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Developing an *in situ* forming polyphosphate coacervate as a new liquid embolic agent: From experimental design to pilot animal study

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ABSTRACT

A radiopaque temporary liquid embolic agent was synthesized from polyphosphate (PP) coacervates and optimized using a design of experiments approach. Variables studied were: strontium substitution (0–15 mol%), barium substitution (0–15 mol%), PP concentration and degree of polymerization of the polyphosphate (D_p). The viscosity, radiopacity and cell viability of the resulting coacervates were measured for 60 formulations and response surface modeling was used to determine the optimum coacervate that maximized radiopacity and cell viability. The optimum coacervate made from PP with a large D_p (9.5 g NaPP/100 mL, 2.2 mol% Sr, 9 mol% Ba and 3.8 mol% Ca) was taken forward to a pilot animal trial. In this rabbit model, PP embolic agent successfully occluded the central auricular artery with promising biocompatibility. Further study is required to optimize the cohesiveness and clinical effectiveness of PP as an *in situ* setting temporary embolic agent.

Statement of significance

This article describes the development of a new radiopaque temporary liquid embolic agent from the optimization using *design of experiments* to a pilot animal study. Embolization is a minimally invasive interventional radiology procedure used to block blood flow in a targeted blood vessel. This procedure is used to treat many conditions including: tumors, aneurysms and arteriovenous malformations. Currently, no inherent radiopaque embolic agents are available in the clinic, which would allow for direct imaging of the material during the procedure and follow up treatment.

1. Introduction

Embolization is a minimally invasive interventional radiology procedure used to block blood flow in a targeted blood vessel. This procedure is performed to infarct tumors, reduce blood supply to highly vascularized lesions before surgical resection, control hemorrhage, treat arteriovenous malformations and fill or exclude aneurysms [1–5]. In all embolization procedures, a catheter is guided under fluoroscopy to the required blood vessel and the embolic agent is then injected to occlude the vessel. Liquid embolic agents conform to the shape of the vessel and solidify *in situ*. Solid embolic agents are carried with the blood stream and lodge in the vessel causing an occlusion; the level of the occlusion is determined by the particle size. Currently, most liquid embolic agents are permanent [6–9], unlike solid embolic agents, which are available for permanent or temporary occlusion. Catheter entrapment has been reported [10,11] with the current liquid embolic agents, some require the use of cytotoxic organic solvents that can cause acute vasospasm [12], and all require careful handling to ensure that the added contrast agent is well dispersed. Temporary embolic agents can be combined with a chemotherapeutic agent in a chemoembolization procedure, causing tumor shrinkage by targeted drug delivery and restricted nutrient supply. This procedure can be repeated if further tumor reduction is required. An intrinsically radiopaque embolic agent would not require radiopacifying

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agents while allowing for non-invasive follow up and monitoring of a temporary occlusion as it resorbs [13]. A new material is required: an aqueous, radiopaque, resorbable, *in situ*-forming embolic agent. Several resorbable aqueous-based liquid embolic agents are currently under investigation, including those based on alginate [14] and chitosan [15], but these require the addition of a contrast agent. We have developed radiopaque coacervates that have the ability to set *in situ* by a cation chelating reaction of polyphosphate and divalent cations.

Polyphosphate (PP) is a linear inorganic polymer formed of phosphate groups linked together by bridging oxygen atoms. This ubiquitous polymer is found in human platelets [16] and has a significant role in the coagulation process [17], which may be beneficial for an embolic agent. PP has been added, for example, to a chitosan based wound dressing to increase its hemostatic potential [18]. We have synthesized large quantities of PP with a wide range of degrees of polymerization (D_p) using phosphate glass reagents [19]. PP has also been shown to have a high ability to bind with divalent cations in solutions and to form coacervates [20]. The term coacervation is used to describe a process in which aqueous colloidal solutions separate upon alteration of the thermodynamic condition of state into two liquid phases: one rich in colloid, i.e. the coacervate, and the other containing little colloid [21]. In this study, we utilize this coacervation process to develop an aqueous, radiopaque, resorbable, *in situ*-forming embolic agent. This liquid embolic agent is comprised of two aqueous solutions; one consisting of sodium polyphosphate (NaPP) and the other containing divalent cations, which form a coacervate on contact. It is anticipated that these two solutions could be separately delivered to the injection site using a dual lumen catheter in order to form the coacervate *in situ*.

