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Synthesis of oligo(poly(ethylene glycol) fumarate)

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Abstract

This protocol describes the synthesis of oligo(poly(ethylene glycol) fumarate) (OPF) (1–35 kDa)(a polymer useful for tissue engineering applications) by a one-pot reaction of poly(ethylene glycol) (PEG) and fumaryl chloride. The procedure involves three parts: dichloromethane and PEG are first dried; the reaction step follows in which fumaryl chloride and triethylamine are added dropwise to a solution of PEG in dichloromethane; and finally the product solution is filtered to remove byproduct salt, and the OPF product is twice crystallized, washed, and dried under vacuum. The reaction is affected by PEG molecular weight and reactant molar ratio. The OPF product is cross-linked by radical polymerization by either a thermally induced or UV-induced radical initiator, and the physical properties of the OPF oligomer and resulting cross-linked hydrogel are easily tailored by varying PEG molecular weight. OPF hydrogels are injectable, polymerize in situ, and undergo biodegradation by hydrolysis of ester bonds. The expected time required to complete this protocol is 6 d.

Keywords

oligo(poly(ethylene glycol) fumarate); OPF; polymer; tissue engineering; polymer synthesis; radical polymerization; hydrogel; PEG

INTRODUCTION

PEG-based polymers for tissue engineering

PEG-based polymers have been developed for tissue engineering applications in the form of cross-linked hydrogels, because PEG is biocompatible, non-immunogenic, and bioinert (to cells, tissues, and drugs especially proteins making PEG-based hydrogels ideal for protein release purposes). Since PEG alone exhibits negligible affinity for cells and biological molecules, numerous specific modulators of cell and tissue behavior can be designed into PEG-based hydrogels by incorporation of cell-binding peptides or cell-modulating growth factors. The incorporation of peptides and/or growth factors can enhance the biological activity of PEG-based biomaterials for tissue-specific applications, an advantage in tissue

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Contributions

L.A.K., F.K.K., and A.G.M. optimized the procedure. L.A.K. drafted the manuscript. F.K.K. and A.G.M. supervised the project and critically revised the manuscript.

Competing financial interests

A.G.M. is inventor on a patent involving the material described in this protocol.

engineering. PEG hydrogels are not intrinsically degradable; however, degradable segments such as fumarates, polyesters, acetals, disulfides, and enzyme-sensitive peptides can be incorporated¹, making it possible to adjust the degradation rate of the material to fit its intended application and providing unique physicochemical properties. OPF is a PEG-based oligomer featuring degradable fumarate ester groups and is the focus of this protocol. Other PEG-based hydrogels featuring fumarate groups include poly(propylene fumarate-co-ethylene glycol)² and poly(lactide-co-ethylene oxide-co-fumarate)³ (The relative advantages and limitations are summarized in Table 1).

OPF and its applications

Oligo(poly(ethylene glycol) fumarate) is a linear polyester based on the condensation polymerization between PEG and fumaryl chloride in the presence of triethylamine as a proton scavenger (Figure 1). The condensation polymerization (carried out with the reactant feed ratio specified herein) preferably results in the formation of OPF with PEG end groups as demonstrated previously¹². The number of PEG molecules incorporated into the OPF oligomer depends strongly on steric considerations since higher molecular weight PEG molecules obstruct the addition of fumarate groups to their ends. Therefore, as PEG molecular weight increases, fewer PEG molecules are incorporated into each oligomer and more PEG remains unreacted. During hydrogel fabrication, OPF forms cross-links between fumarate double bonds in the oligomer backbone. The non-polarized double bond of the fumarate ester group in OPF is far less reactive than acrylic acid esters (e.g. PEG-diacrylate) or amides (e.g. *N,N'*-methylene bisacrylamide). Therefore cross-linker molecules are typically added to facilitate the cross-linking reaction and reduce the cross-linking time. An advantage of using OPF as a starting material for hydrogel fabrication, an advantage that applies to PEG-based polymers in general, is that the finite chain length of each PEG block results in a fixed distance between cross-links in OPF hydrogels with the assumption that all the fumarate segments are consumed in cross-linking. This makes control of the hydrogel structure highly precise and responsive to changes in the molecular weight of the PEG starting material⁴. OPF oligomers are composed of PEG-fumaric acid units that undergo ester hydrolysis to break down into their constituent parts. The body processes the fumaric acid byproducts by natural mechanisms since fumaric acid is intrinsically present as a component of the Krebs cycle. The minimal cytotoxicity of OPF oligomers and degradation products has been demonstrated experimentally⁵. Research studies have investigated the physicochemical properties of cross-linked OPF hydrogels^{4,13}. OPF has shown the capacity to support tissue formation in bone^{14,15}, cartilage¹⁶, osteochondral^{17–19}, tendon²⁰, cardiovascular²¹, ocular²², and neural tissue engineering²³. Furthermore, OPF hydrogels have supported the encapsulation of various cell types including mesenchymal stem cells²⁴, chondrocytes²⁵, embryonic stem cells²¹, primary tendon/ligament fibroblasts²⁶, pigment epithelial cells²², and Schwann cells²³. In addition to cell encapsulation, OPF hydrogels have been tested for the delivery of growth factors^{16–19,25,27–33}, plasmid DNA^{34–37}, small molecules²³, nanoparticles³⁸, and chemotherapeutics³⁹. Additional studies have demonstrated techniques for modifying OPF hydrogels with cell-binding moieties^{40–45} as well as with osteoconductive domains^{45–46}.

