Chapter 1

Controlled Delivery of Chemopreventive Agents by Polymeric Implants

Farrukh Aqil and Ramesh C. Gupta

Abstract

The clinical development of cancer chemopreventive agents has been hampered by poor oral bioavailability issue. Several compounds have low aqueous solubility and undergo extensive first pass metabolism following oral dosing. To overcome this limitation, we developed polymeric implants from biodegradable ε-polycaprolactone (PCL) that can deliver both lipophilic as well as hydrophilic compounds. Implants furnish controlled release of compounds for long duration and provide dose-dependent release. The rate of release in vitro correlated well with the in vivo release. The polymeric implant technology thus overcomes the oral bioavailability issues, lowers the total required dose and minimizes or eliminates toxicity generally associated with high doses.

Key words Drug delivery, Polycaprolactone, Polymeric implants, Controlled release, Bioavailability

1 Introduction

Issues of poor oral bioavailability of chemopreventive and therapeutic agents have hindered the progress in cancer prevention and treatment. Drug delivery systems are engineered technologies for the targeted delivery and/or controlled release of chemopreventive and therapeutic agents. The practice of drug delivery has changed dramatically in the last few decades and even greater changes are anticipated in the near future. The development of new approaches in cancer prevention and treatment could encompass new delivery systems for approved and newly investigated compounds [1, 2]. Moreover, targeted drug delivery is intended to reduce the side effects of drugs with concomitant decreases in drug amount and treatment expenses. It is generally expected that most applicable drug delivery systems be biodegradable, biocompatible, and with minimal adverse effects. The major emphasis of an effective delivery system is to deliver the compound in minimum therapeutic doses with minimal or no toxicity.

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Bioavailability of the drugs and chemopreventive agents can be increased by encapsulation or systemic delivery by various means, including nanoparticles, liposomes, microparticles, micelles, and implants (reviewed in [3]). Encapsulation of agents using polymeric nanoparticles or nanocarriers has emerged as the workhorse solution to manage poor biodistribution and stability of chemopreventives and therapeutics [4]. However, subchronic and chronic toxicity studies with nanoparticle formations are elusive, and could potentially pose problems for toxicity of the carrier over long durations. Incredible choices in the polymeric designs offer a direct route to optimal carrier design. Polymeric implants offer controlled delivery as shown by us [3, 5–8] and others [9, 10]. Unlike oral nanoparticles, polymeric implants provide continuous delivery for long durations (months to >1 year) circumventing repeated dosing thereby eliminating polymer toxicity [3, 6].

Different types of implantable devices have been used, such as poly(lactide-co-glycolide) (PLGA)-based implants and implants of high-melting-point polymers. However, their uses are limited due to development of fibrous encapsulation around PLGA implants [11]; and use of compounds with high thermal stability in the later [12]. We have initially demonstrated the use of silastic tubing implants which, due to their non-biodegradable nature, have encountered issues with their removal after the end of treatment [13].

We recently developed biodegradable polymeric implants using ε-polycaprolactone (PCL):F-68 embedded with chemopreventive agents. These implants provided sustained release for long durations in vivo [3, 14]. This concept has been tested successfully for various agents. A simple procedure has been used to develop the polymeric "extrusion" implants. Polymeric implants are prepared by homogenous entrapment of agents in a polymeric matrix. The implants provide slow-release kinetics with a continuous drug release for long durations (months to >1 year) [14]. The implants can be grafted at various sites and elicit a sustained systemic or localized delivery of agents with complete bioavailability with no observable toxicity. These advantageous attributes of polymeric implants not only improve bioavailability, but can also improve patient compliance by eliminating the need for frequent parenteral dosing [3]. However, implants developed using this formulation ("extrusion" method) generally results in an initial high burst release followed by a gradual decline and also do not apply to heat-labile compounds.

More recently, we have improvised the method and developed multi-layer coated implants that can accommodate almost all types of compounds including compounds of different physicochemical properties. This method involves (1) preparation of blank PCL:F-68 implants (1.4 mm dia), and (2) coating of 20–40 layers

by dipping blank implants, with intermittent drying, in 10–20 % PCL solution in dichloromethane (DCM) containing 0.5–2 % of test agent in DCM or another appropriate solvent. The coated implants of various chemopreventive agents when tested for in vitro release showed that the burst release was substantially reduced, and the release was largely sustained for 3 weeks. The details of the two types of implant technologies (extrusion and multilayer) are provided below.

