Developing a Liquid Embolic Agent: Radiopacity, Injectability and Cytocompatibility Studies

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Introduction:

Embolization is a minimally invasive interventional radiology procedure which results in occlusion of selective arteries. This procedure is performed to infarct tumors, reduce blood supply to highly vascularized lesions before surgical obliteration, control hemorrhage, and treat arteriovenous malformations or aneurysm. Occlusion is achieved by catheterization under fluoroscopy and injection of liquid embolic agents that solidify in-situ. However, available liquid embolic agents have a wide range of safety and effectiveness limitations; they can glue the catheter to the blood vessel, they use highly toxic organic solvents, and they are intrinsically non-radiopaque.

We have recently focused on developing a novel liquid embolic agent. Our liquid embolic agent is comprised of sodium polyphosphate and calcium chloride aqueous solutions which, on contact, form a gel-like material. The objective of this study is to report our methods and results on optimizing the radiopacity, injectability and cytocompatibility of this liquid embolic agent.

Materials and Methods:

Sodium polyphosphates with different degrees of polymerization (D_p) were prepared through a condensation polymerization reaction of NaH_2PO_4 and KH_2PO_4. 1M $CaCl_2$, $SrCl_2$ and $BaCl_2$ solutions were used as the calcium, strontium and barium sources. Addition of these solutions to polyphosphate solution at divalent cation/phosphorus mole ratios higher than ~30% results in gel formation. Using Design-Expert® software, three design spaces were developed for three distinct polyphosphate D_p (short = 190±1, medium = 9,666±400, and long = 19,455±2234). Each design space had 20 design points to assess the effect and significance of three variables on the viscosity of the polyphosphate solutions, the resulting gel radiopacity and cytocompatibility. These three variables were polyphosphate concentration, Sr/P and Ba/P mole ratios. The polyphosphate solutions were preloaded with Ca, Sr and Ba to reach 15% divalent cation/phosphorus mole ratios. At this mole ratio, gel does not form and the viscosity of this preloaded solution was determined by a cone-plate rheometer at 25°C. Then, enough calcium was added to this preloaded polyphosphate solution to reach 50% divalent cation/phosphorus mole ratio, forming the gel. The radiopacity was determined by x-ray imaging of the mould containing the gels in conjunction with an aluminum step-wedge and image pro-plus® software to report the radiopacity of 1.82mm of each gel in millimeters of the aluminum step-wedge. For cytocompatibility studies, an MTT assay was performed on sample extracts that were collected from gels maintained in DMEM cell culture medium for 24 hours [1]. The cytocompatibility for each extract is reported in the %change from the control, i.e. DMEM. ANOVA analysis was carried out where p values lower than 0.05 were considered statistically significant.

Results:

Figure 1(a) shows a typical gel and Figure 1(b) shows an X-ray image of some gels compared to an aluminum step wedge. Brighter samples are more radiopaque, and as expected higher Sr/P or Ba/P mole ratios resulted in more radiopaque gels. The radiopacity results in each of design spaces were fitted by a model. As an example, Figure 1(c) shows the two factor interaction model that was fitted to the radiopacity results in the medium chain design space. In medium and long chain polyphosphates the radiopacity is only significantly affected by Sr/P and Ba/P mole ratios, but in

short chains the effect of polyphosphate concentration is also significant. As expected, the effect of Ba/P is more prominent than Sr/P on radiopacity of the gels.