Overview of the synthesis of OPF

In preparation for the reaction step, various reagents (dichloromethane and PEG) must be treated to remove water in order to improve the reaction conversion and increase the molecular weight of the OPF product. The reaction step is prepared by combining anhydrous dichloromethane and dried PEG (toluene containing PEG melt). The reaction is carried out in an inert atmosphere (system is purged with nitrogen gas) in a single step by adding separate solutions of triethylamine and fumaryl chloride (in anhydrous dichloromethane) dropwise to the stirring solution of PEG at 0°C. After addition is completed, the reaction

mixture is left to stir at room temperature for 48 h as the reaction proceeds. Purification of the OPF product occurs through removal of dichloromethane by rotary evaporation under reduced pressure followed by removal of triethylamine hydrochloride salt by filtration after precipitating the salt in ethyl acetate at 40°C. Triethylamine hydrochloride salt is produced by the reaction between chloride ions from fumaryl chloride and triethylamine. Further purification of the OPF product involves twice crystallization of the oligomer in ethyl acetate by reducing the temperature of the solution to 0°C. Following filtration of the recrystallized mixture, the purified OPF product is washed in ethyl ether. Finally, the OPF product is dried under reduced pressure resulting in the formation of a powder. The timing of the procedure is summarized in Table 2.

Experimental design

This protocol describes the synthesis of OPF in a one-pot reaction. This protocol involves certain critical steps that are required in order to achieve high conversion and high molecular weight OPF oligomer product. The optimum ratio of moles of PEG, fumaryl chloride, and triethylamine should be used as indicated; reactants of high purity should be purchased and further purified as specified, and care should be taken to implement the reaction under an inert atmosphere to the exclusion of water. OPF produced using the methods described herein is suitable for investigational use in biomedical applications including formation of injectable, biodegradable hydrogels for cell encapsulation, as scaffolds for tissue engineering, and as drug delivery vehicles. The OPF oligomer consists only of biocompatible units, and therefore, the degradation products are anticipated to be biocompatible, and this has been demonstrated through *in vivo* studies^{6,14,18,21–23,30,31,34,37}.

The reactant ratios specified in this protocol were optimized experimentally by measuring the degree of oligomerization and conversion of OPF¹². Other reactant ratios could be used to achieve different end groups or alternative molecular weight distributions. Fumaryl chloride and triethylamine were reacted with PEG (1 mol PEG/0.9 mol fumaryl chloride and 1 mol fumaryl chloride/2 mol triethylamine). These ratios provide a 10% molar excess of PEG in the condensation reaction with fumaryl chloride and enough triethylamine to remove two chloride ions from each fumaryl chloride molecule. The molecular weight of the OPF oligomers depends on PEG molecular weight, reactant molar ratio, reactant purity, and reaction time. Typical number average and weight average molecular weights of OPF synthesized according to this protocol are listed in Table 3.