2 Materials

Prepare all solutions using ultrapure water and analytical grade reagents. Prepare and store all reagents at room temperature, except wherever indicated. Diligently follow all waste disposal regulations when disposing waste materials. All the solvents used in the preparation were of HPLC grate unless otherwise specified. Take all other precautions as required.

2.1 Supplies for Implant Formulation

1. The polymers and other materials used were obtained from these sources: PCL mol. wt. 80,000 (P-80), PCL mol. wt. 65,000 (P-65), and PCL mol. wt. 15,000 (P-15) were from Sigma–Aldrich (St. Louis, MO, USA), and polyethylene glycol, mol. wt. 8000 (PEG-8) from Fisher Scientific (Fair Lawn, NJ, USA). Pluronic^{R} F68 (F-68) was a gift from BASF Corporation (Florham Park, NJ, USA). Silastic tubing of different diameters (1.4, 2.0, and 3.2 mm internal diameter) were purchased from Allied Biomedical (Ventura, CA, USA). Test agents used for the implant preparation were purchased from different sources. DCM, tetrahydrofurane (THF) and absolute ethanol were from BDH chemicals (VWR, West Chester, PA), Sigma-Aldrich (St. Louis, MO), and Pharmco-AAPER (Louisville, KY, USA), respectively. All other chemicals were of analytical grade.

2.2 Supplies for Release Media

1. Release of the agents from polymeric implants was done in the release medium containing phosphate-buffered-saline (PBS), pH 7.4 containing 10 % calf serum. We also used 1 % of penicillin-streptomycin solution to suppress any bacterial growth. PBS tablets were from Sigma–Aldrich (St. Louis, MO, USA). Bovine calf serum was from Hyclone (Logan, UT, USA) and stored in aliquots at −20 °C for long durations. Penicillin/streptomycin solution was purchased from Life Technologies (Invitrogen, Carlsbad, CA). Scintillation vials (clear and amber) (20 and 40 ml) were purchased from National Scientific (Rockwood, TN, USA).

3 Methods

3.1 Formulation of "Extrusion" Polymeric Implants

1. Add 4.05 g P-80 (or P-65) and 0.45 g F-68 or polyethylene glycol mol. wt. 8000 (PEG-8K) to 10 ml DCM in a 50 ml glass beaker (*see* **Note 1**) (Fig. 1a).
2. Keep the beaker at room temperature and stir the solution with a glass rod occasionally until polymers solubilize.
3. Dissolve 0.5 g curcumin (or other agent) in 2–3 ml of solvent (ethanol, DCM, or THF) in a glass tube or scintillation vial. Vortex to solubilize the compound (*see* **Notes 2** and **3**) (Fig. 1a).
4. Add drug solution to the polymer solution slowly (*see* **Note 4**).
5. Place a water bath under fume hood, and set it at 70 °C. Transfer the formulation to the water bath. Stir the solution with a glass rod occasionally (*see* **Note 5**). Alternatively, transfer the solution to a glass Petri dish and the solution is evaporated under hood (Fig. 1b, c).
6. Once the solvent is almost completely evaporated, place the beaker/Petri dish in a Savant Speed-Vac (Thermo-Savant, Holbrook, NY) for complete removal of the solvents under reduced pressure (*see* **Note 6**). Formulation should be left in Savant at 65 °C for 6–8 h or overnight for complete removal of residual solvents.
7. Collect the material from the Savant Speed-Vac, and excise into small pieces using a scissor.
8. Take 5 ml plastic syringe (BD, Franklin Lakes, NJ), and attach it to a silastic tubing of desired internal diameter (Fig. 1d) (*see* **Note 7**).