First, using design of experiments (DOE) and response surface modeling, we evaluated the radiopacity, injectability and cell viability of coacervate formulations as a function of PP D_p, PP concentration, and barium and strontium content. The optimum formulation was identified using the model. Subsequently, the clinical relevance of this optimized formulation was evaluated in a pilot animal study using a rabbit model where the *in vivo* biocompatibility analysis was carried out in conjunction with a proof-of-principle embolization of the central artery of the rabbit ear.

2. Materials and methods

2.1. Materials

NaPP with different D_p were prepared fresh and characterized as comprehensively reported by Momeni and Filiaggi [19]. Briefly, NaPP with D_p 190 (determined by liquid ^{31}P NMR; provided as supplementary material) was produced by holding $NaH_2PO_4 \cdot H_2O$ at 700 °C for 1 h to obtain a NaPP glass, followed by acetone fractionation to yield NaPPs with a narrow size distribution. NaPPs with D_p 10,000 and 19,000 (determined by viscosity measurements; provided as supplementary material) were produced by holding KH_2PO_4 at 775 °C, for 2 and 50 h respectively, to obtain a potassium Kurrol salt, followed by an ion exchange process to replace potassium with sodium. Following acetone fractionation or ion exchange, the resulting NaPP solutions were freeze-dried to yield NaPP powder that was subsequently stored at −20 °C for later studies. $CaCl_2 \cdot 2H_2O$, $SrCl_2 \cdot 6H_2O$ and $BaCl_2 \cdot 2H_2O$ were used as the sources for divalent cations for coacervate formation. All materials were of reagent grade and purchased from Sigma–Aldrich.

2.2. Design of experiments and formulation optimization

Using Design-Expert® V9 by StatsEase software, DOE was used to develop three design spaces for NaPP with three distinct D_p (small 190 ± 1, medium 10,000 ± 400 and large 19,000 ± 2000). Each design space had 20 design points (10 model points, 5 points to determine lack of fit and 5 points as replicates) to assess the effect and significance of three design variables (Ba content, Sr content and polyphosphate concentration) on three properties of the resulting coacervates (viscosity of the preloaded PP solutions, radiopacity and cell viability). All design spaces with respect to these variables, including their ranges and any imposed constraints, are described in Table 1. An IV-optimal design, which seeks to minimize the integral of the prediction variance across the design space, was used to determine the design points. After the results were collected, Design-Expert® was used to fit the data to a linear, a two-factor-interaction model or a quadratic model. With the resulting "best-fit" models, the significance of each variable was determined and terms that were not significant were removed. ANOVA was used to determine the significance of fit of the model in two ways; first, the model *F*-value compares model variance with residual variance and was calculated by dividing the model mean square by residual mean square. A large model *F*-value indicates that the data have been captured by the model. Secondly, the lack of fit *F*-value, that compares the residual error from the lack of fit points with the pure error from the replicated design points, was calculated by dividing lack of fit mean square by pure error mean square. A small lack of fit *F*-value indicates that the deviations between the data and model are due to background noise.

The optimum formulation was chosen by Design-Expert® using numerical optimization. Here, a weight was assigned to each goal (i.e. increasing radiopacity and cell viability, and decreasing viscosity) to adjust the shape of its particular desirability function, and the importance of each goal was adjusted in relation to the other goals through a power function. The goals were then combined into an overall desirability function and the software numerically maximized this function to find the optimum formulation for each design space.

2.3. Preparing formulations

NaPP weighing 145.1 mg was dissolved in deionized water and 1 M Sr, Ba and Ca chloride solutions were added to obtain the required formulation as specified by the DOE, reaching a 15% (Ca + Sr + Ba)/P mole ratio. At this molar ratio, the solution remained clear after mixing for 24 h, as the NaPP chains only chelate these cations. The desired PP concentration was achieved by adjusting the final volume with water to form PP solutions with

Table 1
Test parameters for the three design spaces for three different PP D_p.

	Design Space 1	Design Space 2	Design Space 3
Polyphosphate name D_p	Small 190 ± 1	Medium 10,000 ± 400	Large 19,000 ± 2000
Variable A: PP concentration (g/100 mL)	3–15	3–12	3–10
Variable B: Strontium to phosphorus mole ratio (%)	0–15	0–15	0–15
Variable C: Barium to phosphorus mole ratio (%)	0–15	0–15	0–15
Constraints (mol %)	$0 \leqslant B + C \leqslant 15$	$0 \leqslant B + C \leqslant 15$	$0 \leqslant B + C \leqslant 15$

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