While this protocol focuses on the synthesis of OPF, a brief description of cross-linking methods is instructive. OPF hydrogels are formed by radical polymerization in the presence of either a thermally induced or UV-induced radical initiator. Thermally induced radical initiators used for OPF hydrogel cross-linking include the water-soluble redox pairs ammonium persulfate/*N,N,N',N'*-tetramethylethylenediamine^{15,27} and ammonium persulfate/ascorbic acid^{4,12}, but other combinations of oxidizing agents (sodium persulfate) and reducing agents (sodium ascorbate, magnesium ascorbate-2-phosphate) can be envisioned⁴⁷. UV-induced radical initiators used for OPF hydrogel cross-linking include bis(2,4,6-trimethylbenzyl)phenylphosphine oxide^{12,43} and Irgacure 2959^{26,48} although many photoinitiators are available. Additionally, cross-linking molecules are often used in OPF hydrogel fabrication to reduce cross-linking time and provide suitable handling characteristics for injectable applications. Cross-linkers used in OPF hydrogel fabrication include PEG-diacrylate ($M_n = 575$ Da, 3,400 Da)⁵, *N,N'*-methylene bisacrylamide^{27,28}, and *N*-vinylpyrrolidone⁴⁸. In this way, OPF hydrogels can be fabricated using a variety of formulations providing a wide range of tunable properties that can be controlled as needed for certain applications from cell encapsulation to therapeutic molecule delivery. One can find in the literature the hydrogel formulation that would be most appropriate for their specific application.

MATERIALS

Reagents

- Calcium hydride, 95% (Sigma-Aldrich Co., cat. no. 208027-100G)
- Dichloromethane, 99.5%, ACS certified (EMD, cat. no. DX0835-5)
- Ethyl acetate, 99.5%, ACS certified (Sigma-Aldrich Co., cat. no. 319902-4L)
- Ethyl ether (anhydrous), 99.0%, ACS certified (EMD, cat. no. EX0190-5)
- Fumaryl chloride, 94% (Acros, cat. no. 165975000)
- Indicating Drierite (W.A. Hammond Drierite Co., cat. no. 23005, 8 mesh)
- Nitrogen gas (N₂(gas)), ultra-high purity, 99.999% (Matheson Tri-Gas)
- Nitrogen liquid (Matheson Tri-Gas)
- Poly(ethylene glycol) (Sigma-Aldrich Co., cat. no. P3515-500G (1 kDa), P4338-500G (3 kDa), P6667-500G (10 kDa), 81310-1KG (35 kDa))
- Toluene, 99.5%, ACS certified (Fisher Scientific, cat. no. T324-4)
- Triethylamine, 99% (Sigma-Aldrich Co., cat. no. T0886-100ML)

Equipment

- Addition funnel, graduated with stopcock and pressure equalizing arm (125 mL) (Chemglass, cat. no. CG-1710-02) (2)
- Balloon
- Barrett distilling receiver with stopcock (20 mL) (Corning, cat. no. 3622-20)
- Beaker (1 L)
- Beaker (250 mL)
- Buchner funnel (550 mL) (Fisher Scientific, cat. no. 10-356E, CoorsTek no. 60244)
- Condenser (Chemglass, cat. no. CG-1218-07)
- Distillation adapter (Chemglass, cat. no. CG-1024-01)
- Drying tube, “U” shaped (Chemglass, cat. no. CG-1296-01)
- Filter paper (Whatman no. 40 Ashless Circles, cat. no. 1440 110)
- Flow control adapter glass stopcock (90°) (Wilma LabGlass, cat. no. IG-90-104)
- Glass stoppers
- Glass thermometer (10/30) (Wilma LabGlass, cat. no. LG-10515-106)
- GPC instrument (Waters 510 HPLC Pump equipped with a Waters Pump Control Module; Waters 717 Autosampler; Waters 486 Tunable Absorbance Detector; Waters 410 Differential Refractometer) (Waters)
- Graduated cylinder (1 L)
- Graduated cylinder (100 mL)
- Heating mantle (1000 mL) (Glas-Col, cat. no. O408)
- High vacuum pump