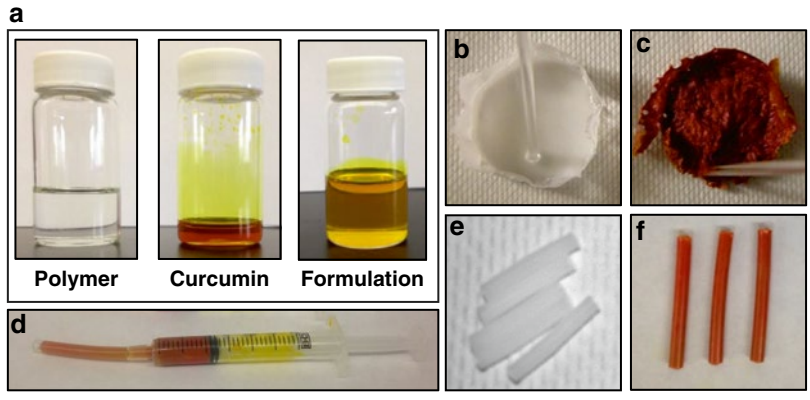

Fig. 1 Solution of ε-polycaprolactone (P-65) and F-68 in dichloromethane, curcumin in tetrahydrofuran, mixture of P-65/F-68 solution and curcumin solution (**a**). Dried sham. (**b**) Polymer-drug formulation (**c**). Dried polymer was exercised into small pieces and heated in syringe attached with a silastic tube (**d**). Photographs of representative sham (**e**) and curcumin (**f**) implants prepared by extrusion method

9. Fill the syringe with dried pieces of polymer-drug formulation.
10. Keep the assembly of syringe attached with silastic tubing (Fig. 1d) at 70 °C in an incubator for 30 min.
11. Remove the assembly from the incubator, and extrude the material immediately but slowly (*see* **Note 8**).
12. After cooling the assembly at room temperature, remove the implant by cutting the silastic tubing mold longitudinally with a scalpel or blade and excise implants into desired sizes (Fig. 1e, f).
13. Store implants in amber vials under argon at 4 °C.

3.2 Formulation of "Coated" Polymeric Implants

To overcome the issues related to burst release and use of heat-labile compounds, we improvised the methodology as "coated implants" as described below.

1. Prepare extruded implants in the absence of any drug as described above using silastic tubing mold of internal diameter 1.4 mm. These are thin implants and referred as inserts (Fig. 2a).

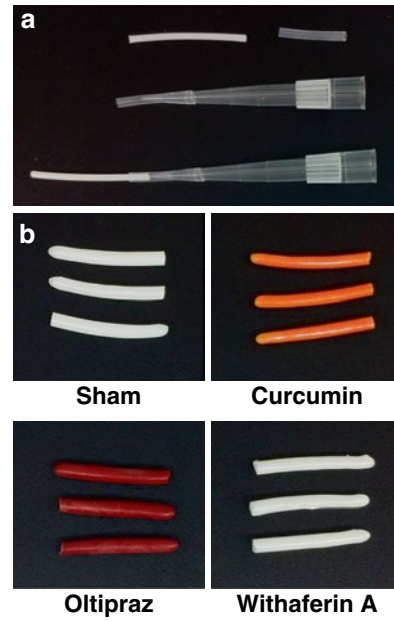

Fig. 2 Assembly of sham insert assembled with pipet tip using silastic tubing for coating (**a**), and photographs of representative coated polymeric implants (**b**). Implants were prepared by coating indicated compounds mixed with P-80 as described in Subheading 3.2. Implant size: 2 cm length, 2.6 mm diameter. Reprinted from Cancer Letters, 326 (1), Aqil et al., Multilayer polymeric implants for sustained release of chemopreventives, 33–40. Copyright (2012), with permission from Elsevier

2. Excise inserts into 2.5–3.5 cm pieces.
3. Cut silastic tubing (1.4 mm internal diameter) in about 6 mm pieces.
4. Attach one end of the silastic tubing plug to a pipet tip while the other end to blank insert (Fig. 2a) (*see* **Note 13**).
5. Polymer-drug solution: Dissolve 4.5 g P-80 in 20 ml DCM in a 50 ml glass beaker (*see* **Note 1**).
6. Dissolve curcumin (or other test agent) in 2–3 ml solvent (ethanol, DCM, or THF) in a glass tube or scintillation vial (*see* **Notes 2**, **3**, and **14**).
7. Add drug solution to the polymer solution slowly and mix the two solutions thoroughly, stirring with a glass rod (*see* **Note 4**). This solution is referred to as coating solution.
8. Set up clamp under the hood and attach a commercial hair dryer with cool air setting.
9. For coating, hold the implant assembly and dip quickly into the coating solution (*see* **Note 15**).
10. Place the coated implants into a rack and place under the hair dryer for drying for 2–3 min (*see* **Note 16**).
11. Repeat the coating process 25–30 times. These coatings generally increase the size of coated implants from 1.4 to 2.6 mm diameter as measured by a digital caliper (Fig. 2b).
12. Place the assembly under hood overnight to remove the residual DCM.
13. Excise the implants in 1 or 2 cm lengths and store in amber vials under argon at −20 °C until use (*see* **Note 17**).

