA-QT instilled was over-estimated during the procedure, and overfilling was not occurring.

Although this delivery strategy resulted in 100% animal survival, 2 of 3 aneurysms in Group 3 showed PPODA-QT present in the parent vessel upon sample explantation. This occurrence may be attributed to the coil acting as a “scaffold” for the polymer upon delivery. Takao et al. (2009) described the

scaffolding action of coils when delivering a thermo-gelling liquid polymer to an aneurysm, in which the thermo-gelling polymer adhered to coils first, allowing a faster and easier delivery. Upon contacting the coil, PPODA-QT may have stuck onto the metal, as alluded to previously. In the ostium, polymer adherence to coils could lead to PPODA-QT displacement into the parent vessel.

In this study, while PPODA-QT migration was observed when used in conjunction with a coil, there were no instances of material migration when PPODA-QT was used alone. Migration into the parent vessel is one of the most common problems with Onyx embolization, occurring both alone and when used with a framing coil (Murayama et al. 2000; Struffert et al. 2008; Piske et al. 2009; Simon, Eskioglu, et al. 2010). Although the small animal numbers prevent firm conclusions, the absence of PPODA-QT migration when used alone has positive implications for using this material clinically in the future.

4.5 Conclusion

The experiments performed in this study were aimed at determining initial biocompatibility of PPODA-QT in an appropriate large animal aneurysm model, as well as experimenting with different delivery strategies in hopes of providing insight for future use of the polymer. These one-month studies indicated that PPODA-QT has favorable biocompatibility, given its ability to facilitate NI tissue overgrowth in a swine aneurysm model.

Analysis of different delivery strategies provided valuable insight regarding potential concerns with using PPODA-QT, yet also unveiled a delivery strategy suitable for the polymer system. Filling experimental aneurysms to 100% capacity proved detrimental, given the propensity for aneurysm model stretching,

which resulted in failure of the experimental aneurysm in two of 5 cases, likely due to over-stressed aneurysm walls. Placement of a framing coil followed by aneurysm volume filling with PPODA-QT may counter the over-stretching problem, but resulted in the presence of excess PPODA-QT in the parent vessel in two of 3 samples. Sub-complete filling, to 80-90% of the aneurysm volume, resulted in the best filling strategy. Sub-completely filled aneurysms showed progressive occlusion, resulting in complete obliteration one month after embolization. It is possible that sub-complete filling of an aneurysm after 3D coil placement would minimize excess PPODA-QT in the parent vessel, yet still allow for progressive occlusion, but requires discriminatory visibility of materials during the procedure.

This study successfully examined biocompatibility and delivery strategy when using PPODA-QT to embolize experimental aneurysms. However, this study did not assess long-term efficacy. In order to evaluate the initial effectiveness of PPODA-QT, a more clinically appropriate animal model must be employed, such as a canine aneurysm model (Raymond, Metcalfe, et al. 2003, Bouzeghrane et al. 2011). Furthermore, survival duration should be longer in order to capture data on healing and recanalization (Sluzewski, van Rooij, et al. 2003). These initial studies indicate that PPODA-QT holds potential material advantages in a clinical realm where few similar materials have been successfully investigated, let alone employed for patient benefit.

Chapter 5: IN VIVO ANEURYSM EMBOLIZATION IN A CANINE LATERAL WALL ANEURYSM MODEL: A 6-MONTH PILOT STUDY

5.1 Introduction

The use of liquid embolics for cerebral aneurysm embolization has recently gained clinical acknowledgement. A liquid embolic material can be delivered through a microcatheter endovascularly, providing a non-invasive treatment option. Endovascular delivery of platinum coils is considered the “gold standard” of non-invasive cerebral aneurysm treatment, but coils tend to compact inside the aneurysm dome resulting in re-perfusion of the aneurysm in roughly 15%-35% of cases (Cognard et al. 1998; Cognard et al. 1999; Molyneux 2002; Murayama, Nien, et al. 2003; Raymond, Guilbert, et al. 2003; Henkes et al. 2004; Kurre and Berkefeld 2008; Ries and Groden 2009).

Liquid embolics have the advantage of achieving greater aneurysm volume filling than coils, because a liquid can conform to the contours of an aneurysm and the degree of filling is not limited by spatial and physical hinderances as it is with coils (Murayama et al. 2000; Mawad et al. 2002; Tamatani et al. 2002; Molyneux et al. 2004). Liquid embolics may be particularly useful for large/giant aneurysms as well as wide-necked aneurysms, which have recanalization rates after coil embolization between 35-70% and 25-50%, respectively (Cognard et al. 1999; Hope, Byrne, and Molyneux 1999; Hayakawa et al. 2000; Murayama, Nien, et al. 2003; Sluzewski, Menovsky, et al. 2003; van Rooij and Sluzewski 2007; Youn et al. 2010).

During delivery, a liquid embolic requires the use of balloon protection across the aneurysm neck in order to prevent the material from flowing out of the

aneurysm. After delivery, the material must transition from a liquid to solid in order to occlude the aneurysm and prevent further blood flow into the defect.

The only clinically available liquid embolic on the market is Onyx® (eV3, Irvine, CA), a precipitating copolymer system consisting of ethylene-co-vinyl alcohol (EVOH) and the organic solvent dimethyl sulfoxide (DMSO). The copolymers are dissolved in DMSO so that the system can be used endovascularly. Injection of DMSO has been linked to angiotoxicity and vasospasm when delivered too quickly, a serious drawback to using this material (Murayama et al. 1998; Chaloupka et al. 1999; Raftopoluos et al. 2000; Jahan et al. 2001; Pamuk et al. 2005).

While initial studies with Onyx have shown reduced recanalization rates for large, giant, and wide-necked aneurysms, there have been recent reports of uncontrolled migration of EVOH into the parent vessel, heightening the risk of parent artery occlusion (Molyneux et al. 2004; Struffert et al. 2008; Piske et al. 2009). In some instances, the cause of migration has been attributed to continued delivery of EVOH after injection has ceased (the “toothpaste effect”), resulting in delivering more material than intended (Molyneux et al. 2004).

The Onyx delivery procedure requires the material to be delivered in stages, where EVOH is injected for a short period of time with the balloon inflated, followed by balloon deflation so DMSO can diffuse away. Onyx delivery is resumed when deemed safe and the EVOH mass has sufficiently solidified, and the process is repeated until a sufficient amount of EVOH is delivered (Mawad et al. 2002; Molyneux et al. 2004). This delivery technique is not only extremely technical (Song et al. 2004; Simon, Eskioglu, et al. 2010), but it is also associated with very long procedure times, with a reported average procedure

time of 95 minutes (Molyneux et al. 2004), potentially increasing the patient's exposure to radiation. Furthermore, oscillating balloon inflation and deflation may also increase the risk of local vascular damage.

We have developed a liquid-to-solid gelling polymer system made of polypropylene (glycol diacrylate) and pentaerythritol tetrakis(3-mercaptopropionate) (PPODA-QT) for cerebral aneurysm embolization as an alternative to currently available treatments. The PPODA-QT material is composed of liquid monomer precursors that undergo chemical cross-linking in a basic, aqueous environment. When the precursors are mixed with aqueous initiating solution, the monomers have been shown to form a cross-linked gel over time in a predictable and controllable manner (Riley et al. 2011).

This system can be delivered endovascularly as a true liquid and has the additional benefit of incorporating a water-based initiator instead of being formulated with organic solvents. The gel time of the material is tailorable and able to solidify in a few minutes, affording shorter procedure times than either Onyx or coils. Furthermore the delivery procedure has proven to be straightforward, requiring only a one-time delivery of the PPODA-QT, followed by a short wait time for solidification, then catheter removal.

Previous work with PPODA-QT has resulted in identification of an optimal formulation for use in initial applications. The optimal formulation was used in a small *in vivo* pilot study to compare delivery techniques in surgically created lateral wall aneurysms in swine. Results from this previous investigation showed that PPODA-QT was biocompatible, resulted in complete angiographic occlusion after one month, and was able to produce robust neointimal tissue growth over aneurysm necks in swine. Analysis of different delivery strategies also provided

valuable information. Overfilling of experimental aneurysms was detrimental, resulting in overstressing the surgically created sac and leading to aneurysm model failure. Furthermore, we found that placing a single “framing” platinum coil followed by PPODA-QT embolization was promising, but was associated with increased instance of material in the parent vessel (discussed in Chapter 4).

These initial findings have encouraged further investigation of PPODA-QT as an embolic material. Because swine are known for robust healing after aneurysm embolization (Raymond, Metcalfe, et al. 2003; Dai, Ding, et al., “Histopathologic,” 2005; Kadirvel et al. 2007; Raymond et al. 2007), the work presented here investigates embolization of experimental lateral wall aneurysms created in canines. In response to coil embolization, canines have a similar healing response as seen in coiled human aneurysms (Bouzeghrane et al. 2010). Therefore, the canine model is more clinically relevant for investigating the potential efficacy of an embolic material.

5.2 Materials and Methods

5.2.1 Study Outline

This long-term pilot study evaluated surgically created sidewall aneurysms in canines, embolized with either sub-complete filling of PPODA-QT (PPODA-QT group, n=3) or with one three-dimensional (3D) coil followed by sub-complete filling of the remaining volume with PPODA-QT (Coil+PPODA-QT group, n=3). The embolization procedure was performed by first housing the filling and balloon catheters in an 8 French guide catheter. The tip of the filling catheter (Renegade^{TM} Hi-Flo, Boston Scientific) was placed in the aneurysm. The

balloon catheter (30 mm, HyperGlide™, eV3) was inflated across the aneurysm neck immediately before delivery of PPODA-QT. For animals that received a 3D framing coil (Cashmere™ 14, Micrus Endovascular), the balloon was not inflated during coil placement, but was inflated before PPODA-QT delivery.

Aneurysm embolization was monitored by fluoroscopic visualization of PPODA-QT during delivery. Embolization was performed such that the aneurysm volume was filled to a target of 85% to 95% by fluoroscopic visualization, leaving a small unfilled region at the aneurysm neck. A target filling degree of 100% was not chosen because previous work has shown that this may lead to overfilling and stretching of the surgically created aneurysm, resulting in failure of the aneurysm model (reported in Chapter 4).

The control cases of aneurysm embolization with platinum coils alone, or surgically created aneurysms with no treatment, were not included in this study. Previous studies have reported results of these specific cases in canine lateral wall aneurysms. Therefore, repeating these control cases for this small pilot study (only n=6 animals) was deemed repetitive and would provide little, if any, new insight. The discussion section addresses PPODA-QT treatment versus to the “gold standard” of coil embolization using the results of this work compared to published studies.

5.2.2 PPODA-QT Formulation

PPODA-QT was formulated using two multi-functional hydrophobic monomers, poly(propylene glycol) diacrylate (PPODA) and pentaerythritol tetrakis(3-mercaptopropionate) (QT) (both from Sigma, St. Loius, MO). These monomers, along with their reaction scheme, are shown in Figure 5.1.

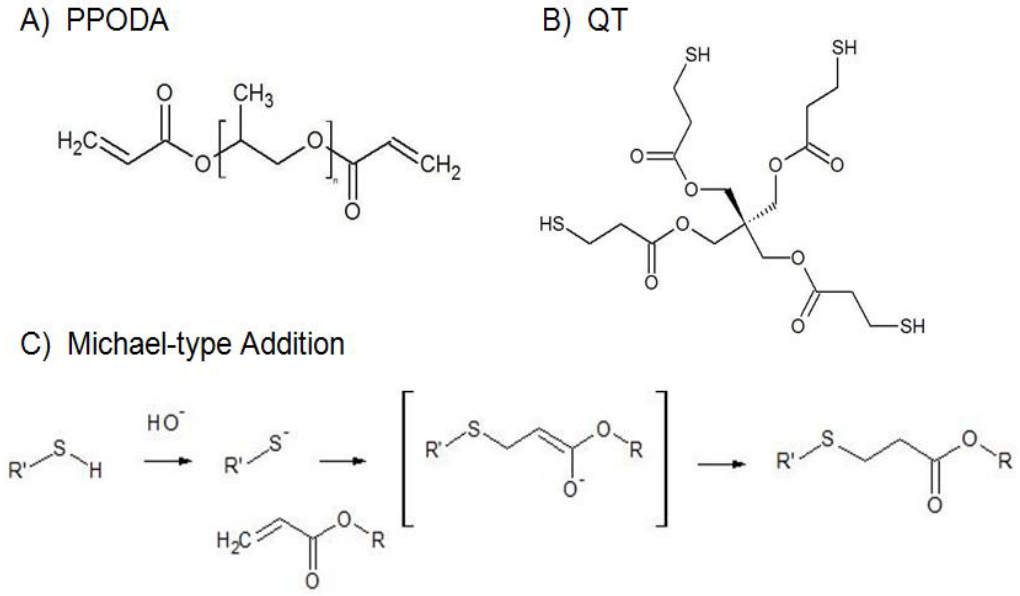


Figure 5.1 Components and reaction scheme of PPODA-QT. (A) Poly(propylene glycol) diacrylate, also called PPODA, M_w≈900, n~13; (B) Pentaerythritol tetrakis(3-mercaptopropionate), also called QT, M_w≈488; (C) Michael-type addition reaction scheme. Deprotonated thiol group of QT performs nucleophilic attack on acrylate group of PPODA.