- Ice bath
- Insulating wool
- Laboratory clamps
- Large magnetic stir bars (PTFE)
- Magnetic stir plate
- One-necked round-bottomed flask (1 L) (2)
- One-necked round-bottomed flask (100 mL)
- Pipette tips (1 mL)
- Pipetter (1 mL)
- Plastic syringe (1 mL)
- Rotary evaporator (Buchi Rotavapor R-200, Buchi Vacuum Controller V-800, Buchi Vac V-500, Buchi Heating Bath B-490) (Buchi)
- Rubber fitting for Buchner funnel
- Rubber tubing
- Syringe needle (27G1/2)
- Three-necked round-bottomed flask (1 L)
- Three-way 120° angle connecting adapter (Corning, cat. no. 9021-24)
- Transformer (Warner Electric, cat. no. 3PN116C)
- Vacuum flask (1 L)
- Vacuum flask (2 L)
- Vacuum grease
- Vacuum rated desiccator (Corning, cat. no. 3121-150)
- Vacuum trap (Chemglass, cat. no. CG-4514-01)

Equipment Setup

Preparation of glassware

- Thoroughly clean and dry all glassware overnight (12 h) in an oven (100°C).

PROCEDURE

Synthesis of OPF: drying of dichloromethane TIMING ~3 h

- 1 Add calcium hydride (20 g) and dichloromethane (700 mL) into a one-necked round-bottomed flask (1 L) and fit the round-bottomed flask with a condenser and drying tube filled with drierite (Figure 2).

CAUTION Calcium hydride and dichloromethane are irritating and/or harmful if exposed to the skin or inhaled. Calcium hydride releases flammable gases upon contact with water. Conduct all work in a chemical fume hood. Proper personal protective equipment (lab coat, nitrile gloves, and safety glasses) should be worn throughout the procedure.

- 2 Stir the mixture using a magnetic stir bar.

- 3 Heat the reaction flask to 60°C using a heating mantle and reflux at 60°C for 2 h. Under these conditions, calcium hydride reacts with water to form calcium hydroxide and hydrogen gas. The hydrogen gas is allowed to escape from the system through a drying tube.
- 4 After 2 h, cool the reaction flask to room temperature (20–25°C).
PAUSE POINT Once cooled, the dichloromethane/calcium hydride mixture may be sealed and stored overnight in the fume hood, if necessary.

Synthesis of OPF: distillation of dichloromethane TIMING ~6 h

- 5 Attach the flask from Step 4 to the apparatus shown in Figure 3 with a 100 mL receiving flask.
- 6 Stir the mixture using a magnetic stir bar and heat the reaction flask to 80°C using a heating mantle.
- 7 Collect the first 30 mL distillate in the 100 mL receiving flask and then replace with a 1000 mL receiving flask.
CRITICAL STEP The first 30 mL distillate may contain residual water and should be discarded.
- 8 Continue heating until ~600 mL anhydrous dichloromethane has been collected in the receiving flask.
- 9 Once the desired amount of anhydrous dichloromethane has been collected, cool the reaction flask to room temperature.
- 10 Once cooled, seal the flask with the remaining mixture from step 1 and store in the fume hood awaiting either reuse of unreacted calcium hydride or disposal.
PAUSE POINT The receiving flask containing the anhydrous dichloromethane distillate may be sealed and stored in the fume hood until Step 17, if necessary.

Synthesis of OPF: drying of PEG TIMING ~2 h

- 11 Add PEG (50 g) (1 kDa, 3 kDa, 10 kDa, or 35 kDa depending on the synthesis plan) and toluene (200 mL) into a one-necked round-bottomed flask (1 L) (Figure 4). (Note: This protocol will produce ~40 g OPF.)
CAUTION Toluene is irritating and/or harmful if exposed to the skin or inhaled. Conduct all work in a chemical fume hood. Proper personal protective equipment (lab coat, nitrile gloves, and safety glasses) should be worn throughout the procedure.
- 12 Stir the mixture using a magnetic stir bar.
- 13 Heat the reaction flask to 170°C using a heating mantle.
- 14 Measure the distillate using the Barrett distilling receiver and collect the distillate into a 250 mL beaker for disposal.
- 15 Continue heating until 180 mL distillate has been collected.
- 16 Once the desired amount of distillate has been collected, cool the reaction flask to room temperature and collect any subsequent distillate.
PAUSE POINT Once cooled, the reaction flask may be sealed and stored in the fume hood until Step 17, if necessary.