3.3 In Vitro Release

1. Release media: Add 8.9 ml PBS in amber color 20 ml glass scintillation vial. Add 0.1 ml of penicillin–streptomycin solution (Invitrogen, Carlsbad, CA) (1 %, v/v) to minimize the growth of microorganisms. Finally, add 1 ml (10 %, v/v) of bovine calf serum to simulate the in vivo scenario.
2. Release study: Place 1 or 2 cm implants in media placed in 20 ml amber vials to determine the rate of release of test agents.
3. Incubate vials containing the media and implant at 37 °C with constant agitation in a water bath (Julabo SW 23, Seelback, Germany) for 24 h.
4. Transfer the media from the vial to a fresh scintillation vial and add 1 ml ethanol (10 % final concentration) to the release medium to completely solubilize the compound. Add fresh release media and continue incubation.

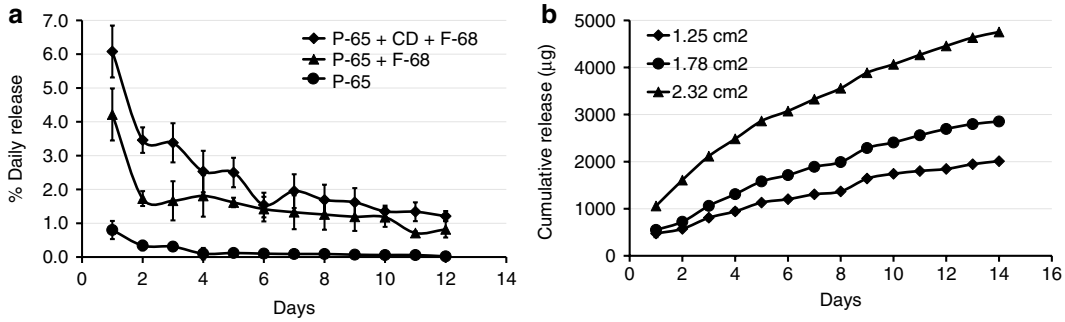

Fig. 3 Effect of water-soluble polymer on the percent daily release of punicalagins. Addition of water-soluble polymers [cyclodextrin (CD) and F-68] increase the release as it facilitates entry of release media in the polymer matrix and allows drug to dissolve and come out (**a**). In vitro cumulative release of punicalagins from different size (1 cm, 1.5 cm and 2 cm) implants providing total surface area of 1.25 cm^2, 1.78 cm^2, and 2.32 cm^2, respectively. As expected the release was found directly proportional to the surface area. The release was measured by incubating implants in a shaker incubator in PBS supplemented with 10 % bovine serum as described in Subheading 3.3. SD was generally 5–10 % (**b**)

5. To measure the compound released, transfer 1 ml of the solution to an Eppendorf tube, centrifuge at 10,000 × *g* for 10 min.
6. Collect the supernatant and measure the release spectrophotometrically at 430 nm, the absorbance maxima for curcumin.
7. Generate a standard curve using curcumin and calculate the concentration against the standard curve.
8. Rate of release (extrusion implants): We observed that (1) the inclusion of the water-soluble polymer(s) facilitates the release from the implants (Fig. 3a), (2) the release is proportionately increased with the increase of surface area (Fig. 3b), and (3) the release is largely sustained for long duration when tested for various compounds (Table 1) including chemopreventive agents [3, 6, 8, 15], carcinogens [16, 17], and chemotherapeutic drug.
9. Rate of release (coated implants): Multi-layer coated implants generally provide sustained release as shown for oltipraz, curcumin, and withaferin A [6] for long durations. When withaferin A implants coated with 6 and 10 times with blank polymer, it minimize the burst release and provided sustained release (Fig. 4). The effect was even more pronounced with eight coatings and release was almost sustained as tested for curcumin.