When reactive groups are mixed in equimolar ratios in the presence of a basic initiating solution, the monomers undergo Michael-type addition and form a cross-linked network (Vernon et al. 2003). Radio-opacity was incorporated into the PPODA-QT system by using a liquid contrast agent as the basic initiating solution, adjusted appropriately with 5N sodium hydroxide to provide the basic conditions required for the chemical reaction. For this study, the liquid contrast agent Conray™ (Mallinckrodt, St. Louis, MO) adjusted to pH 11.0 was incorporated into the PPODA-QT system. Previous studies with Conray™ have

shown potentially beneficial biocompatibility behavior of PPODA-QT gels when formulated with this contrast agent (reported in Chapter 3). The optimized formulation of the polymer system investigated previously (Riley et al. 2011) and used for this work is composed of PPODA and QT, with Conray^{TM} adjusted to pH 11.0, as described below.

PPODA, QT, and pH-adjusted Conray^{TM} were each weighed and aliquoted in a sterile environment after sterile filtration through 0.2 µm syringe filters. Components were aliquoted into 1cc syringes such that the final composition was 75% (wt.) organic components (PPODA and QT) and 25% (wt.) Conray^{TM}. For these experiments, 0.293g of QT was syringe-mixed with 1.08g of PPODA using a luer-loc syringe coupler to attach component syringes. PPODA and QT were pre-mixed for 30 seconds, followed by introduction of 0.458g of Conray^{TM} at pH 11.0. This final composition (deemed PPODA-QT) was mixed for 2 minutes.

The gel time was analyzed using parallel plate rheology. Samples (n=3) were mixed as described above, then placed on a Physica MCR 101 rheometer (Anton Paar, Graz, Austria) at 25^{o}C. An oscillation time sweep was performed with a constant stress of 10 Pa and constant frequency of 1 Hz. Gel time is the time at which the phase angle, δ, reaches 45^{o}. A phase angle of 90^{o} represents a viscous liquid, while 0^{o} is considered an elastic solid. Therefore, a phase angle of 45^{o} represents the point at which the PPODA-QT material has an equal proportion of solid-like and liquid-like properties. This convention is commonly used to define the gel point.

5.2.3 Surgical and Endovascular Procedures

The animal studies done here were approved by the Institutional Animal Care and Use Committee at Barrow Neurological Institute prior to the study. Lateral wall aneurysms were surgically created in the right common carotid artery of canines as previously described (German and Black 1954; Guglielmi et al. 1994; Becker et al. 2007). Animals were intubated then anesthetized with and maintained on 2% isoflurane and oxygen during the procedure. A lateral wall carotid artery aneurysm was created by making a 10 cm incision on the right side of the animal's neck to access the right common carotid artery and external jugular vein (EJV). A 2 cm section of the EJV was removed and sewn over a 5 mm opening made in the common carotid artery in order to form the aneurysm sac. Experimental aneurysms were embolized immediately after creation via standard endovascular access procedure through the right femoral artery. Prior to embolization, animals were given a bolus IV injection of 3000 IU heparin, followed by maintenance of 500 IU by IV every 30 minutes during the procedure, to control clotting. Post-operatively, animals were given aspirin orally at a dose of 81.25 mg/day.

Aneurysm dimensions (neck length, maximum width, and dome height) were recorded at the time of aneurysm creation. The dome-to-neck ratio and volume were calculated for each aneurysm. The dome-to-neck ratio was the measured dome height divided by the neck length. Aneurysm volumes were calculated as reported previously (Dimmick et al. 2009), using the equation for an ellipse, given below. The measured maximum width is W, and the measured dome height is H:

$$Aneurysm\ Volume = \frac{4\pi}{3} \times \frac{W}{2} \times \frac{H}{2} \times \left(\frac{W+H}{4}\right)$$

5.2.4 Analysis

5.2.4.1 Angiography

Aneurysm occlusion *in vivo* was monitored by fluoroscopic angiography at various time points. Angiography was performed immediately after embolization to assess initial aneurysm volume filling, as well as at 3 months and 6 months after embolization. Angiographic images were processed with MATLAB image analysis tools to calculate the degree of 2-dimensional angiographic filling at each time point. Along with image analysis calculations, occlusion was also analyzed using classification by the Raymond-Roy scale (Roy, Milot, and Raymond 2001). This system uses features of angiograms to classify occlusion: class 1 indicates total obliteration, including the aneurysm neck; class 2 means a residual neck is present, but there is no opacification of the aneurysm sac; and class 3 indicates that a residual aneurysm is present due to opacification of the aneurysm sac.

5.2.4.2 Explanted Aneurysms

Explanted aneurysms were observed for neointimal (NI) tissue growth over the aneurysm neck region (ostium). Images of neck regions were macroscopically analyzed to calculate the percent of the ostium covered by visible tissue. Given the contrasting colors of PPODA-QT (white, opaque), and NI tissue (pink) the degree of tissue coverage in the aneurysm neck region after 6 months was estimated using MATLAB image analysis tools.

5.2.4.3 Histology

Histological processing and analysis was performed for PPODA-QT filled aneurysms. Samples were fixed in 10% formaldehyde and sent to the Medical College of Georgia (Augusta, GA) for paraffin embedding, sectioning, and staining with hematoxylin and eosin (H&E). Samples were sectioned in three distinct regions: the proximal, middle, and distal regions of the aneurysm, where blood flows into the proximal side and out of the distal end of the aneurysm. For each PPODA-QT treated aneurysm, one slide from each region was analyzed at 25X magnification for tissue thickness, with 7 measurements per slide. One-way ANOVA was used to determine differences in tissue thickness between each aneurysm neck region and between samples. Post-hoc Tukey's multiple comparison test with a 95% confidence interval was used to identify differences between samples when appropriate.

5.3 Results

5.3.1 PPODA-QT Formulation

Previous studies with this formulation of PPODA-QT have shown that the gelling kinetics are reproducible and have low sample-to-sample variability (Riley 2011). Rheological analysis reconfirmed these findings, with gel times of 10.2 ± 0.5 minutes (n=3). The phase angle profiles for PPODA-QT replicates are shown in Figure 5.2.

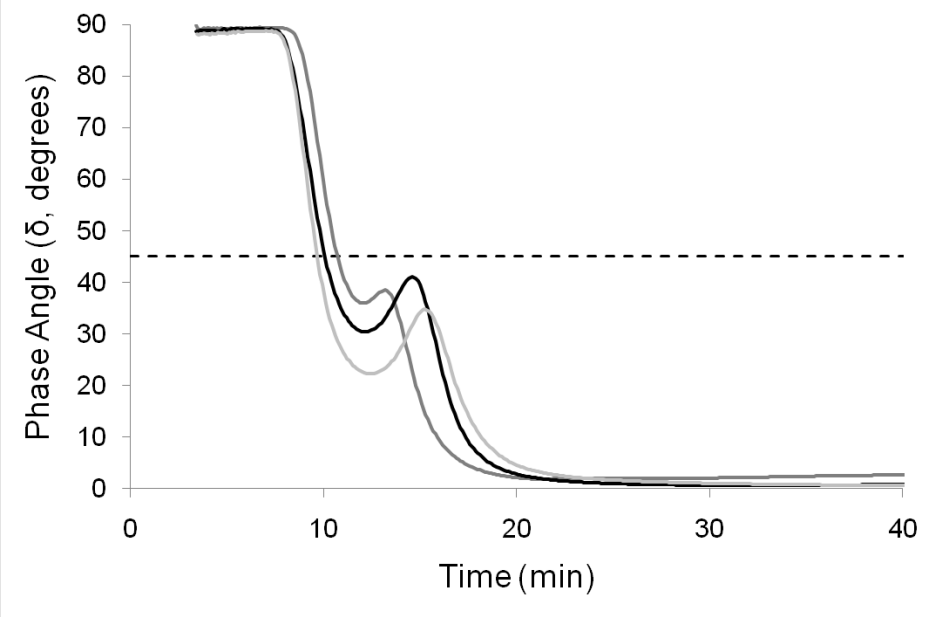


Figure 5.2 Rheology measurements. Phase angle (δ) profile of PPODA-QT over time at 25^{o}C, each solid line represents one replicate. Gel time is considered the time when the phase angle reaches 45^{o} (dashed line). Average gel time for PPODA-QT (n=3) is 10.2 ± 0.5 minutes.

5.3.2 Surgical and Endovascular Procedures

Lateral wall aneurysms were created in the right common carotid arteries of 6 canines. The dimensions of created aneurysms are shown in Table 5.1.

Table 5.1 Dimensions of Surgically Created Aneurysms

Treatment	Rep.	Neck (mm)	Dome Height (mm)	Width (mm)	Volume (mm^3)	D/N Ratio
PPODA-QT	1	5.0	6.0	7.0	143	1.20
	2	5.5	7.0	8.0	220	1.27
	3	5.0	6.0	6.0	113	1.20
Coil + PPODA-QT	1	5.0	7.0	6.5	161	1.40
	2	5.0	6.0	7.0	143	1.20
	3	5.0	6.5	8.0	197	1.30

Aneurysm volumes were calculated using the formula for an ellipse, using the dome height and maximum width dimensions. Dome-to-neck ratio is a measurement of the aneurysm's dome height divided by the neck dimension. Using a t-test, calculated aneurysm volumes between the PPODA-QT group and the Coil+PPODA-QT group were not found to be significantly different (p=0.83). Similarly, the dome-to-neck ratio of each group was also not significantly different (p=0.29).

5.3.3 Angiography

Angiography was performed immediately after embolization, and at 3 and months post embolization in order to determine degree of occlusion. X-ray fluoroscopy was also performed to verify visibility of PPODA-QT within the aneurysm. Figure 5.3 shows non-subtracted fluoroscopic images of embolized aneurysms at 6 months post embolization. In all PPODA-QT group aneurysms, the material is visible at 6 months, indicating that the contrast agent is present within the material. It is more difficult to see the polymer material in the Coil+PPODA-QT group due to the radiograpically denser framing coil, which obscures polymer visibility.

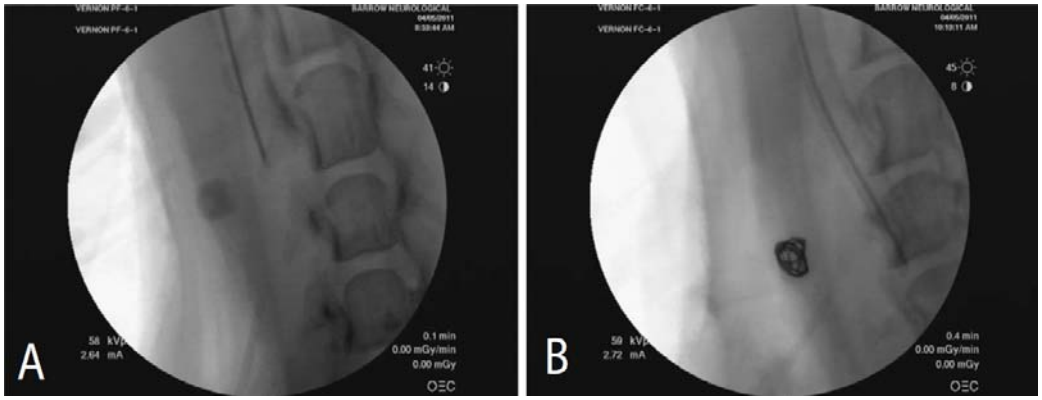


Figure 5.3 Fluoroscopic visibility at 6 months. (A) Radio-opaque PPODA-QT is clearly visible in the PPODA-QT group 6 months post-embolization, but is obscured in the Coil+ PPODA-QT group (B) due to the presence of the radiographically denser coil.

At each time point, angiograms provided a means of calculating and scoring occlusion. Table 5.2 shows the results of the 2D MATLAB analysis, as well as Raymond-Roy classification scores. All model aneurysms showed initial scores of incomplete obliteration, given that the filling technique was not meant to completely occlude the entire aneurysm immediately after delivery. Over time, all but one aneurysm in the PPODA-QT group achieved 100% occlusion, while all of the Coil+PPODA-QT aneurysms were 100% occluded at 6 months. A Raymond-Roy classification of 3 (indicating dome recanalization) was given to one sample (replicate #1) in the PPODA-QT group at 3 and 6 months. Angiograms for this sample are shown in Figure 5.4, where reperfusion of blood flow can be seen around the periphery of the aneurysm.

Table 5.2 Angiographic Filling Percentages and Raymond-Roy Scores: Initially, 3 Months, and 6 Months Post-Embolization.

Time	Treatment	Rep.	2D Filling %	Raymond-Roy Score
Initial	PPODA-QT	1	93.0%	2
		2	86.2%	2
		3	94.7%	2
	Coil + PPODA-QT	1	94.5%	2
		2	97.1%	2
		3	94.7%	2
3 Months	PPODA-QT	1	78.4%	3
		2	100%	1
		3	100%	1
	Coil + PPODA-QT	1	100%	1
		2	100%	1
		3	100%	1
6 Months	PPODA-QT	1	81.6%	3
		2	100%	1
		3	100%	1
	Coil + PPODA-QT	1	100%	1
		2	100%	1
		3	100%	1

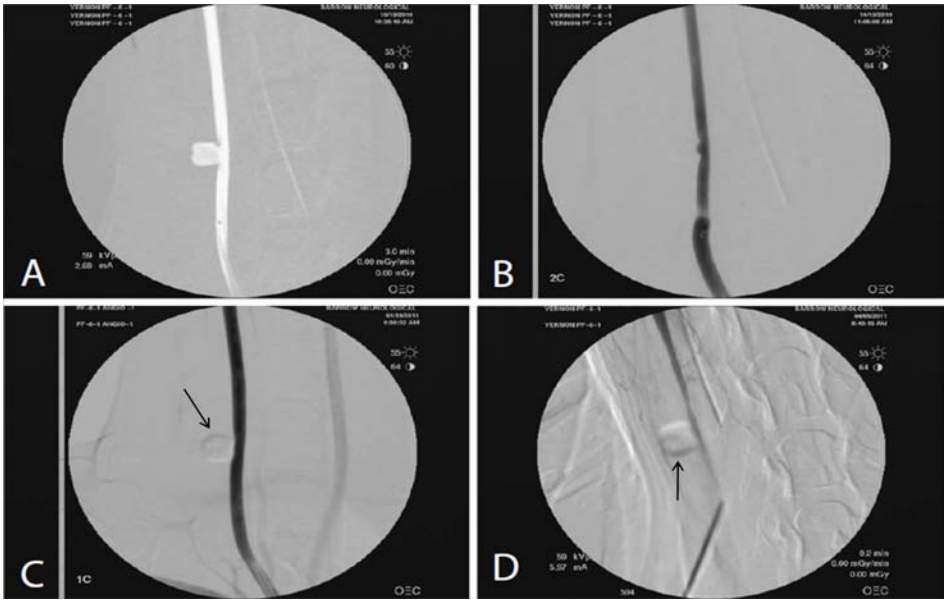


Figure 5.4 Recanalized aneurysm in the PPODA-QT group. Angiography was performed (A) before embolization and (B) immediately after embolization. (C) At 3 months post embolization, angiography showed slight recanalization due to blood flow along the outer edge of the aneurysm (arrow). (D) Recanalization was visible at 6 months as well. The location of aneurysm recanalization looks different in images C and D because of C-arm positioning.

5.3.4 Explanted Aneurysms

Experimental aneurysms were explanted at 6 months, and analyzed for neointimal tissue coverage in the ostium. The explanted samples are shown in Figure 5.5. From this figure, PPODA-QT samples show a smooth interface surface at the ostium, where NI tissue can easily be identified. While tissue coverage is not complete in all replicates, evidenced by NI tissue coverage calculations shown in Figure 5.6, PPODA-QT does seem to provide a suitable surface for overgrowth when delivered alone. The Coil+PPODA-QT group shows rougher neck regions due to PPODA-QT protruding into the parent vessel

(replicate #1 and #2). However, replicate #3 in the Coil+PPODA-QT group provided a smooth surface for tissue growth.

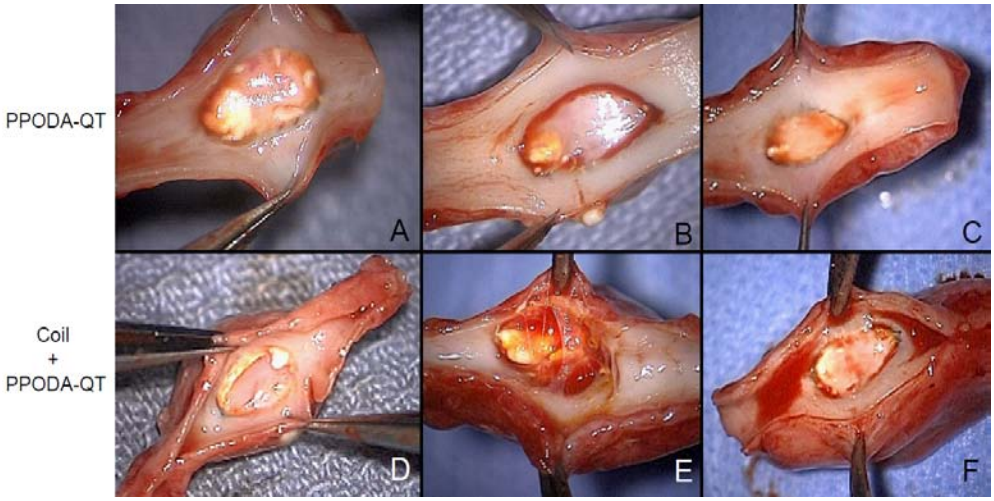


Figure 5.5 Explanted aneurysm samples. PPODA-QT filled aneurysm samples (A-C) all showed a smooth surface in the ostium. Coil+PPODA-QT -filled aneurysms (D-F) showed excess PPODA-QT protruding into the parent vessel in two samples resulting in rough surfaces (D, E), while one sample displayed no PPODA-QT protrusion and a smooth surface (F).

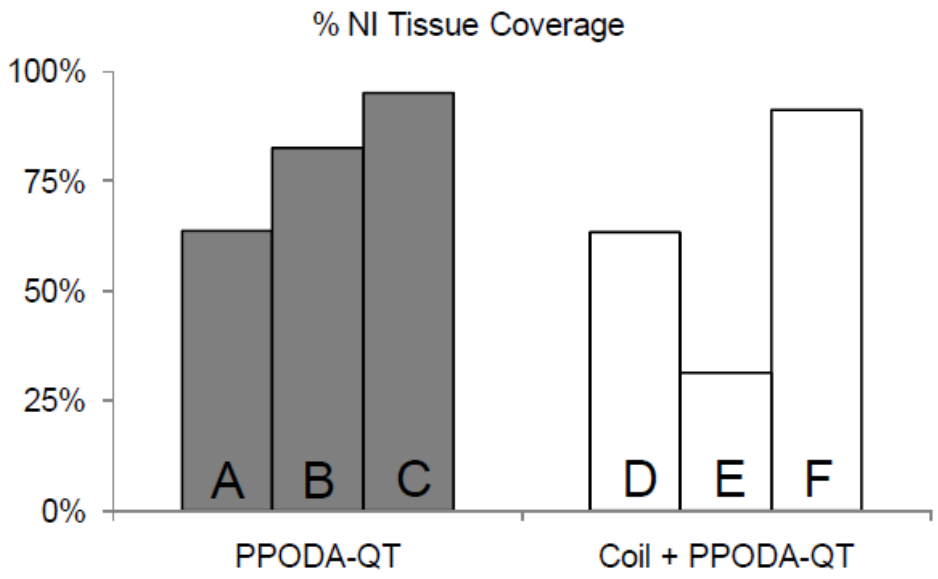


Figure 5.6 Percent of NI tissue coverage in the ostium. Average tissue coverage values between PPODA-QT group and Coil+PPODA-QT group are not statistically significant, with 80% ± 16% and 63% ± 31%, respectively. When shown individually, replicates in the PPODA-QT group (A-C) show consistently higher NI tissue coverage values, while there is greater sample-to-sample variability in the Coil+PPODA-QT group (D-F). A-F correspond to the same aneurysm samples shown in Figure 5.5.

Neointimal tissue coverage calculations, shown above in Figure 5.6, indicate that both the PPODA-QT and Coil+PPODA-QT groups have, on average, similar tissue coverage with 80 ± 16% for the PPODA-QT group and 62 ± 30% for the Coil+PPODA-QT group (p=0.399). While the averages are not statistically different, the PPODA-QT group shows consistently higher NI tissue coverage values than the Coil+PPODA-QT group, given the lower sample-to-sample variability in the PPODA-QT group. Furthermore, both replicates in the Coil+PPODA-QT group (#1 and #2) with a rough surface had the two lowest

calculated NI tissue coverage percentages overall, 63% and 31%, respectively. The next lowest value (64%) was found in replicate #1 of the PPODA-QT group.

5.3.5 Histology

Histological analysis and tissue thickness measurements were performed on PPODA-QT filled aneurysms only. Tissue thickness measurements were confounded for samples in the Coil+PPODA-QT group with non-smooth neck regions, and thus not able to provide suitable comparison between the two groups.

Histology images of a representative PPODA-QT sample (replicate #2) in the aneurysm neck region stained with H&E are shown in Figure 5.7. Robust NI tissue growth in this image is characterized by a clear neoendothelial layer of cells over the new tissue with organized tissue nearer to the parent-vessel interface and more amorphous tissue at the PPODA-QT interface.

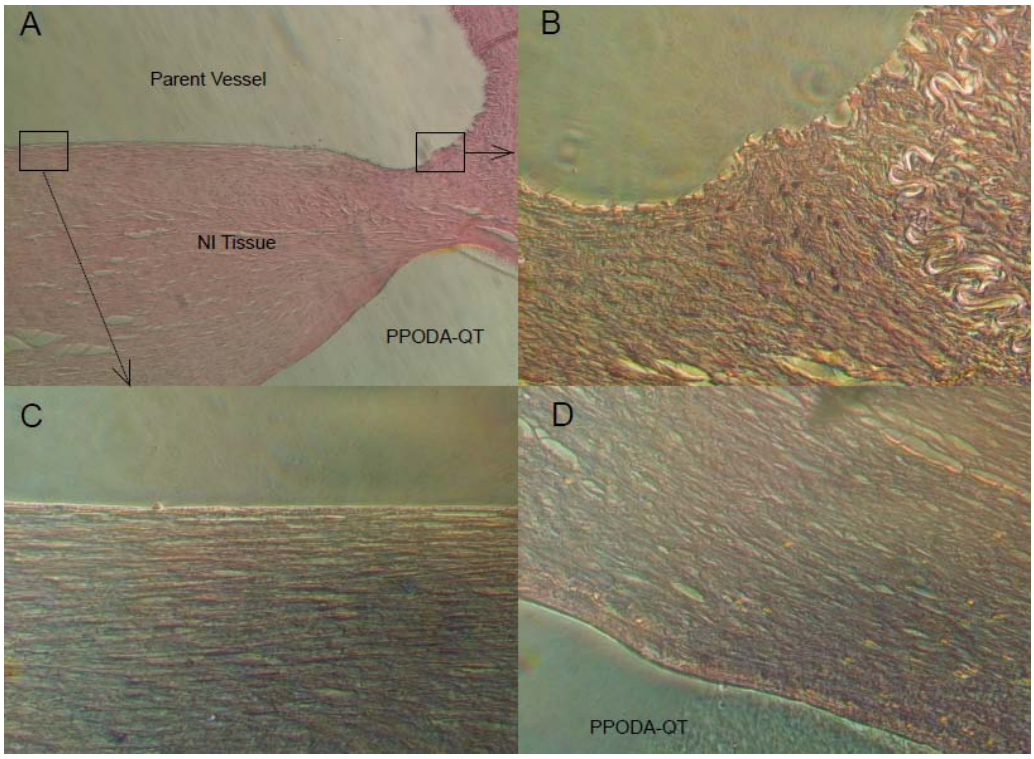


Figure 5.7 Histology images of PPODA-QT filled aneurysms. H&E staining of replicate #2 from the PPODA-QT group at 100X (A) and 400X magnifications (B-D). (A) The region of neointimal tissue growth is shown interfacing with both the parent vessel and the PPODA-QT embolic material. (B) Near the surgical site, a single layer of neoendothelial cells has formed over new tissue. (C) In the center of the aneurysm near the parent vessel interface, NI tissue is dense and well-aligned. (D) At the interface of NI tissue and PPODA-QT, NI tissue is more amorphous and less dense.