Synthesis of OPF: initiate reaction TIMING ~4.5 h

- 17 Add 320 mL anhydrous dichloromethane to the dried PEG (Figure 5).
- 18 Stir the mixture using a magnetic stir bar until the PEG dissolves.
- 19 Transfer the PEG solution to a three-necked round-bottomed flask (1 L).
- 20 Mix anhydrous fumaryl chloride (1 mol PEG/0.9 mol fumaryl chloride) with 30 mL anhydrous dichloromethane in an addition funnel with a stopcock and pressure equalizing arm. (PEG [mol] = 50 g / M_n [g/mol]) (Note: Fumaryl chloride is always used in the anhydrous state since it reacts with water to form HCl and fumaric acid. Fumaryl chloride is distilled at 161°C, purified from its byproducts, and stored under nitrogen prior to its use.)

CAUTION Fumaryl chloride is irritating and/or harmful if exposed to the skin or inhaled. Conduct all work in a chemical fume hood. Proper personal protective equipment (lab coat, nitrile gloves, and safety glasses) should be worn throughout the procedure.

- 21 Mix triethylamine (1 mol fumaryl chloride/2 mol triethylamine) with 30 mL anhydrous dichloromethane in a second addition funnel with a stopcock and pressure equalizing arm.

CAUTION Triethylamine is irritating and/or harmful if exposed to the skin or inhaled. Conduct all work in a chemical fume hood. Proper personal protective equipment (lab coat, nitrile gloves, and safety glasses) should be worn throughout the procedure.

CRITICAL STEP The recommended molar ratios of PEG, fumaryl chloride, and triethylamine have been optimized for high OPF conversion and should be followed precisely to achieve the expected results from this protocol.

- 22 Fill the balloon attached to the flow control adapter with N₂(gas).
- 23 Purge the system with N₂(gas) from the balloon by opening the valve on the flow control adapter and allowing the ungreaed glass stoppers on the addition funnels to lift gently.
- 24 Initiate rapid stirring (~9) of the PEG solution using a magnetic stir bar.
- 25 Cool the reaction flask in an ice bath.
- 26 Initiate a slow drip of triethylamine (~0.2 Hz).
- 27 Initiate a slow drip of fumaryl chloride to match the drip rate of triethylamine as closely as possible.

CRITICAL STEP In order to achieve precise control of the addition rate, the addition funnel can be outfitted with a stopcock featuring a threaded needle valve. With this apparatus the stopcock can be left in the open position and all flow adjustments can be made using the needle valve to avoid excess addition at the beginning of the reaction and tedious adjustment thereafter.

- 28 Continue the addition of fumaryl chloride and triethylamine for 3 h.

CRITICAL STEP An equal and slow rate of addition of fumaryl chloride and triethylamine improves OPF conversion. Continued addition for at least 3 h results in OPF oligomers with approximate molecular weights to those listed in Table 3.

- 29 Once dripping has completed, replace the addition funnels with glass stoppers and continue cooling the reaction flask in the ice bath for 30 min.

CRITICAL STEP The color of the reaction solution will transition from colorless to black during addition of fumaryl chloride and triethylamine. While this protocol describes our preferred method of OPF synthesis, an alternative approach has been developed that benefited from a report using potassium carbonate (K_2CO_3) as a proton scavenger in PPF synthesis⁴⁹. The alternative approach uses K_2CO_3 instead of triethylamine as the proton scavenger⁵⁰, which avoids the formation of colored complexes between triethylamine and fumaryl chloride⁵¹.

- 30 After stirring for 30 min, remove the reaction flask from the ice bath and continue stirring at room temperature for 48 h.

Purification of OPF: workup TIMING ~9.5 h

- 31 Transfer the reacted OPF product to a one-necked round-bottomed flask (1 L).
- 32 Remove dichloromethane from the oligomer solution through rotary evaporation at reduced pressure (500 mbar) using a rotary evaporator and heated water bath (30°C).

- 33 Continue rotary evaporation until ~400 mL dichloromethane is collected.

PAUSE POINT The flask containing the reacted OPF product may be sealed and stored overnight in the fume hood, if necessary.

- 34 Add ethyl acetate (700 mL) to the oligomer solution.