Table 1
Compounds successfully tested for release

(1) Chemopreventive agents		
Curcumin	Curcumin I	Demethoxycurcumin
Bisdemethoxycurcumin	Green tea polyphenols	Punicalagins
Resveratrol	Withaferin A	Tanshinone II
Cucurbitacin B	Luteolin	Oltipraz
Diindolylmethane	Ellagic acid	Anacardic acid
(2) Carcinogenic agents		
PCB-126	PCB-153	Benzo[*a*]pyrene
Dibenzo[*a*,*l*]pyrene		
(3) Chemotherapeutic agents		
Paclitaxel		

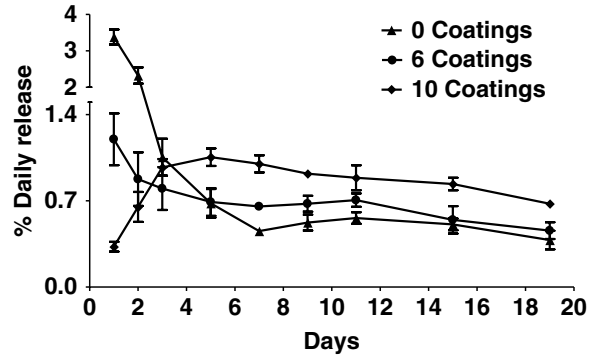

Fig. 4 Effect of coatings with blank P-80 on withaferin A polymeric implants to minimize the burst release. Withaferin A implants were coated 6 and 10 times with 10 % solution of P-80 in dichloromethane with intermittent drying. The in vitro release was measured as described in Subheading 3.3. Data represent average of 3 implants ± SD

4 Notes

1. P-80 or P-65 or P-15 provides almost similar release from the implants. However, the release rate changes if PCL material is of higher or lower mol. wts.
2. Solvents to dissolve test agents should be chosen based on miscibility with DCM. Curcumin is used here as a model compound which has high solubility in THF. The volume of the drug

solvent can vary based on drug's solubility. In our experience the drug solvent to DCM ratio is about 1:3 to avoid crystallization of drug or polymer.

3. There are three major components of the implant formulation: First, drug percent should be calculated based on drug and polymer weight. Second, ratio of two polymers (PCL and F-68) can vary based on the use. Usually we use 10 % F-68 or 10–30 % PEG-8K. The ratio of polymer weight should be calculated based on total weight minus drug weight.
4. While adding drug to the polymer solution, continuous stirring helps to obtain uniform drug distribution into matrix.
5. Alternatively, formulation can be dried by pouring the material in a Petri dish and leaving it under the hood. Once solvent is evaporated, the Petri dish is transferred to Savant Speed-Vac for more complete removal of residual solvents. All precautions like wearing gloves should be exercised.
6. High drying rate should be selected as it provides around 65 °C temperature and keep formulation in molten form for more complete removal of residual solvents. Use lower temperature for heat-labile compounds but increase evaporation time.
7. Size of silastic tubing should be chosen based on the requirement of implant size. The release of compounds from the implants is based on the surface area. We observed that a diameter of 3.2 mm is desirable for rat studies and 1.4–2.6 mm for mice.
8. Slow and steady extrusion is needed as rapid extrusion sometimes leaves air bubbles in implants. Long processing time may result in solidification of formulation; in the event the matrix solidifies, the assembly is heated again for 20–30 min.
9. In our experience, we observed 120 RPM is optimum for shaking the implants in release media. At this speed we do not observe any adverse effect on implants.
10. Centrifugation step is included to remove any precipitate due to the addition of ethanol.
11. Curcumin is a mixture of three curcuminoids. These curcuminoids are structural analogs and absorb at similar wavelength (430 nm). Wavelength should be selected based on the compounds used.
12. Calibration curves for each compound should be generated by spiking PBS containing 10 % bovine serum, 1 % penicillin-streptomycin solution and 10 % ethanol with known concentrations of test compound.
13. Make sure that silastic tubing holds both insert and pipette tip tightly. Implants can fall off from the loose assembly.

14. Polymer and drug ratio can be selected based on the requirement. For example 20 % drug loading can be achieved by mixing 4 g of polymer with 1 g of drug (w/w).
15. Implant assembly should be rotated after dipping to provide uniform coating.
16. A single coat usually takes 2–3 min for complete drying. However, time can be increased to confirm the drying. Do not dry implants under hot air as it can melt the polymeric coating.
17. Implants thus formulated will have a 10 % drug load of the test agents.

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