Tissue thickness was measured using 3 histology slides at 25X magnification per sample, with 7 measurements per slide. Histology slides were taken within the ostium region at the proximal end, in the middle, and at the distal end. The average tissue thickness in each replicate of the PPODA-QT group is shown in Figure 5.8A. The lowest average tissue thickness was found in replicate

#1 at 123 µm ± 191µm, which is statistically different than average tissue thickness in replicate #2 (520 µm ± 314 µm) and replicate #3 (566 µm ± 373 µm), found by Tukey's comparison following a one-way ANOVA. Average tissue thickness of replicates #2 and #3 are not statistically different.

Comparison of NI tissue thickness in individual regions of each sample (proximal, middle, and distal) is shown in Figure 5.8B. In this representation, the proximal regions have the smallest tissue thicknesses, which are not statistically different between replicates, and the middle and distal regions are the thickest. A statistical difference in tissue thickness was found between replicate #1 and #3 in the middle region. For NI tissue in the distal region, replicates #2 and #3 were both found to be statistically thicker than replicate #1.

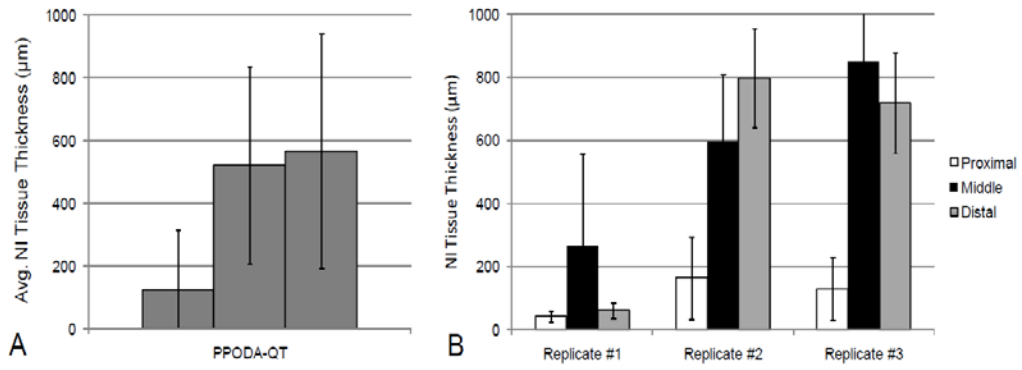


Figure 5.8 Neointimal tissue thickness measurements. Tissue thickness measurements were taken for replicates in the PPODA-QT group. Three slides per group were analyzed, with 7 measurements per slide. Each slide was taken from a distinct region of the aneurysm ostium along the vessel: the proximal (blood inflow), middle, and distal (blood outflow) regions. (A) Average NI tissue thickness measurements show that replicate #1 has the lowest tissue thickness, statistically significantly lower than the other two replicates. (B) Tissue thickness

measurements separated by aneurysm region. Thickness in the proximal region (white bars) is not significantly different among replicates. Thickness in the middle region (black bars) is significantly different only between replicates #1 and #3. Distal tissue thickness, however, is significantly lower in replicate #1 than in the other two replicates. All statistical comparisons were done using one-way ANOVA followed by post-hoc Tukey's multiple comparison tests with a 95% confidence interval.

5.4 Discussion

5.4.1 Pilot Study Limitations

This study was designed as a long-term (6-month) pilot study to investigate the potential effectiveness of using PPODA-QT as an embolic agent in experimental aneurysms. The study design included two experimental groups: PPODA-QT used alone, as well as PPODA-QT delivered after placement of a 3D framing coil. Because this study was designed to be a small "proof of concept" investigation in a rigorous animal model, experimental controls were not included. Previous studies have reported the use of platinum coils alone (positive control) or without embolization at all (negative control) in the canine lateral wall aneurysm model (Mawad et al. 1995; Macdonald et al. 1998; Kallmes et al. 1999). Although including controls would have allowed more in depth statistical comparison, this pilot study had so few subjects to begin with (n=6), that statistical comparison would not have provided conclusive information regarding efficacy. Therefore, the results of this study are qualitative in nature, yet statistical comparisons are included within study groups where appropriate.

The canine lateral wall aneurysm model was chosen because it is considered more rigorous than the swine lateral wall model, due to the fact that canines undergo aneurysm healing post-coil embolization in a similar manner to human aneurysms (Raymond, Metcalfe, et al. 2003; Bouzeghrane et al. 2010). The 6-month study also reflects a clinically relevant time frame, since in humans most aneurysm recanalization occurs within the first 6 months after embolization (Sluzewski, van Rooij, et al. 2003). While the surgically created aneurysms in this study are not considered to be large in size, in which the largest dimension must measure >12 cm in diameter by Chyatte's classification (Chyatte 2003), they are all considered to be wide-necked. Wide-necked aneurysms have been characterized by a neck opening diameter >4 mm or a D/N ratio <1.5 (Piske et al. 2009 Youn et al. 2010), which all aneurysms in this study achieve. Therefore, these experimentally created aneurysms provide a clinically relevant embolization model to assess feasibility of using PPODA-QT for cerebral aneurysms.

5.4.2 Treatment Groups

5.4.2.1 Coil+PPODA-QT Group

The Coil+PPODA-QT treatment group showed promising angiographic results initially, as well as at 3 and 6 months post-embolization. None of the experimental aneurysms showed recanalization at any time point, with corresponding volume filling percentages at 100% and Raymond-Roy scores of 1 at both 3 and 6 months.

While the 3D framing coil was always seen easily during fluoroscopy, PPODA-QT was more difficult to identify when used in conjunction with the coil, due to the difference in radio-opacity between the coil and PPODA-QT. Visibility issues with liquid embolics used in combined treatments with coils have been reported previously (Murayama et al. 2000; Takao et al. 2009). Poor monitoring of PPODA-QT during delivery as a result of visibility obstruction from the coil was a significant problem. Upon explanation, 2 out of 3 aneurysms in the Coil+PPODA-QT group showed PPODA-QT protrusion into the parent vessel, yet this was not observed angiographically during delivery.

The delivery technique of sub-complete initial filling (<100%) was done specifically to prevent the occurrence of PPODA-QT in the parent vessel. Previous studies in swine indicated that attempting complete filling after 3D coil placement was associated with excess PPODA-QT in the parent vessel (reported in Chapter 4). However, results reported here show that deliberate sub-complete filling did not prevent PPODA-QT protrusion either.

In the replicates showing PPODA-QT in the parent vessel, animals did not experience parent artery occlusion (PAO) during the 6-month study window. However, the excess PPODA-QT did seem to hinder tissue coverage, since these two replicates also had the lowest percent area of tissue overgrowth in the neck region (63% and 31%). The one replicate in the Coil+ PPODA-QT group that had a smooth neck region displayed 91% tissue coverage in the ostium. The inability to monitor PPODA-QT when used in conjunction with a coil severely limits the clinical applicability of the Coil+ PPODA-QT treatment method.

5.4.2.2 PPODA-QT Group

Experimental aneurysms treated with PPODA-QT alone underwent filling such that <100% of the aneurysm volume was initially occluded. While this delivery method may seem contrary to the goal of treatment (complete aneurysm obliteration), this study showed that it may actually be safer than aiming for 100% volume filling initially. In previous work with the carotid artery aneurysm model in swine, when the goal was to initially fill the aneurysm volume to 100% capacity, 2/5 animals died within a week due to failure of the aneurysm model. Attempting a 100% fill of the volume actually resulted in overfilling with PPODA-QT beyond the aneurysm's initial volume. When PPODA-QT solidified, the aneurysm was maintained in an overstressed state, eventually causing it to fail (discussed in Chapter 4).

In the current canine study, all aneurysms were initially filled to 85%-95% of their 2D angiographic volume and there were no instances of aneurysm model failure. The limited number of animals in both studies means that no definite conclusions can be drawn, but the trend of higher model success when sub-complete filling (85%-95%) is performed may influence how PPODA-QT is delivered in the future in a clinical setting.

The hesitation with using a sub-complete delivery technique with PPODA-QT is the assumption that an aneurysm may be more prone to recanalization if it is only occluded to 85% of its angiographic volume rather than 100%. In this study, one of the PPODA-QT -filled aneurysms did show recanalization at 3 months, but it was not the aneurysm that had the lowest initial filling percentage. The recanalized aneurysm (replicate #1) was filled to 93% of its angiographic volume, while the two successfully treated aneurysms (100% occluded at 3 and 6 months), were initially filled to 86% and 95%. As a result, recanalization was not

correlated with lower filling percentages in the 85%-95% range. Furthermore, these results show that initial sub-complete aneurysm filling in the 85%-95% range can allow for complete healing over time, given that 2/3 aneurysms were 100% angiographically occluded after 3 months.

Explantation of PPODA-QT embolized aneurysms showed that when PPODA-QT is delivered alone, no excess material was found in parent vessels and all aneurysm neck regions were smooth. In contrast to the Coil+PPODA-QT group, PPODA-QT delivered without the coil was easily monitored during delivery. While there is a correlation between rough surface and lower percentage of NI tissue coverage in the neck region, replicate #1 in the PPODA-QT group shows that a smooth surface does not necessarily guarantee robust tissue coverage. Even with a smooth surface for tissue overgrowth, the recanalized aneurysm showed the next lowest degree of tissue coverage, at 64% of the aneurysm neck area. The other two replicates in this group had NI tissue coverage percentages of 82% and 95%.

Histological analysis of the PPODA-QT group aneurysms allowed NI tissue thickness measurements at different spatial locations through the aneurysm neck region (Figure 5.8). The recanalized aneurysm, replicate #1, showed the lowest average tissue thickness over the whole aneurysm. While the proximal and middle region thicknesses were not statistically different than both of the other samples, the distal region NI tissue thickness was significantly lower in replicate #1 than in the other samples. Based on these results, aneurysm recanalization may be correlated with lower overall average NI tissue thickness as well as thinner NI tissue in the distal region. Again, with such a small study group, definitive conclusions cannot be drawn about the relationship between

tissue thickness and recanalization in all lateral wall model aneurysms, but these results may provide rational for investigating tissue thickness in different aneurysmal regions in future aneurysm embolization studies.

The relationship between calculated NI tissue coverage in the neck region (Figure 5.6) and tissue thickness measurements (Figure 5.8) between PPODA-QT and the parent vessel should be addressed. From the tissue coverage calculations (area percent of ostium covered by tissue), one might expect that there should be no tissue thickness in areas that were deemed “uncovered” by this analysis. However, using macroscopic visualization of the neck region means that regions with very thin tissue coverage will not be distinguished from uncovered PPODA-QT, as evidenced by thin NI tissue in the proximal region of all PPODA-QT aneurysms, which was omitted from NI tissue coverage calculations. Therefore, these calculations represent the percent of ostium area visibly covered by NI tissue. As such, this calculation is more of a gross observational tool than a strict quantitative representation of tissue coverage. Tissue thickness measurements, on the other hand, do represent discretely measured quantities of microscopically visualized NI tissue, and are quantitative in nature.

While the results presented here do not explain why recanalization occurred in replicate #1 and not in the other PPODA-QT group samples, angiographic findings were successfully correlated with histologic findings. In the PPODA-QT group, aneurysms that showed complete angiographic obliteration (no recanalization) at 6 months, and are thus considered “successfully treated”, showed a greater percentage of NI tissue coverage in the neck region and greater average NI tissue thickness. On the contrary, the aneurysm that showed

angiographic recanalization had the lowest NI tissue coverage and lowest average NI tissue thickness values in the PPODA-QT group. Being able to correlate angiographic findings with histological analyses has been considered a major limitation of animal model studies when testing efficacy of coil embolization (Bouzeghrane et al. 2010). In studies where both outcomes are discussed, there are often contradicting indicators between angiographic occlusion and histological findings, where angiography generally overestimates the degree of aneurysm healing (Macdonald et al. 1998). In this small scale study with PPODA-QT, angiographic results were correlated with histology findings without implying contradictory conclusions.

5.4.3 PPODA-QT vs. Platinum Coil Embolization

Due to the small scale of this study, control animal groups were not performed alongside treatment groups. In order to make qualitative comparisons between PPODA-QT as an embolic material and currently available aneurysm treatments, previously published studies were examined. Only studies in which canine carotid artery lateral wall aneurysms were embolized with platinum coils will be discussed in terms of positive controls.