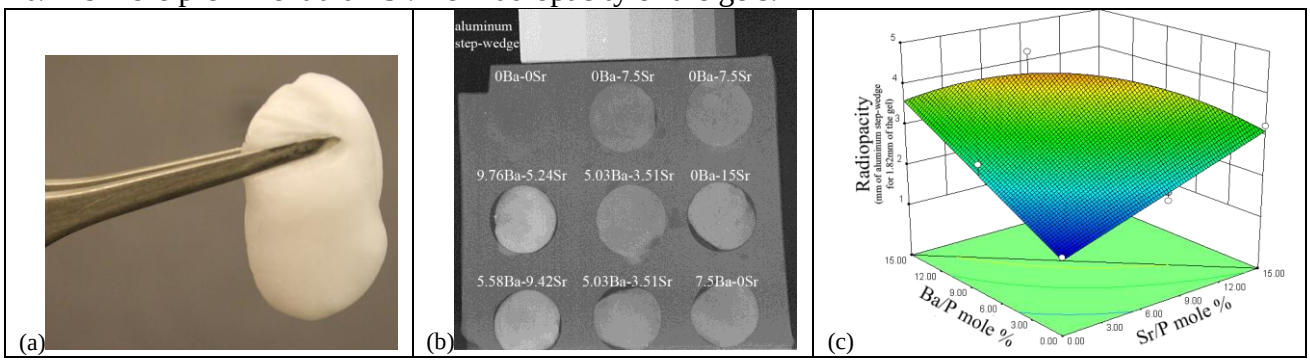

Figure 1: (a) Image of a gel, (b) X-ray image of gels compared to an aluminum step-wedge and (c) Radiopacity model produced by Design-Expert® for medium chain length polyphosphate at fixed [polyphosphate] of 3 g/mL.

Viscosity results of preloaded polyphosphate solutions were also fitted by models. Viscosity was significantly affected by all three factors; however, as could be seen in Figure 2(a), the effect of polyphosphate concentration is much more prominent. The viscosity was also highly dependent on the polyphosphate chain length; the highest viscosity for short, medium and long chain samples were 2.65, 28.29 and 67.05 cP, respectively.

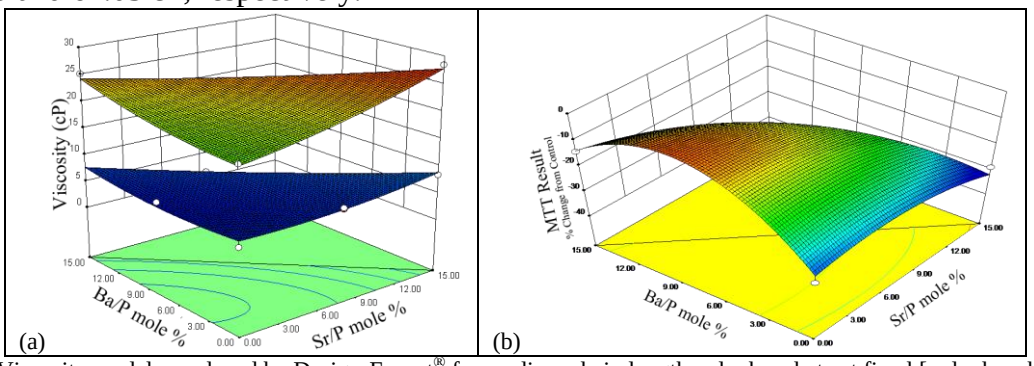

Figure 2: (a) Viscosity models produced by Design-Expert® for medium chain length polyphosphate at fixed [polyphosphate] of 3 and 12 g/mL (lower and upper 3D surfaces, respectively), (b) Cytocompatibility model produced by Design-Expert® for medium chain length polyphosphate at fixed [polyphosphate] of 12 g/mL.

In practically all cases, these gels were not deemed to be cytotoxic by ISO standard [1]. Figure 2(b) shows the quadratic model that was fitted to the MTT results of the medium chain samples. In all polyphosphate chain lengths, MTT results show that increasing Ba/P mole ratio significantly decreases the cytotoxicity of gels. In contrast, Sr/P mole ratio has only a significant effect on cytotoxicity in long chain samples. The effect of polyphosphate concentration on cytotoxicity is not significant. For medium and long chain gels the highest cytocompatibility is achieved around 9% Ba/P but in short chain gels the highest cytocompatibility is achieved at the highest Ba/P, i.e. 15%.

Discussion:

Viscosity results confirm that polyphosphate solutions are injectable through available catheters which are able to inject solutions with viscosities up to 120 cP. Addition of strontium and barium to the calcium polyphosphate liquid embolic agent significantly improves its radiopacity, distinguishing this system from other available embolic agents that require contrast agents. Nevertheless, we had a concern with the level of cytotoxicity that barium and strontium might induce into the system. Paradoxically, MTT results showed that replacing calcium with barium or strontium improves the cytocompatibility of the gels. We believe this observation is related to the higher stability of barium or strontium loaded gels, making their degradation rate studies the future focus of our research.

References: [1] ISO 10993-5:2009; Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity.