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Controlled release of NFκB decoy oligonucleotides from biodegradable polymer microparticles

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Abstract

The objective of this study was to evaluate a poly(DL-lactic-*co*-glycolic acid)/poly(ethylene glycol) (PLGA/PEG) delivery system for nuclear factor-kappa B (NFκB) decoy phosphorothioated oligonucleotides (ODNs). PLGA/PEG microparticles loaded with ODNs were fabricated with entrapment efficiencies up to 70%. The effects of PEG contents (0, 5, and 10 wt%), ODN loading densities (0.4, 4, and 40 μg/mg), and pH of the incubation medium (pH 5, 7.4, and 10) on ODN release kinetics from the PLGA/PEG microparticles were investigated in vitro for up to 28 days. The release profiles in pH 7.4 phosphate buffered saline (PBS) were characterized by an initial burst during the first 2 days, a linear release phase until day 18, and a final release phase for the rest of the period. Up to 85% of the ODNs were released after 28 days in pH 7.4 PBS regardless of the ODN loading density and PEG content. Higher ODN loading densities resulted in lower entrapment efficiencies and greater initial burst effects. The bulk degradation of PLGA was not significantly affected by the PEG content and ODN loading density, but significantly accelerated at acidic buffer pH. Under acidic and basic conditions, the aggregation of microparticles resulted in significantly lower cumulative mass of released ODNs than that released at neutral pH. The effects of pH were reduced by the incorporation of PEG into PLGA microparticles. Since the PLGA degradation products are acidic, PLGA/PEG microparticles might provide a better ODN delivery vehicle than PLGA microparticles. These results suggest that PLGA/PEG microparticles are useful as delivery vehicles for controlled release of ODNs and merit further investigation in cell culture and animal models of glioblastoma.

Keywords: Phosphorothioated decoy oligonucleotides (ODNs); Poly(DL-lactic-*co*-glycolic acid) (PLGA); Poly(ethylene glycol) (PEG); Controlled release; Biodegradable microparticles; Nuclear factor-kappa B (NFκB)

1. Introduction

Glioblastoma (GBM) prognosis is poor with a median survival of one year from the time of diagnosis [1]. Previous studies have focused on identifying the distinct biological features of high-grade human GBM [2–10]. Nuclear factor-kappa B (NFκB) activation has recently been shown to be important in regulating cell proliferation in a variety of tumor cells [11–15]. Activation of NFκB involves phosphorylation and dissociation of the inhibitory IκB protein from a cytoplasmic complex with NFκB [16–18]. After serine phosphorylation of IκB, the inhibitory protein is ubiquinated and degraded by the proteasome pathway [19,20]. Liberated NFκB is translocated into the nucleus where it induces transcription of responsive genes by binding to DNA κB motifs. The functional consequence of NFκB activation was confirmed by the generation of *cis* "decoys" against NFκB binding sites [21]. Binding of liberated NFκB to phosphorothioated decoy oligonucleotides (ODNs) within the cytoplasm could prevent nuclear translocation and DNA binding, and therefore, inhibit the tumor cell proliferation.

Such tumor specific therapy requires delivery of ODNs to the tumor sites. One conceptual approach has been to use gene therapy transfected cells that produce and secrete the appropriate molecules to the tumor site [22]. This method is complex and may be

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haphazard when exploring new therapies. A novel therapeutic approach is to deliver inhibitory ODNs from biodegradable microparticles [23]. This is an alternative to cell based delivery systems during the phase of drug development. Microparticles made of biodegradable polymers have been widely utilized as vehicles for drug delivery [23–29]. They can be implanted at an afflicted site during surgery, injected as a suspension to a wound area, or impregnated into polymer scaffolds and then transplanted [30]. A major advantage of biodegradable synthetic microparticles is the ability to control the rate of drug release to obtain the desired local therapeutic concentrations by changing the structure and biodegradation rate of the polymers. Using such a delivery system may also offer other advantages such as site-specific delivery and protection of ODNs from degradation prior to release. ODNs have been previously shown to retain their activities during encapsulation in poly(DL-lactic-*co*-glycolic acid) (PLGA) microparticles and upon release [31]. Our recent work also showed that NFκB decoy ODNs released from PLGA microparticles were biologically active and inhibited the proliferation of glioblastoma cell lines [32].

PLGA biodegradable delivery systems have been used successfully to deliver a number of bioactive molecules, and the lack of toxicity of PLGA polymers has been established by a long history of use as absorbable suture materials [33]. The degradation times of PLGA can be altered to suit a particular application by varying the polymer molecular weight, the ratio of lactic to glycolic acid in the copolymer, or the structure of the microparticles [25,34–36]. Poly(ethylene glycol) (PEG), a hydrophilic and water-soluble polymer, was shown to modulate drug release when incorporated into PLGA microparticles [28,37]. Compounds incorporated into these PLGA/PEG blend microparticles showed extended linear release profiles.

The drug release rates from PLGA microparticles are affected by various factors including the structure, size, loading, and solubility of the encapsulated molecules, as well as the structure and degradation properties of the polymer. The increased drug loading was shown to result in an increased release rate, especially in the initial burst phase [38,39]. It is attributed to the larger amount of drug near the microparticle surface and, consequently, available for initial release. The drug release profiles are also dependent on the release medium that may have profound effects on the structure, solubility, diffusivity, and activity of the compound. Most of the in vitro release studies in the literature are carried out in pH 7.4 phosphate buffered saline (PBS) to simulate body fluids. However, an acidic environment can occur naturally in vivo. For example, tumor sites may contain necrotic areas that may be acidic [40]. The release of acidic degradation products from PLGA microparticles can result in a decrease in local pH [28]. In addition, basic pH conditions were also shown to affect ODN release kinetics [31]. It is therefore of great importance to assess the effects of environmental pH on the degradation of and protein release from biodegradable polymers.

In this study, experiments were designed to evaluate a PLGA/PEG delivery system for NFκB decoy phosphorothioated ODNs. We determined: (1) whether phosphorothioated ODNs could be released in a controlled manner using PLGA/PEG blend microparticles, (2) whether the ODN loading densities, PEG contents, and buffer pH would affect the ODN release kinetics, and (3) whether the ODN loading densities, PEG contents, and buffer pH would affect the degradation of PLGA.

2. Materials and methods

2.1. Raw materials

End-capped PLGA of 50:50 lactic to glycolic acid copolymer ratio (Medisorb®, Alkermes, Cincinnati, OH) was used in this study. The weight average molecular weight (M_w) of PLGA was $72{,}000 \pm 360$ ($n = 4$), as determined by gel permeation chromatography (GPC). The polydispersity index (PI), equal to the ratio of the weight average to the number average molecular weight (M_w/M_n), was 1.73 ± 0.05. PEG ($M_w = 10{,}700$) and poly(vinyl alcohol) (PVA, 88% mole hydrolyzed, $M_w = 13{,}000-23{,}000$) were purchased from Aldrich Chemical (Milwaukee, WI).

NFκB phosphorothioated decoy ODNs 5′-GGGATTTCCC-3′ and mutated ODNs 5′-GGGCTTTCCC-3′ were purchased from Oligos Etc., Inc. (Wilsonville, OR) on a 50 μM scale, which were purified to $\geqslant 90\%$ with gel electrophoresis and HPLC purification [41]. Chloroform, dichloromethane, 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS), isopropyl alcohol (IPA), NaOH and acetic acid were acquired from Sigma (St. Louis, MO). The PBS at pH 7.4 was obtained from Gibco (Grand Island, NY).

2.2. Microparticle preparation

Microparticles of PLGA/PEG blends containing 0, 5, or 10 wt% initial PEG were fabricated using a double-emulsion-solvent-extraction technique ((water-in-oil)-in-water or W/O/W) as previously described [23,28]. Briefly, PLGA and PEG with a total weight of 250 mg were first dissolved in 1 ml dichloromethane. Then, the appropriate amount of ODNs was dissolved in 100 μl distilled deionized water (ddH_2O) and injected into a flint glass tube containing the polymer solution. The entire mixture was emulsified on a vortexer (Vortex Genie 2, Scientific Industries, Bohemia, NY) for 40 s.

This solution was then re-emulsified in 100 ml of 0.3% aqueous PVA solution resulting in a double emulsion. The second emulsion was added to 100 ml of 2% aqueous IPA solution and maintained on a magnetic stirrer for 1 h. The extraction of the dichloromethane to the external alcoholic phase resulted in precipitation of the dissolved polymer and subsequent formation of microparticles. The supernatant was decanted off and settled microparticles were washed three times with double distilled water. The fabricated microparticles were finally collected, centrifuged, lyophilized to dryness, and stored at −70°C before use.

2.3. Microparticle characterization

The morphology of the fabricated microparticles was assessed by scanning electron microscopy (SEM). Freeze-dried microparticles were mounted on metal stubs with double-sided carbon tapes and coated with a 50/50 mixture of gold and platinum. The samples were then observed with a Hitachi S4700 Field Emission Scanning Microscope (San Jose, CA).

The distribution of fluorescein-labelled ODNs within the microparticles was examined by fluorescence microscopy. Dry microparticles were dispersed on glass coverslips and representative fluorescence images were taken using a LSM510 confocal laser scanning microscope (Carl Zeiss, Oberkochen, Germany).

The actual compositions of microparticles were determined by integration data of ^{1}H nuclear magnetic resonance (NMR) spectra, which were obtained on a Brucker DRX-600 NMR spectrometer (600 MHz) using CD_2Cl_2 as solvent.

Size distribution of the microparticles was measured with a Coulter counter multisizer III (Coulter Electronics, Hialeah, FL) after suspending microparticles in an Isoton II solution (Coulter Electronics).

The entrapment efficiency of the ODNs was determined by normalizing the amount actually entrapped to the starting amount, using an established solvent extraction technique [28]. Approximately 20 mg of microparticles were dissolved in 1 ml dichloromethane for 6 h at 37°C. The entrapped ODNs were then extracted from the organic phase to the aqueous phase by incubation with 1 ml PBS for an additional 24 h. The ODN concentrations were determined by absorption at 260 nm in a Beckman DU-600 UV spectrometer.

2.4. Buffer preparation

The pH 5 buffer for in vitro release studies was prepared by adding 1 N NaOH to 0.0042 mol/l acetic acid solution. The pH 10 buffer was prepared by adding 1 N NaOH to 0.042 mol/l CAPS solution. NaCl was added to both buffers at a final concentration of 9.0 g/l. The resulting ionic strength ($I = 0.16$) of the pH 5 and 10 buffers was the same as the pH 7.4 PBS. The pH of buffer solutions was measured using a pH meter (Accumet AR15 pH meter, Fisher Scientific, Pittsburgh, PA).

2.5. Phosphorothioated oligonucleotide release kinetics

The release kinetics of ODNs from PLGA/PEG microparticles were studied under 15 experimental conditions. For each ODN loading density (0.4, 4, or 40 μg/mg), microparticles of approximately 20 mg with varied initial PEG contents (0, 5, or 10 wt%) were placed into 1.8 ml microvials containing 1 ml pH 7.4 PBS. Additional microparticles containing 0, 5, or 10% PEG and 4 μg/mg ODNs were incubated in buffers of pH 5 and 10. All samples were maintained at 37°C with shaking (~100 rpm) for various time periods up to 28 days.

At the end of each time point: 4, 8, 12, 16 h, 1, 2, 3, 4, 5, 6, 7, 10, 14, 18, 22, and 28 days, the microparticle suspension was centrifuged and the supernatant was collected for analysis. The pellet was re-suspended in fresh media. The amount of ODNs released from microparticles was determined by quantifying the UV absorption at 260 nm of collected sample solutions.

2.6. In vitro degradation of microparticles

The degradation of PLGA/PEG microparticles (loaded with ODNs) was studied under the same conditions as in the ODN release experiments. After 0, 1, 4, 7, 10, 14, 18, 22, and 28 days, the microparticle samples were collected, frozen, and vacuum-dried for 24 h before further analysis.