CAUTION Ethyl acetate is irritating and/or harmful if exposed to the skin or inhaled. Conduct all work in a chemical fume hood. Proper personal protective equipment (lab coat, nitrile gloves, and safety glasses) should be worn throughout the procedure.

- 35 Stir the mixture using a magnetic stir bar and heat the solution to 40°C using a heating mantle.

- 36 After the solution has reached 40°C, continue heating for 30 min.

- 37 Set up an apparatus for vacuum filtration using a vacuum flask (1 L) and Buchner funnel with no. 40 filter paper.

- 38 Filter the reacted OPF product to collect salt in the filter and desalted product in the flask.

- 39 Place the flask in an ice bath and allow the OPF product to crystallize for 2 h.

- 40 Filter the crystallized product using vacuum filtration (1 L vacuum flask, no. 40 filter paper) to collect the product in the filter.

- 41 Recrystallize the OPF product according to Steps 39–40: dissolve in ethyl acetate (1 L) under stirring at 40°C for 20 min, precipitate on ice for 2 h, and collect by filtration.

- 42 Transfer the product to a beaker (1 L) and add ethyl ether (500 mL).

CAUTION Ethyl ether is irritating and/or harmful if exposed to the skin or inhaled. Conduct all work in a chemical fume hood. Proper personal protective equipment (lab coat, nitrile gloves, and safety glasses) should be worn throughout the procedure.

- 43 Stir the mixture using a magnetic stir bar for 10 min.
- 44 Filter the product using vacuum filtration (2 L vacuum flask, no. 40 filter paper) to collect the product in the filter.
- 45 Wash the product three times with ethyl ether (1 L total).
- 46 Following the final wash step let the product air dry for 10 min, and transfer the OPF product to a glass jar.

CRITICAL STEP The OPF product should appear as a light-brown, dry powder.

TROUBLESHOOTING

PAUSE POINT The product may be sealed and stored overnight in the fume hood, if necessary.

Purification of OPF: drying TIMING ~10 h

- 47 Complete drying of the product in a vacuum rated desiccator under high vacuum for at least 10 h.

CAUTION The vacuum apparatus should be equipped with a liquid nitrogen-cooled vacuum trap to collect potentially harmful volatile compounds.

- 48 After ~10 h of drying, purge the jar with N₂(gas).

CRITICAL STEP Purging the OPF product with N₂(gas) prevents hydrolytic degradation of the OPF oligomers while in storage.

PAUSE POINT Store the OPF product at –20°C.

Determination of the molecular weight of OPF TIMING ~8 h

- 49 Determine the molecular weight of the OPF product using GPC with an appropriate column at 30°C and a flow rate of 1.0 mL min^{–1} with degassed chloroform as the eluent. For systems using a refractive index detector, the molecular weight distributions should be determined relative to a calibration curve generated from PEG standards. (Note: Both GPC and NMR can be used to determine OPF molecular weight, and the values are typically in agreement¹².)

NMR analysis of OPF TIMING ~1 h

- 50 Confirm the incorporation of fumarate monomers into the OPF oligomer using ¹H NMR (Bruker 400 MHz NMR spectrometer) in CDCl₃. A representative ¹H NMR spectrum for OPF is shown in Figure 6. (Note: For a more thorough NMR analysis including an analysis of the polymer end groups, one should refer to a previously published report¹².)

CRITICAL STEP ¹H NMR should be used to confirm the absence of obvious contaminants in the OPF product.

TROUBLESHOOTING

TIMING

The expected timeline for this protocol is presented in Table 2.

TROUBLESHOOTING

Troubleshooting advice can be found in Table 4.

TROUBLESHOOTING

ANTICIPATED RESULTS

- Typical yields are ~40 g OPF per 50 g PEG precursor.
- Anticipated number average and weight average molecular weights of OPF relative to PEG standards are listed in Table 3.
- A typical ^1H NMR spectrum for OPF3K is shown in Figure 6.

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Figure 1. Synthesis of oligo(poly(ethylene glycol) fumarate) from poly(ethylene glycol) and fumaryl chloride in the presence of triethylamine.

Figure 2.
Apparatus for Synthesis of OPF: drying of dichloromethane (used in Steps 1–4).

Figure 3.

