A Cross-linking Polymer System for Cerebral Aneurysm Embolization: Formulation, Characterization, and Testing

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Statement of Purpose: Cerebral aneurysms, characterized by a weakened and dilated portion of an artery in the brain, represent a significant risk of subarachnoid hemorrhage and death upon rupturing. In fact, subarachnoid hemorrhage results in death 50% of the time [1]. With 1 in every 15 Americans at risk of developing a cerebral aneurysm, better treatments for this condition are in high demand. We have developed an *in situ* cross-linking polymer system for aneurysm embolization. The studies reported here detail the formulation, characterization, and initial *in vivo* testing that have brought this system closer to a clinical reality.

Methods:

Formulation: Poly(propylene glycol) diacrylate (PPODA, Mw 900) and pentaerythritol (tetrakis 3-mercaptopropionate) (QT) both from Sigma (St. Louis, MO) were aliquoted in equimolar ratios into 3cc or 1cc syringes, and syringe mixed. For these molecules to react via Michael Type addition, a high-pH basic solution was incorporated at 25% wt. A liquid contrast agent was pH-adjusted to with NaOH to an appropriate level. Conray (Mallinckrodt, St. Louis, MO) at pH 11.2 or Omnipaque 300 (GE Healthcare, Princeton, NJ) at pH 12.6 was syringe-mixed with the organic monomers. Addition of Conray or Omnipaque provided radiographic contrast, which is essential for clinical use of an embolic material.

Characterization: In order to determine each formulation's suitability for aneurysm embolization, we investigated water uptake characteristics and *in vitro* cytotoxicity. Water uptake measurements consisted of monitoring the increase in gel weight when samples were kept in 37°C PBS, replaced every two weeks. Water uptake ratio (u) was calculated by: (wet weight – initial weight) ÷ initial weight. *In vitro* cytotoxicity was performed by preparing PPODA-QT gels with Conray or Omnipaque, mixed for 0.5 and 1.5 minutes. Samples were then injected into 0.8µm inserts, and placed in contact with 3T3 fibroblasts. A cell proliferation assay was performed after 3 days to determine the how gels affected cell growth.

Testing: Initial *in vivo* testing was performed in swine using the carotid artery sidewall aneurysm model [2]. Only the least toxic polymer formulation was used for *in vivo* studies. Embolization was performed with balloon protection, and angiograms were taken immediately post-embolization. After the survival period of 1 month, another angiogram was done to determine degree of occlusion. Samples were explanted and examined histologically with H&E and Masson's Trichrome stains.

Results: Results from the water uptake analysis showed that Conray-formulated gels take up more water than Omnipaque-formulated gels when mixed for 0.5 minutes (50±0.9% vs. 34±0.6%) and 1.5 minutes (44±0.2% vs. 38±0.7%), respectively. Results from cytotoxicity indicate that Omnipaque-formulated gels are more detrimental to 3T3 fibroblast growth, shown in Figure 1.

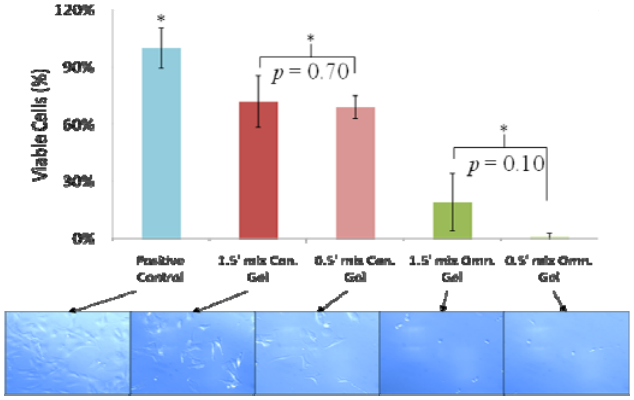

Figure 1: Results from cytotoxicity assay. Conray-formulated gels result ~70% cell viability, while Onmipaque-formulated gels result in <20% cell viability.

Initial 1-Month *in vivo* testing found that using the polymer system formulated with Conray is effective for occluding experimental carotid artery aneurysms in swine. The table below highlights overall findings.

Total # Treated	Total # Surviving	Survival Rate	Raymond-Roy Classification		Cell Layer (Y/N)
			30-min	1 Mo.	
5	3	60%	1, 1, 1	1, 1, 1	Y, Y, Y

While only 60% of the animals survived, the premature loss of 2 animals was a result of filling technique. In the two non-surviving animals, aneurysms were accidentally overfilled, resulting in stretching and eventual rupture (within 3 days of procedure) after material gelation. Subsequent procedures were done with particular care to not overfill aneurysms. Of the 1-month survivals, all showed a Raymond-Roy classification of 1, meaning complete occlusion, at 1 month [3]. Histology samples showed the presence of a neointimal cell layer over the aneurysm neck in all animals.

Conclusions: Characterization of the polymer system formulated with Conray and Omnipaque showed that while Conray-formulated gels swell more, they are less cytotoxic to 3T3 fibroblasts, and are therefore a better candidate for *in vivo* testing. Initial *in vivo* testing showed promising results, with experimental aneurysms maintaining complete occlusion after 1 month and developing neointimal tissue covered by a layer of endothelial cells, which will protect the aneurysm from recanalization and rupture [4]. Future studies will include longer-term (6-month) survival studies to further characterize biocompatibility and *in vivo* response.

References:

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