It is necessary to mention the negative controls as well. A negative control for this aneurysm model would be a lateral wall aneurysm that is surgically created in the carotid artery of a canine, but does not undergo embolization of any kind. The patency of the aneurysm would be evaluated over time, in order to ensure that the surgically created aneurysm does not undergo spontaneous thrombosis and render itself “healed”. If this does occur, the animal model is not appropriate to assess efficacy of the embolic material because the

aneurysm will heal whether or not the treatment is administered, as is common with swine aneurysm models (Guglielmi et al. 1994, Byrne et al. 1997). Negative control outcomes for surgically created carotid artery lateral wall aneurysms in canines have been reported previously. In general, this aneurysm model undergoes spontaneous thrombosis less than 10% of the time, meaning that the model is fairly robust and appropriate for assessing endovascular treatment methods (Kallmes et al. 1999).

A few studies have been published documenting the outcomes of coil embolization in the canine lateral wall carotid artery aneurysm model. Even though PPODA-QT and platinum coils occlude aneurysms in different ways, important outcomes can still be compared. Many coil embolization studies report the nature of the thrombus within the aneurysm dome, indicating whether it has been reorganized into tissue or if a residual clot is still present, etc. These outcomes are not applicable to PPODA-QT treatment because there is no clot formation in the aneurysm dome—it is occluded with the polymer. However, angiographic recanalization can be compared. Mawad et al. (1995) showed that of 10 coiled lateral wall aneurysms in canines, 4 (40%) showed recanalization at 6 months. Macdonald et al. (1998) reported 1 in 5 (20%) lateral wall aneurysms were incompletely occluded 2 months after platinum coil embolization in the canine model. Results from the current investigation show that 1 in 3 (33.3%) PPODA-QT embolized aneurysms recanalized by 3 months. Even though our study numbers are low, PPODA-QT embolization seems to fall within the recanalization rate range of experimental aneurysms embolized with platinum coils, when considering the same animal/aneurysm model.

NI tissue thickness is not often reported for coiled aneurysms. This is likely because it is almost impossible to remove coils prior to sectioning without disrupting the neointimal tissue layer, since newly formed tissue adheres strongly to coils (Mawad et al. 1995; Macdonald et al. 1998). Coils can be removed after sectioning, but this process is tedious and not commonly performed (Dai, Ding, et al., “Modified histologic,” 2005). Most reported results qualitatively describe tissue coverage, but generally not thickness. Macdonald et al. (1998) reported that of the 5 experimental aneurysms treated, 3 showed some amount of NI tissue coverage in the neck region, but that it was not flush with the parent vessel walls. The other 2 coiled aneurysms showed no NI tissue coverage at all. In the present study with PPODA-QT embolization (PPODA-QT group only), all 3 aneurysms showed some degree of NI tissue coverage, with 1 aneurysm (replicate #1) displaying significantly less coverage than others. While less quantitative in nature than angiography findings, the PPODA-QT treatment showed visible NI tissue coverage in all replicates, yet NI tissue coverage over coils tends to be more variable in this animal model.

Overall, quantitative NI tissue coverage and thickness comparisons may provide insight into potential effectiveness of an embolic aneurysm treatment. However, scarcity of published reports containing these measurements after coil embolization will limit their usefulness for comparison across different treatment types. Within a treatment group, however, as in the PPODA-QT group of this study, these comparisons may provide another way, besides angiography, to characterize successful treatments versus recanalized aneurysms.

5.5 Conclusion

This study reports results of the first long-term *in vivo* study using the novel liquid-to-solid gelling polymeric material, PPODA-QT for embolization of experimental aneurysms. The canine, lateral wall aneurysm model used here represents a clinically applicable model for initial testing of the efficacy of PPODA-QT in wide-necked aneurysms. This study evaluated the response of two different treatment methods: PPODA-QT delivered alone (n=3), as well as PPODA-QT delivered after placement of one 3D framing coil into the aneurysm (n=3). Findings from the Coil+PPODA-QT group indicate that this treatment method is prone to PPODA-QT protrusion into the parent artery, due to difficulty in monitoring PPODA-QT delivery in the presence of a radiographically denser coil. While PPODA-QT in the parent vessel did not lead to PAO over the 6-month study duration, it did result in a rougher surface in the ostium, which was correlated with less NI tissue coverage.

Aneurysms in the PPODA-QT group displayed no instances of protruding PPODA-QT in the parent vessel, and ostium surfaces were smooth. Recanalization was seen in 1 of 3 aneurysms via angiography at 3 and 6 months. Histotogy results showed that the recanalized aneurysm had the lowest calculated NI tissue coverage area as well as the lowest average NI tissue thickness of all samples in the treatment group. Furthermore, NI tissue was found to be significantly thinner in the distal ostium region of the recanalized aneurysm when compared to the two completely occluded aneurysms. The recanalization rate found in this study compares well with other studies reporting results of coiled lateral wall carotid artery aneurysms in canines. Only larger scale animal studies will be able to determine if PPODA-QT is significantly more advantageous than coil embolization. NI tissue coverage compared favorably to

published studies, although quantitative reports of NI tissue coverage and thickness are lacking.

Overall, this study indicates that using PPODA-QT alone as an embolic material for aneurysm treatment is promising. Future work with PPODA-QT should encompass a greater number of PPODA-QT embolized animals, as well as a sufficient number of coiled aneurysms as controls. Due to the lack coiled experimental aneurysm studies that report NI tissue coverage and thickness measurements in published literature, it would be beneficial to perform such control experiments in parallel so that these embolic materials can be properly compared. Furthermore, these analyses may allow for greater success when attempting to correlate of angiographic results with histology findings post-coil embolization, as was shown for PPODA-QT filled aneurysms in this study.

Chapter 6: CONCLUSIONS AND FUTURE WORK

6.1 Polymer System Development for Cerebral Aneurysm Embolization

The work presented in this thesis reports the development of a liquid-to-solid gelling polymer system for cerebral aneurysm embolization. The polymer system is based on the hydrophobic liquid monomers, poly(propylene glycol) diacrylate (PPODA) and pentaerythritol tetrakis(3-mercaptopropionate) (QT), which undergo a chemical reaction resulting in cross-linking of monomers and formation of an elastic gel. This work focuses on developing the PPODA-QT system from a clinical standpoint, in order to adapt the system for application in cerebral aneurysms.

6.2 Formulation: PPODA-QT Gels Made with Different Contrast Agents

The PPODA-QT system was first formulated with different liquid contrast agents in order to provide the polymer with radio-opacity required of an embolic material, as these materials must be visible under X-ray during delivery. The objective of these formulation experiments was to identify if different types of commercially available contrast agents (Conray™ and Omnipaque™ 300 were examined) affect the gelling process of PPODA-QT in different ways. Complete knowledge of gelling kinetics is imperative, especially when the material under investigation will be delivered into the brain. In such a vital area, insufficient understanding of the material could have devastating consequences.

Results from the formulation experiments indicated that different types of contrast agents alter the gelling process of PPODA-QT gels. Formulating PPODA-QT with Conray, an ionic contrast agent, resulted in gels that underwent

gellation at an earlier time, yet exhibited in a long post-gellation phase where cross-linking continued to occur, as evidenced by the slowly increasing polymer viscosity after the material had gelled. These characteristics indicate that Conray-formulated gels undergo early, wide-spread network formation, in which components become immobilized in the gel network even though the gel is not fully cross-linked. After global network formation, reactive monomers that are near each other in proximity can still cross-link, but this occurs slowly.

Gels formulated with Omnipaque showed very different gelling kinetics. Omnipaqie-formulated gels had a long initial period of low viscosity, followed by rapid network formation in which all cross-linking was completed. This gelling profile suggests that Omnipaque-formulated gels begin to react by localized cross-linking in discrete areas, which allows the overall solution viscosity to remain low. Eventually, locally cross-linked regions become large enough to "link-up" with other cross-linked regions, resulting in global network formation. The material viscosity does not increase further after network formation in Omnipaque-formulated gels, because local sites are already densely cross-linked by the time network formation occurs.

Sample-to-sample variability in gel time is of great clinical importance because gelling behavior must be predictable. Therefore, a formulation with lower sample-to-sample variability in the gel time is more favorable for clinical use. Conray-formulated gels showed lower variability in gel time, while Omnipaque-formulated gels had much higher sample-to-sample variability. This difference can be attributed to gelling kinetics between formulations, where early network formation in Conray-formulated gel is not dependent on reactions in local regions—it is a wide-spread event. Further cross-linking occurs after the gel is

already formed. The gel time of Omnipaque-formulated materials, however, is first dependent on cross-linking in small regions, which may be subject to more variability due to mixing conditions, etc. Only after localized regions react can the entire gel undergo network formation. The gelling sequence exhibited by Omnipaque-formulated gels is therefore more prone to gel time variability than Conray-formulated gels.

6.3 Characterization: *In Vitro* Behavior of PPODA-QT Formulated Gels

After identifying differences in gelling kinetics based on incorporation of Conray or Omnipaque, the next step was to determine how these different formulations translated into material behaviors. Specifically of interest were material behaviors that would be important in an aneurysm, such as toxicity of the material, swelling properties, and degradation characteristics. These clinically relevant properties were examined under benchtop conditions to gain initial information as to how PPODA-QT gels may behave *in vivo*.

Cytotoxicity, or the affect of each gel formulation on cultured cells, was examined as a proxy for biocompatibility. Results showed that Conray-formulated gels were more biocompatible than Omnipaqie-formulated gels, due to a significantly higher percentage of living cells after exposure to gels over a three-day period.

Swelling and degradation were also investigated in a simulated physiologic environment. Conray gels were found to swell more than Omnipaque gels at 37^{o}C over a 10 month period. Maximal swelling in Conray-formulated gels resulted in a ~60% volume increase, while Omnipaque-formulated gels showed a ~35% volume increase. These volume increases actually translate into only

moderate differences in geometric diameter increases. In general, the long term swelling behavior of both gels at 37^{o}C does not suggest significant problems for aneurysm embolization, but characterizing this behavior is valuable. Degradation was also monitored, and both gels showed very little degradation at 37^{o}C until 8 months, after which Conray-formulated gels exhibited a measurable decline in compressive strength.

In vitro swelling and degradation experiments represented "worst-case-scenario" conditions. In both experiments, the entire surface area of gels was exposed to aqueous penetration. In an aneurysm, however, the only gel surface that would be subject to as much aqueous penetration would be in the aneurysm neck region immediately after the PPODA-QT gel is delivered, where blood in the parent vessel contacts the material. As a result, *in vitro* swelling and degradation results presented here are likely exaggerations of material behavior *in vivo*.

Cytotoxicity experiments also represented a challenging test case, given that cells were exposed to formulated gels without the media being flushed from cell surfaces, as would likely happen *in vivo*. However, given that good biocompatibility is essential for aneurysm healing, such that neointimal tissue can grow over the gel surface once delivered, more weight was given to *in vitro* cytotoxicity results than swelling or degradation experiments. From these results, as well as previous experiments indicating less variability in gel times, PPODA-QT gels formulated with Conray at pH 11.0 were used for subsequent *in vivo* testing.

6.4 Testing: Biocompatibility and Delivery Strategy

Initial *in vivo* studies used a swine lateral wall aneurysm model to assess biocompatibility of PPODA-QT formulated with Conray in a live animal model over the course of one month. This study also examined different delivery strategies for administration of PPODA-QT into an aneurysm model. This small scale *in vivo* study was not designed to assess efficacy of the PPODA-QT material, but did provide valuable information regarding how the material behaves *in vivo*, and with different delivery strategies. This study showed that PPODA-QT did not result in any local toxicity to nearby tissue in the aneurysm model, and provided a suitable substrate for neointimal tissue overgrowth, as a robust tissue layer was observed over aneurysm necks one month after embolization.

The delivery strategy analysis allowed for comparison between different administration methods. Delivery strategies examined were: completely filling the aneurysm with PPODA-QT to 100% capacity, sub-complete filling of the aneurysm (80-90% capacity) with PPODA-QT, as well as placement of a "framing" platinum coil followed by complete filling PPODA-QT. Results from these experiments indicated that sub-completely filling experimental aneurysms with PPODA-QT was the most suitable delivery strategy, with progressive occlusion of the aneurysm volume to reach 100% by the end of the one month time frame. Complete (100%) filling resulted in instances of overstretching and rupturing of the model aneurysm. Placement of a coil followed by complete embolization with PPODA-QT did not result rupture, but did show two out of three instances of excess polymer found in the parent vessel upon explantation, which is a clinically undesirable outcome.