Apparatus for Synthesis of OPF: distillation of dichloromethane (used in Steps 5–10). Note that the apparatus features a 100 mL receiving flask in Steps 5–7 and a 1000 mL receiving flask in Steps 8–10.

Figure 4.
Apparatus for Synthesis of OPF: drying of PEG (used in Steps 11–16).

Figure 5.
Apparatus for Synthesis of OPF: initiate reaction (used in Steps 17–30).

Figure 6.

A typical ^1H NMR spectrum (400 MHz, CDCl_3 , ambient temperature) of OPF3K showing chemical shifts of important peaks.

Table 1

Comparison of typical PEG-based, fumarate-containing hydrogels under investigation for biomedical applications.

Polymer	Advantages	Limitations
Oligo(poly(ethylene glycol) fumarate)	<ul style="list-style-type: none"> Finite molecular weight between cross-links and finite mesh size based on PEG molecular weight assuming total consumption of fumarate groups during cross-linking⁴ Control of tensile modulus by varying PEG molecular weight⁴ Minimal cytotoxicity of OPF oligomers and hydrogel degradation products⁵ Excellent biocompatibility <i>in vivo</i>⁶ 	<ul style="list-style-type: none"> Methods to control physical properties less multifarious than PEG-based fumarate di-block and tri-block copolymers
Poly(propylene fumarate-co-ethylene glycol)	<ul style="list-style-type: none"> Control of hydrophilic/hydrophobic balance through copolymer ratio and block length⁷ Control of ultimate tensile stress and tensile modulus by varying copolymer ratio and block length⁸ Excellent biocompatibility <i>in vivo</i>⁹ Ability to undergo temperature-responsive gel formation at body temperature¹⁰ 	<ul style="list-style-type: none"> Variable molecular weight between cross-links and variable mesh size due to variation in PPF block length Increased cytotoxicity and acute inflammation with lower copolymer ratio of PEG to PPF or with lower molecular weight PEG⁹
Poly(lactide-co-ethylene oxide-co-fumarate)	<ul style="list-style-type: none"> Control of hydrophilic/hydrophobic balance through copolymer ratio and block length³ 	<ul style="list-style-type: none"> Variable molecular weight between cross-links and variable mesh size due to variation in PLA block length Tensile mechanical properties not quantified Moderate cytotoxicity of media conditioned with hydrogel sol fraction¹¹ <i>In vivo</i> biocompatibility not investigated

Table 2

Expected timing for the synthesis and purification of OPF.

Day	Task	Time (h)
1	Synthesis of OPF: drying of dichloromethane	3
	Synthesis of OPF: distillation of dichloromethane	6
	Synthesis of OPF: drying of PEG	2
2	Synthesis of OPF: initiate reaction	4.5
2–4	Synthesis of OPF: reaction	48
4	Purification of OPF: workup	9.5
5	Purification of OPF: drying	10
6	Determination of the molecular weight of OPF	8
	NMR analysis of OPF	1

Table 3

Typical number average and weight average molecular weights of the PEG precursor and of the OPF product synthesized according to this protocol as determined by gel permeation chromatography based on a calibration curve from PEG standards³².

	PEG		OPF	
	M_n (Da)	M_w (Da)	M_n (Da)	M_w (Da)
1K	860 ± 30	1,000 ± 30	2,930 ± 90	15,560 ± 490
3K	2,900 ± 90	3,390 ± 100	4,290 ± 140	39,540 ± 1,250
10K	8,870 ± 280	11,570 ± 360	9,230 ± 300	64,970 ± 2,110
35K	38,380 ± 1,310	62,170 ± 2,110	41,520 ± 1,570	125,580 ± 4,750

Table 4

Troubleshooting table.

Step	Problem	Possible cause	Solution
46	OPF product is in the form of small, rigid chunks instead of a fine, dry powder	Residual solvents not removed during workup are retained in polymer	Repeat purification steps (34–50) using ubiquitous amounts of ethyl ether to wash the polymer in step 45 while also physically crushing polymer chunks
50	Residual toluene present in the final OPF product ¹ H NMR [300 MHz, CDCl ₃]: σ 2.36 (s, 3H, -CH ₃), 7.17 (m, 3H, -CH- <i>o/p</i>), 7.25 (m, 2H, -CH- <i>m</i>)	Toluene not completely removed during purification	Repeat purification steps (34–50)