6.5 Testing: Pilot Study to Gauge Effectiveness

Further *in vivo* testing was performed to gauge long-term effectiveness of PPODA-QT in a more clinically relevant animal model. The canine lateral wall aneurysm model was used, because canines have reportedly shown a more human-like healing response aneurysm treatment. Model aneurysms in swine tend to heal over regardless of embolization material due to an aggressive clotting response. However, model aneurysms in canines do not show such amenable healing, and are therefore more representative of human aneurysms.

This pilot study was designed to examine sub-complete PPODA-QT delivery as well as coil placement followed by sub-complete delivery of PPODA-QT, because these delivery methods proved the most promising in the one month swine model study. While swine studies showed that attempting complete filling with PPODA-QT after coil placement resulted in excess polymer in the parent vessel, it was hypothesized that intentional sub-complete filling after coil placement may eliminate this problem.

Because of the small nature of the pilot study (n=3 animals per group), control groups, such as model aneurysms that were only embolized with coils or model aneurysms that were not embolized at all, were not included. Outcomes from these control cases in the canine model have been reported previously in the literature, and including them in the small pilot study would not provide sufficient new information to justify the use of these additional animals.

The results from the 6 month pilot study indicated that even though sub-complete filling was performed, aneurysms in the Coil+PPODA-QT group still exhibited excess PPODA-QT in the parent vessel. Due to the difficulty in seeing

PPODA-QT while the radiographically denser coil is in place means that monitoring polymer delivery is hindered. This difficulty suggests that delivering PPODA-QT after coil placement may have limited clinical applicability.

However, PPODA-QT delivered by itself, to 85-95% of the aneurysm volume, resulted in progressive occlusion and complete aneurysm obliteration after 6 months, in two of three aneurysms. The two successfully healed aneurysms displayed near-complete neointimal tissue coverage in the neck region, as well as robust tissue thickness. One aneurysm in this group showed recanalization by 3 months, which was sustained, but not worsened, at 6 months post-embolization. This aneurysm also showed the lowest percent of tissue coverage over the aneurysm neck, and the lowest average tissue thickness measurements, correlating angiographic recanalization with poorer histology outcomes. Even though one of three aneurysms recanalized in the PPODA-QT group, this study reflects a recanalization rate within the range reported of coiled lateral wall aneurysms in canines. While this study alone does not fully answer efficacy questions, it does provide justification for further investigation of PPODA-QT as a treatment for cerebral aneurysms.

6.6 Future Work

The work presented here reflects efforts to develop a liquid-to-solid gelling polymer system optimized for cerebral aneurysm embolization. The results of this work suggest that PPODA-QT may be a clinically viable alternative to currently available treatments, and further investigation of this material is warranted. In order to adequately compare the PPODA-QT system with current treatment techniques, a larger scale pre-clinical animal study must be performed.

The animal model again must be rigorous, showing aneurysm healing similar to humans. The canine Y-type bifurcation aneurysm model may be even more clinically stringent than the lateral wall model, because it displays an anatomical situation representative of difficult-to-treat aneurysms in humans (Raymond et al. 2002; Raymond et al. 2008).

Such a study should compare PPODA-QT embolization with current treatments, namely standard coil embolization as well as Onyx embolizatin. The main outcomes to be measured should be relevant to clinical effectiveness and safety. Angiographic occlusion and recanalization rates should be assessed, as well as neointimal tissue coverage and thickness measurements, which are currently not tabulated on a regular basis for coiled and Onyx-treated aneurysms in animal models.

This larger pre-clinical study could also monitor other outcomes that are not related to effectiveness, but do carry important implications. For example, procedure time, ease of use, and material cost analysis could be reported. Long procedure times are undesirable for patients and hospitals. Monitoring this outcome could suggest a more alluring treatment option if procedural safety and efficacy results are comparable. Ease of use could be assessed by interviewing the neurointerventionalist after the procedure, which would give qualitative information about potential clinical acceptance. Finally, a cost comparison could be made similar to that done by Simon, Reig, et al. (2010), where the total cost of all consumables were tallied. This type of comparison would not address costs such as hospital stay or inpatient recovery time, which are applicable to human patients, but may also elucidate a more cost-effective treatment option if safety and efficacy outcomes are similar.

Overall, PPODA-QT has proven to be worthy of further investigation as a treatment option for cerebral aneurysms. The future study outlined here represents one way to showcase the benefits or uncover the drawbacks of using PPODA-QT in this manner. In any case, further preclinical studies with PPODA-QT should be undertaken because this system may provide a better treatment option for people with cerebral aneurysms.

REFERENCES

Abrahams JM, MS Forman, S Grady, and SL Diamond. 2001. Delivery of human vascular endothelial growth factor with platinum coils enhances wall thickening and coil impregnation in a rat aneurysm model. *American Journal of Neuroradiology* 22:1410-17.

Alexander MJ, and ME Tolbert. 2006. Targeting cerebral arteriovenous malformations for minimally invasive therapy. *Neurosurgery* 59 (S3):178-83.

Arthur AS, SA Wilson, S Dixit, and JD Barr. 2005. Hydrogel-coated coils for the treatment of cerebral aneurysms: Preliminary results. *Neurosurgical Focus* 18 (2):E1.

Becker TA, and DR Kipke. 2001. Flow properties of liquid calcium alginate polymer injected through medical microcatheters for endovascular embolization. *Journal of Biomedical Materials Research* 61:533-40.

Becker TA, MC Preul, WD Bichard, DR Kipke, and CG McDougall. 2005. Material for endovascular arteriovenous malformation embolization: Six-month results in an animal model. *Neurosurgery* 56:793-801.

Becker TA, MC Preul, WD Bichard, DR Kipke, and CG McDougall. 2007. Preliminary investigation of calcium alginate gel as a biocompatible material for endovascular aneurysm embolization *in vivo*. *Neurosurgery* 60:1119-28.

Bendszus M, AJ Bartsch, and L Solymosi. 2007. Endovascular occlusion of aneurysms using a new bioactive coil: A matched pair analysis with bare platinum coils. *Stroke* 38:2855-57.

Bendszus M, and L Solymosi. 2006. Cerecyte coils in the treatment of intracranial aneurysms: A preliminary clinical study. *American Journal of Neuroradiology* 27:2053-57.

Birdno M, and BL Vernon. 2004. Mechanical optimization of an arteriovenous malformation embolization material: A predictive model analysis. *Annals of Biomedical Engineering* 33:191-201.

Blakely B, BH Lee, CM Riley, R McLemore, CP Pathak, and BL Vernon. 2010. Formulation and characterization of radio-opaque conjugated *in situ* gelling materials. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 93B:9-17.

Boecher-Schwarz HG, K Ringel, L Kopacz, A Heimann, and O Kempski. 2000. *Ex vivo* study of the physical effect of coils on pressure and flow dynamics in experimental aneurysms. *American Journal of Neuroradiology* 21:1532-36.

Bouzeghrane F, O Naggara, DF Kallmes, A Berenstein, and J Raymond. 2010. *In vivo* experimental intracranial aneurysm models: A systematic review. *American Journal of Neuroradiology* 31:418-23.

Brilstra EH, GJ Rinkel, Y van der Graff, and WJ van Rooij. 1999. Treatment of intracranial aneurysms by embolization with coils: A systematic review. *Stroke* 30:470-76.

Busetti F, KL Linge, JW Blythe, and A Heitz. 2008. Rapid analysis of iodinated X-ray contrast media in secondary and tertiary treated wastewater by direct injection liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A* 1213:200-208.

Byrne JV, JKA Hope, N Hubbard, and J Morris. 1997. The nature of thrombosis induced by platinum and tungsten coils in saccular aneurysms. *American Journal of Neuroradiology* 18:29-33.

Byrne JV, AJ Molyneux, and PR Brennan. 1995. Embolisation of recently ruptured intracranial aneurysms. *Journal of Neurology, Neurosurgery, and Psychiatry* 59:616-20.

Cekirge S, I Saatci, S Geyik, K Yavuz, H Ozturk, and G Pamuk. 2006. Intrasaccular combination of metallic coils and Onyx liquid embolic agent for the endovascular treatment of cerebral aneurysms. *Journal of Neurosurgery* 105:706-12.

Chaloupka JC, DC Huddle, J Alderman, S Fink, R Hammond, and HV Vinters. 1999. A reexamination of the angiotoxicity of superselective injection of DMSO in the swine rete embolization model. *American Journal of Neuroradiology* 20:401-10.

Chun-Ho Yu S, and H Kin-Ming Cheng. 2004. Recent advances in neuro-endovascular therapy. *Annals of the College of Surgeons of Hong Kong* 8:49-54.

Chyatte D. 2003. Pathology of intracranial aneurysms. In *Management of Cerebral Aneurysms*, eds. PD Le Roux, HR Winn, and DW Newell, 89-98. Philadelphia: Saunders.

Cloft HJ. 2006. HydroCoil for endovascular aneurysm occlusion (HEAL) study: Perprocedural results. *American Journal of Neuroradiology* 27:289-92.

Cloft HJ. 2007. HydroCoil for endovascular aneurysm occlusion (HEAL) study: 3-6 month angiographic follow-up results. *American Journal of Neuroradiology* 28:152-54.

Cloft HJ, and DF Kallmes. 2004. Aneurysm packing with HydroCoil embolic system versus platinum coils: Initial clinical experience. *American Journal of Neuroradiology* 25:60-62.

Cognard C, A Weill, L Castaings, A Rey, and J Moret. 1998. Intracranial berry aneurysms: Angiographic and clinical results after endovascular treatment. *Radiology* 206:499-510.

Cognard C, A Weill, L Spelle, M Piotin, L Castaings, A Rey, and J Moret. 1999. Long-term angiographic follow-up of 169 intracranial berry aneurysms occluded with detachable coils. *Radiology* 212:348-56.

Dai D, YH Ding, MA Danielson, R Kadirvel, DA Lewis, HJ Cloft, and DF Kallmes. 2005. Histopathologic and immunohistochemical comparison of human, rabbit, and swine aneurysms embolized with platinum coils. *American Journal of Neuroradiology* 26:2560-68.

Dai D, YH Ding, MA Danielson, R Kadirvel, DA Lewis, HJ Cloft, and DF Kallmes. 2005. Modified histologic technique for processing metalic coil-bearing tissue. *American Journal of Neuroradiology* 26:1932-36.

Debrun GM, V Aletich, JI Ausman, F Charbel, and M Dujovny. 1997. Embolization of the nidus of brain arteriovenous malformations with n-butyl cyanoacrylate. *Neurosurgery* 40:112-21.

de Gast AN, A Soepboer, M Sluzewski, WJ van Rooij, and GN Beute. 2008. How long does it take to coil an intracranial aneurysm? *Neuroradiology* 50:53-56.

Dimmick S, M Jones, B Steinfort, C Pines, and K Faulder. 2009. Accuracy and interobserver reliability of three-dimensional rotational angiography versus mathematical models for volumetric measurement of intracranial aneurysms. *Journal of Clinical Neuroscience* 16:1195-98.

Duffner F, R Ritz, A Bornemann, D Freudenstein, H Wiendl, and R Siekmann. 2002. Combined therapy of cerebral arteriovenous malformations: Histological differences between a non-adhesive liquid embolic agent and n-butyl 2-cyanoacrylate (NBCA). *Clinical Neuropathology* 21:13-17.

Fanning NF, Z Berentei, PR Brennan, and J Thornton. 2007. HydroCoil as an adjuvant to bare platinum coil treatment of 100 cerebral aneurysms. *Neuroradiology* 49:139-48.

Fiorella D, FC Albuquerque, and CG McDougall. 2006. Durability of aneurysm embolization with Matrix detachable coils. *Neurosurgery* 58:51-59.

Fiorella D, FC Albuquerque, HH Woo, CG McDougall, and PA Rasmussen. 2006. The role of neuroendovascular therapy for the treatment of brain arteriovenous malformations. *Neurosurgery* 59 (S3):163-77.

Friedman JA, DA Nichols, FB Meyer, MA Pichelmann, JI McIver, LGI Toussaint, PL Axley, RDJ Brown. 2003. Guglielmi detachable coil treatment of ruptured saccular cerebral aneurysms: Retrospective review of a 10-year single-center experience. *American Journal of Neuroradiology* 24:526-33.

Gallas S, A Pasco, JP Cottier, J Gabrillargues, J Drouineau, C Cognard, and D Herbreteau. 2005. A multicenter study of 705 ruptured intracranial aneurysms treated with Guglielmi detachable coils. *American Journal of Neuroradiology* 26:1723-31.

Gao X, G Liang, Y Li, and Z Wu. 2010. Neuroform stent-assisted coiling of large and giant intracranial aneurysms: Clinical outcomes in 71 consecutive patients. *Neurology India* 58 (6):825-32.

German WJ, and SP Black. 1954. Experimental production of carotid aneurysms. The *New England Journal of Medicine* 250:104-106.

Geyik S, K Yavuz, S Cekirge, and I Saatci. 2007. Endovasular treatment of basilar and ICA termination aneurysms: Effects of the use of HydroCoils on treatment stability in a subgroup of patients prone to a higher recurrence rate. *Neuroradiology* 49:1015-21.

Guglielmi G, C Ji, TF Massoud, A Kurata, SP Lownie, F Vinuela, and J Robert. 1994. Experimental saccular aneurysms. *Neuroradiology* 36:547-50.

Hayakawa M, Y Murayama, GR Duckwiler, YP Gobin, G Guglielmi, and F Vinuela. 2000. Natural history of the neck remnant of a cerebral aneurysm treated with the Guglielmi detachable coil system. *Journal of Neurosurgery* 93:561-68.

Henkes H, S Fischer, W Weber, E Miloslavski, S Felber, S Brew, and D Kuehne. 2004. Endovascular coil occlusion of 1811 intracranial aneurysms: Early angiographic and clinical results. *Neurosurgery* 54:268-85.

Higashida RT, GB Hieshima, and W Halbach. 1991. Advances in the treatment of complex cerebrovascular disorders by interventional neurovascular techniques. *Circulation* 83 (2):196-206.

Hop JW, GJ Rinkel, A Algra, and J van Gijn. 1997. Case-fatality rates and functional outcome after subarachnoid hemorrhage: A systematic review. *Stroke* 28:660-64.

Hope JKA, JV Byrne, and AJ Molyneux. 1999. Factors influencing successful angiographic occlusion of aneurysms treated by coil embolization. *American Journal of Neuroradiology* 20:391-99.

Jahan R, Y Murayama, YP Gobin, GR Duckwiler, HV Vinters, and F Vinuela. 2001. Embolization of arteriovenous malformations with Onyx: Clinicopathological experience in 23 patients. *Neurosurgery* 48:984-97.

Kadirvel R, D Dai, YH Ding, MA Danielson, DA Lewis, HJ Cloft, and DF Kallmes. 2007. Endovascular treatment of aneurysms: Healing mechanisms in a swine model are associated with increased expression of matrix metalloproteinases, vascular cell adhesion molecule-1, and vascular

endothelial growth factor, and decreased expression of tissue inhibitors of matrix metalloproteinases. *American Journal of Neuroradiology* 28:849-56.

Kallmes DF, TA Altes, DA Vincent, HJ Cloft, HM Do, and ME Jensen. 1999. Experimental side-wall aneurysms: A natural history. *Neuroradiology* 41:338-41.

Kanaan Y, D Kaneshiro, K Fraser, D Wang, and G Lanzino. 2005. Evolution of endovascular therapy for aneurysm treatment. *Neurosurgical Focus* 18 (2):E2.

Kang HS, MH Han, TH Lee, YS Shin, HG Roh, BJ Kwon, SY Kim, and HS Byun. 2007. Embolization of intracranial aneurysms with hydrogel-coated coils: Result of a Korean multicenter trial. *Neurosurgery* 61 (1):51-59.

Kawanabe Y, A Sadato, W Taki, and N Hashimoto. 2001. Endovascular occlusion of intracranial aneurysms with Guglielmi detachable coils: Correlation between coil packing density and coil compaction. *Acta Neurochirurgica* 143:451-55.

Khurana VG, I Meissner, and FB Meyer. 2004. Update on genetic evidence for rupture-prone compared to rupture-resistant intracranial saccular aneurysms. *Neurosurgical Focus* 17 (5):E7.

Klisch J, A Turk, R Turner, HH Woo, and D Fiorella. 2011. Very late thrombosis of flow-diverting constructs after the treatment of large fusiform posterior circulation aneurysms. *American Journal of Neuroradiology* 32:627-32.

Koebbe CJ, E Veznedaroglu, P Jabbour, and RH Rosenwasser. 2006. Endovascular management of intracranial aneurysms: Current experience and future advances. *Neurosurgery* 59 (S3):93-102.

Kulcsár Z, E Houdart, A Bonafé, G Parker, J Millar, AJP Goddard, S Renowden, G Gál, B Turowski, K Mitchell, F Gray, M Rodriguez, R van den Berg, A Gruber, H Desal, I Wanke, and DA Rüfenacht. 2011. Intra-aneurysmal thrombosis as a possible cause of delayed aneurysm rupture after flow-diversion treatment. American *Journal of Neuroradiology* 32:20-25.

Kurre W, and J Berkefeld. 2008. Materials and techniques for coiling of cerebral aneurysms: How much scientific evidence do we have? *Neuroradiology* 50:909-27.

Lanzino G, Y Kanaan, P Perrini, H Dayoub, and K Fraser. 2005. Emerging concepts in the treatment of intracranial aneurysms: Stents, coated coils, and liquid embolic agents. *Neurosurgery* 57:449-59.

Liang G, X Gao, Z Li, X Wei, and H Xue. 2010. Neuroform stent-assisted coiling of intracranial aneurysms: A 5 year single-center experience and follow-up. *Neurological Research* 32:721-27.

Lieber BB, AK Wakhloo, R Siekmann, and MJ Gounis. 2005. Acute and chronic swine rete arteriovenous malformation models: Effect of ethiodol and glacial acetic acid on penetration, dispersion, and injection force of N-butyl 2-cyanoacrylate. *American Journal of Neuroradiology* 26:1707-14.

Linfante I, and AK Wakhloo. 2007. Brain aneurysms and arteriovenous malformations: Advancements and emerging treatments in endovascular embolization. *Stroke* 38:1411-17.

Lohani B. 2004. Fusiform middle cerebral artery aneurysm. *Journal of Neuroscience* 1:50-52.

Lylyk P, C Miranda, R Ceratto, A Ferrario, E Scrivano, HR Luna, AL Berez, Q Tran, PK Nelson, and D Fiorella. 2009. Curative endovascular reconstruction of cerebral aneurysms with the Pipeline embolization device: The Buenos Aires experience. *Neurosurgery* 64:632-43.

Macdonald RL, S Mojtahedi, L Johns, A Kowalczuk, and JP Muizelaar. 1998. Randomized comparison of Guglielmi detachable coils and cellulose acetate polymer for treatment of aneurysms in dogs. *Stroke* 29:478-86.

Mawad ME, S Cekirge, E Ciceri, and I Saatci. 2002. Endovascular treatment of giant and large intracranial aneurysms by using a combination of stent placement and liquid polymer injection. *Journal of Neurosurgery* 96:474-82.

Mawad ME, JK Mawad, J Cartwright, and Z Gokaslan. 1995. Long-term histopathologic changes in canine aneurysms embolized with Guglielmi detachable coils. *American Journal of Neuroradiology* 16:7-13.

Meng H, Z Wang, Y Hoi, L Gao, E Metaxa, DD Swartz, and J Kolega. 2007. Complex hemodynamics at the apex of an arterial bifurcation induces vascular remodeling resembling cerebral aneurysm initiation. *Stroke* 38:1924-31.

Metcalfe A, AC Desfaits, I Salazkin, LH Yahia, WM Sokolowski, and J Raymond. 2003. Cold hibernated elastic memory foams for endovascular interventions. *Biomaterials* 24:491-97.

McKissock W, A Richardson, and L Walsh. 1965. Anterior communicating aneurysms: A trial of conservative and surgical treatment. *Lancet* 1:873-76.

McLemore R, EJ Kim, TA Brandon, G Aerni, KH Roy, and B Vernon. 2005. Tubal sterilization with a waterborne polyethylene glycol *in situ* cross-linking material: A minimally invasive approach. *Fertility and Sterility* 83 (S1):1284-92.

McLemore R, BH Lee, and B Vernon. 2009. Surfactant effects on the kinetics of Michael-type addition reaction in reverse emulsion polymeric systems. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 89B:191-98.

McLemore R, MC Preul, and B Vernon. 2006. Controlling delivery properties of a waterborne, *in-situ*-forming biomaterial. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 79B:398-410.

Molyneux AJ. 2002. International Subarachnoid Aneurysm Trial (ISAT) of neurosurgical clipping versus endovascular coiling in 2143 patients with ruptured intracranial aneurysms: A randomised trial. *Lancet* 360:1267-74.

Molyneux AJ, S Cekirge, I Saatci, and G Gal. 2004. Cerebral aneurysm multicenter European Onyx (CAMEO) trial: Results of a prospective observational study in 20 European centers. *American Journal of Neuroradiology* 25:39-51.

Moret J, C Cognard, A Weill, L Castaings, and A Rey. 1997. Reconstruction technic in the treatment of wide-neck intracranial aneurysms: Long-term angiographic and clinical results. *Journal de Neuroradiologie* 24 (1):30-44.

Moret J, L Pierot, and A Boulin. 1994. "Remodeling" of the arterial wall of the parent vessel in the endovascular treatment of intracranial aneurysms. *Neuroradiology* 36 (S1):S83.

Murayama Y, YL Nien, GR Duckwiler, YP Gobin, R Jahan, J Frazee, N Martin, F Vinuela. 2003. Guglielmi detachable coil embolization of cerebral aneurysms: 11 years' experience. *Journal of Neurosurgery* 98:959-66.

Murayama Y, S Tateshima, N Gonzalez, and F Vinuela. 2003. Matrix and bioabsorbable polymeric coils accelerate healing of intracranial aneurysms: Long-term experimental study. *Stroke* 34:2031-37.

Murayama Y, F Vinuela, S Tateshima, F Vinuela Jr., and Y Akiba. 2000. Endovascular treatment of experimental aneurysms by use of a combination of liquid embolic agents and protective devices. *American Journal of Neuroradiology* 21:1726-35.

Murayama Y, F Vinuela, A Ulhoa, Y Akiba, GR Duckwiler, YP Gobin, HV Vinters, and RJ Greff. 1998. Nonadhesive liquid embolic agent for cerebral arteriovenous malformations: Preliminary histopathological studies in swine rete mirabile. *Neurosurgery* 43:1164-72.

Nemir S, and JL West. 2010. Synthetic materials in the study of cell response to substrate rigidity. *Annals of Biomedical Engineering* 38 (1):2-20.

Niimi Y, J Song, M Madrid, and A Berenstein. 2006. Endosaccular treatment of intracranial aneurysms using Matrix coils: Early experience and midterm follow-up. *Stroke* 37:1028-32.

Ortega J, D Maitland, T Wilson, W Tsai, O Savas, and D Saloner. 2007. Vascular dynamics of a shape memory polymer foam aneurysm treatment technique. *Annals of Biomedical Engineering* 35:1870-84.

Pamuk AG, I Saatci, S Cekirge, and U Aypar. 2005. A contribution to the controversy over dimethyl sulfoxide toxicity: Anesthesia monitoring results in patients treated with Onyx embolization for intracranial aneurysms. *Neuroradiology* 47:380-86.

Piotin M, S Mandai, KJ Murphy, K Sugiu, P Gailloud, JB Martin, and DA Rufenacht. 2000. Dense packing of cerebral aneurysms: An *in vitro* study with detachable platinum coils. *American Journal of Neuroradiology* 21:757-60.

Piske RL, LH Kanashiro, E Paschoal, C Agner, SS Lima, and PH Aguiar. 2009. Evaluation of Onyx HD-500 Embolic System in the treatment of 84 wide-neck intracranial aneurysms. *Neurosurgery* 64:E865-E875.

Prestigiacomo C. 2006. Surgical endovascular neuroradiology in the 21st century: What lies ahead? *Neurosurgery* 59 (S3):48-55.

Raftopoulos C, P Mathurin, D Boscherini, RF Billa, M Van Boven, and P Hantson. 2000. Prospective analysis of aneurysm treatment in a series of 103 consecutive patients when endovascular embolization is considered the first option. *Journal of Neurosurgery* 93:175-82.

Raymond J, T Darsaut, I Salazkin, G Gevry, and F Bouzeghrane. 2008. Mechanism of occlusion and recanalization in canine carotid bifurcation aneurysms embolized with platinum coils: An alternative concept. *American Journal of Neuroradiology* 29:745-52.

Raymond J, F Guilbert, A Weill, SA Georganos, L Juravsky, A Lambert, J Lamoureux, M Chagnon, and D Roy. 2003. Long-term angiographic recurrences after selective endovascular treatment of aneurysms with detachable coils. *Stroke* 34:1398-1403.

Raymond J, A Metcalfe, AC Desfaits, E Ribourtout, I Salazkin, K Gilmartin, G Embry, and RJ Boock. 2003. Alginate for endovascular treatment of aneurysms and local growth factor delivery. *American Journal of Neuroradiology* 24:1214-21.

Raymond J, I Salazkin, SA Georganos, F Guilbert, AC Desfaits, G Gevry, A Weill, and D Roy. 2002. Endovascular treatment of experimental wide neck aneurysms: Comparison of results using coils or cyanoacrylate with the assistance of an aneurysm neck bridge device. *American Journal of Neuroradiology* 23:1710-16.

Raymond J, I Salazkin, G Gevry, and TN Nguyen. 2007. Interventional neuroradiology: The role of experimental models in scientific progress. *American Journal of Neuroradiology* 28:401-405.

Ries T, and C Groden. 2009. Endovascular treatment of intracranial aneurysms: Long-term stability, risk factors for recurrences, retreatment and follow-up. *Clinical Neuroradiology* 19:62-72.

Riley CM, R McLemore, MC Preul, and BL Vernon. 2011. Gelling process differences in reverse emulsion, *in situ* gelling polymeric materials for intracranial aneurysm embolization, formulated with injectable contrast agents. *Journal of Biomedical Materials Research - Part B: Applied Biomaterials* 96B:47-56.

Roy D, G Milot, and J Raymond. 2001. Endovascular treatment of unruptured aneurysms. *Stroke* 32:1998-2004.

Rudnick MR, S Goldfarb, L Wexler, PA Ludbrook, MJ Murphy, EF Halpern, JA Hill, M Winniford, MB Cohen, and DB VanFossen. 1995. Nephrotoxicity of ionic and nonionic contrast media in 1196 patients: A randomized trial. *Kidney International* 47:254-61.

Schirmer CM, V Zerris, and AM Malek. 2006. Electrocautery-induced ignition of spark showers and self-sustained combustion of onyx ethylene-vinyl alcohol copolymer. *Neurosurgery* 59:ONS413-ONS418.

Simon SD, E Eskioglu, AS Reig, and RA Mericle. 2010. Endovascular treatment of side wall aneurysms using a liquid embolic agent: A US single-center prospective trial. *Neurosurgery* 67:855-60.

Simon SD, AS Reig, RF James, P Reddy, and RA Mericle. 2010. Relative cost comparison of embolic materials used for treatment of wide-necked intracranial aneurysms. *Journal of NeuroInterventional Surgery* 2:163-67.

Slob M, M Sluzewski, and WJ van Rooij. 2005. The relation between packing and reopening in coiled intracranial aneurysms: A prospective study. *Neuroradiology* 47:942-45.

Sluzewski M, T Menovsky, WJ van Rooij, and D Wijnalda. 2003. Coiling of very large or giant cerebral aneurysms: Long-term clinical and serial angiographic results. *American Journal of Neuroradiology* 24:257-62.

Sluzewski M, WJ van Rooij, GJ Rinkel, and D Wijnalda. 2003. Endovascular treatment of ruptured intracranial aneurysms with detachable coils: Long-term clinical and serial angiographic results. *Radiology* 227:720-24.

Sluzewski M, WJ van Rooij, M Slob, JO Bescos, CH Slump, and D Wijnalda. 2004. Relation between aneurysm volume, packing, and compaction in 145 cerebral aneurysms treated with coils. *Radiology* 231:653-58.

Small W, PR Buckley, T Wilson, WJ Benett, J Hartman, D Saloner, and DJ Maitland. 2007. Shape memory polymer stent with expandable foam: A new concept for endovascular embolization of fusiform aneurysms. *IEEE Transactions on Biomedical Engineering* 54:1157-60.

Soga Y, MC Preul, M Furuse, TA Becker, and CG McDougall. 2004. Calcium alginate provides a high degree of embolization in aneurysm models: A specific comparison to coil packing. *Neurosurgery* 55:1401-1409.

Song DL, B Leng, LF Zhou, YX Gu, and XC Chen. 2004. Onyx in treatment of large and giant cerebral aneurysms and arteriovenous malformations. *Chinese Medical Journal* 117:1869-72.

Stedman T. 2000. *Stedman's Medical Dictionary.* 27th ed. Baltimore: Lippincott Williams & Wilkins.

Struffert T, C Roth, B Romeike, IO Grunwald, and W Reith. 2008. Onyx in an experimental aneurysm model: Histological and angiographic results. *Journal of Neurosurgery* 109:77-82.

Taha MM, I Nakahara, T Higashi, Y Iwamuro, M Iwaasa, Y Watanabe, K Tsunetoshi, and T Munemitsu. 2006. Endovascular embolization vs surgical clipping in treatment of cerebral aneurysms: Morbidity and mortality with short-term outcome. *Surgical Neurology* 66:277-84.

Takao H, Y Murayama, I Yuki, T Ishibashi, M Ebara, K Irie, H Yoshioka, Y Mori, F Vinuela, and T Abe. 2009. Endovascular treatment of experimental aneurysms using a combination of thermoreversible gelation polymer protection devices: Feasibility study. *Neurosurgery* 65:601-609.

Tamatani S, Y Ito, T Abe, T Koike, S Takeuchi, and R Tanaka. 2002. Evaluation of the stability of aneurysms after embolization using detachable coils: Correlation between stability of aneurysms and embolized volume of aneurysms. *American Journal of Neuroradiology* 23:762-67.

Taschner CA, X Leclerc, H Rachdi, AM Barros, and JP Pruvo. 2005. Matrix detachable coils for the endovascular treatment of intracranial aneurysms: Analysis of early angiographic and clinical outcomes. *Stroke* 36:2176-80.

van Rooij WJ, and M Sluzewski. 2007. Coiling of very large and giant basilar tip aneurysms: Midterm clinical and angiographic results. *American Journal of Neuroradiology* 28:1405-1408.

van Rooij WJ, and M Sluzewski. 2010. Perforator infarction after placement of a pipeline flow-diverting stent for an unruptured A1 aneurysm. *American Journal of Neuroradiology* 31:E43-E44.

Velat GJ, JF Reavey-Cantwell, C Sistrom, D Smullen, GL Fautheree, J Whiting, SB Lewis, RA Mericle, CS Firment, and BL Hoh. 2008. Comparison of n-butyl cyanoacrylate and ONYX for the embolization of intracranial arteriovenous malformations: Analysis of fluoroscopy and procedure times. *Neurosurgery* 63:ONS75-ONS82.

Vernon B, F Fusaro, B Borden, and KH Roy. 2004. Partition-controlled progesterone release from waterborne, *in situ*-gelling materials. *International Journal of Pharmaceutics* 274:191-200.

Vernon B, N Tirelli, T Bachi, D Haldimann, and JA Hubbell. 2003. Water-borne, *in situ* crosslinked biomaterials from phase-segregated precursors. *Journal of Biomedical Materials Research* 64A:447-56.

Wakhloo AK, MJ Gounis, JS Sandhu, N Akkawi, AE Schenck, and I Linfante. 2007. Complex-shaped platinum coils for brain aneurysms: Higher packing density, improved biomechanical stability, and midterm angiographic outcome. *American Journal of Neuroradiology* 28:1395-4000.

Walcott BP, JM Pisapia, BV Nahed, KT Kahle, and CS Ogilvy. 2011. Early experience with flow diverting endoluminal stents for the treatment of intracranial aneurysms. *Journal of Clinical Neuroscience* 18:891-94.

Weber W, B Kis, R Siekmann, P Jans, R Laumer, and D Kuhne. 2007. Preoperative embolization of intracranial arteriovenous malformations with Onyx. *Neurosurgery* 61:244-54.

Weber W, R Siekmann, B Kis, and D Kuhne. 2005. Treatment and follow-up of 22 unruptured wide-necked intracranial aneurysms of the internal carotid artery with Onyx HD 500. *American Journal of Neuroradiology* 26:1909-15.

Wells-Roth D, A Biondi, V Janardhan, K Chapple, YP Gobin, and HA Riina. 2005. Endovascular procedures for treating wide-necked aneurysms. *Neurosurgical Focus* 18(2):E7.

White PM, and J Raymond. 2008. Endovascular coiling of cerebral aneurysms using "bioactive" or coated-coil technologies: A systematic review of the literature. *American Journal of Neuroradiology* 30:219-26.

Wolf GL, RL Arenson, and AP Cross. 1989. A prospective trial of ionic vs nonionic contrast agents in routine clinical practice: Comparison of adverse effects. *American Journal of Roentgenology* 152:939-44.

Youn SO, JI Lee, JK Ko, TH Lee, and CH Choi. 2010. Endovascular treatment of wide-necked intracranial aneurysms using balloon-assisted technique with HyperForm balloon. *Journal of Korean Neurosurgical Society* 48:207-12.

APPENDIX A

IACUC PROTOCOL APPROVAL

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
Tempe, Arizona 85287-1103
Phone: (480) 965-2179 *FAX:* (480) 965-7772

Animal Protocol Review

ASU Protocol Number: **11-1152TK**
Protocol Title: **"Liquid-to-Solid Gelling Polymer System for Intracranial Aneurysm Embolization"**
Principal Investigator: **Brent Vernon**
Date of Action: **09/03/2010**

The animal protocol review was considered by the Committee and the following decisions were made:

- ☐ The original protocol was APPROVED as presented.
- ☐ The revised protocol was APPROVED as presented.
- ☐ The protocol was APPROVED with RESTRICTIONS or CHANGES as noted below. The project can only be pursued, subject to your acceptance of these restriction or changes. If you are not agreeable, contact the IACUC Chairperson immediately.
- ☐ The Committee requests CLARIFICATIONS or CHANGES in the protocol as described in the attached memorandum. The protocol will be considered when these issues are clarified and the revised protocol is submitted.
- ☐ The protocol was approved, subject to the approval of a WAIVER of provisions of NIH policy as noted below. Waivers require written approval from the granting agencies.
- ☐ The protocol was DISAPPROVED for reasons outlined in the attached memorandum.
- ☐ The Committee requests you to contact _____ to discuss this proposal.
- ☐ A copy of this correspondence has been sent to the Vice President for Research.
- ☒ This tracking protocol references research in collaboration with Celeste Riley (ASU) and Mark Preul (BNI). BNI Protocol Number 388.

RESTRICTIONS, CHANGES OR WAIVER REQUIREMENTS:
Approval Period: **2/26/2010 – 2/25/2013**

Signature: _____ Date: 9/2/10
IACUC Chair or Designee

Original: Principal Investigator
Cc: IACUC Office
IACUC Chair

APPENDIX B

BNI PROTOCOL NUMBER 388

St. Joseph's Hospital and Medical Center
A member of CHW

Institutional Animal Care and Use Committee

APPROVAL FORM

Protocol # 388 Animal Welfare Assurance # A3519-01

Grant #

Investigator(s): Dr. Mark Preul

Title of Project "Advanced polymer for endovascular embolization

Species and Numbers of Animals: Swine - 21
Canines - 21

This is to certify that the project identified above has been reviewed by the Institutional Animal Care and Use Committee which has considered specifically the compliance with applicable requirements of the Animal Welfare Act and pertinent state and local laws, regulations and adherence to the PHS Policy, and NIH Guide.

The proposed study has been approved by the IACUC and complies with the institutional assurance certification of the Barrow Neurological Institute of St. Joseph's Hospital and Medical Center.

Unless otherwise stated, this protocol has been approved by the Committee for a period of three years from the date noted below. The protocol is also subject to annual review on or before this date by this Committee.

Date of Committee Approval: February 26, 2010

COMMITTEE APPROVAL:

Thomas M. Hamm, Ph.D. Chairman - Institutional Animal Care and Welfare Committee

INSTITUTIONAL APPROVAL:

Ronald J. Lukas, Ph.D. Vice President of Research

c: Principal Investigator: You are reminded that modifications of any type in the above research project pertaining to animal experimentation requires re-review by this Committee.

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APPENDIX C

STATEMENT OF PERMISSTION FROM CO-AUTHORS

Co-authors on the previously published or submitted articles “Gelling Process Differences in Reverse Emulsion, *In Situ* Gelling Polymeric Materials for Intracranial Aneurysm Embolization, Formulated with Injectable Contrast Agents” and “*In Vitro* Delivery, Cytotoxicity, Swelling, and Degradation Behavior of a Liquid-to-Solid Gelling Polymer System for Cerebral Aneurysm Embolization” have granted their permission for use of the articles in this dissertation.