The PLGA molecular weight distribution of microparticles was determined by GPC (Waters, Milford, MA). The samples were dissolved in chloroform and eluted through a Phenogel 5 guard column (Model 1063376, 50 × 7.8 mm, 5 μm particle diameter, Phenomenex, Torrance, CA) and a Phenogel 5 linear column (Model 106338, 300 × 7.8 mm, 5 μm particle diameter, Phenomenex) at a flow rate of 1 ml/min. Polystyrene standards (Polysciences, Warrington, PA) were used to obtain a primary calibration curve. The Mark–Houwink constants for PLLA, $K = 5.45 \times 10^{-3}$ ml/g and $\alpha = 0.73$, were used to determine the molecular weights of PLGA samples. The half-life of PLGA under each experimental condition was calculated by fitting the data for M_w to an exponential function of time. The morphology of the degrading microparticles at 14, and 28 days was assessed by SEM as described in Section 2.1.

2.7. Statistical analysis

All data are reported as means ± standard deviations (SD) for $n = 4$, except for size distribution measurements where the SD was calculated based on normal distribution. Single factor analysis of variance (ANOVA) was used to assess the statistical significance of results. Scheffé's method was employed for multiple comparison tests at significance levels of 95% and 99%.

3. Results and discussion

This study was conducted to answer the following questions: (1) Could phosphorothioated ODNs be released in a controlled manner from PLGA/PEG blend microparticles? (2) Did the ODN loading density, PEG content, and buffer pH affect the protein release kinetics? (3) Did these parameters also affect the degradation of PLGA in vitro?

3.1. Initial microparticle characterization

3.1.1. Morphology

Using an established double-emulsion-solvent-extraction technique, PLGA/PEG microparticles with a spherical shape and smooth, non-porous surfaces were fabricated, as shown by the SEM (Fig. 1a). The distribution of ODNs in the PLGA/PEG microparticles was examined using fluorescein-labelled ODNs. Fluorescein-ODNs were dispersed fairly uniformly throughout the microparticles based on confocal imaging (Fig. 1b). Some areas with clumps of ODNs were also observed.

3.1.2. PEG content

The actual weight percentage of PEG incorporated into the microparticles was determined as 4.0% and 9.1% for initial PEG contents of 5.1% and 10.3%, respectively (Table 1). The exact PEG contents in PLGA/PEG microparticles were approximately 1% lower than the theoretical values. This discrepancy was due to the loss of PEG during the microparticle fabrication process, since PEG is a hydrophilic and water-soluble polymer [42]. The PEG contents mentioned in this study refer to the initial PEG contents unless otherwise noted.

3.1.3. Microparticle size distribution

Average sizes of the microparticles containing 0, 5, and 10 wt% initial PEG were 23.3 ± 11.9, 21.4 ± 11.2, and 24.6 ± 13.7 μm, respectively. Average sizes of the PLGA microparticles containing 0.4, 4, and 40 μg/mg ODNs were 23.3 ± 11.9, 20.0 ± 12.9, and 18.0 ± 9.7 μm, respectively. Microparticles with larger loading densities were prepared by reducing the polymer concentration in the organic phase, a circumstance that may cause a reduction in microparticle size.

3.1.4. Entrapment efficiency

The entrapment efficiencies of ODNs in the microparticles were similar at different PEG contents but significantly different at different loading densities (Fig. 2). After fabrication, 66.4 (±1.6)%, 50.9 (±8.1)%, and 21.9 (±1.9)% of ODNs were entrapped for the loading densities of 0.4, 4, and 40 μg/mg, respectively. The entrapment efficiencies of ODNs at high loading density (40 μg/mg) was significantly lower ($p < 0.01$) than both lower loading densities (0.4 and 4 μg/mg). With the conventional W/O/W method we used, the entrapment efficiency decreased with increasing ODN loading. Several methods may be used to improve the entrapment efficiency, such as replacing dichloromethane with ethyl acetate, using micronized drug powder instead of an internal aqueous phase, and adding electrolytes to the external phase [43].

Table 1
Compositions of fabricated microparticles as determined by ^{1}H NMR integration data

Composition	PEG content (%)	PLA content (%)	PGA content (%)
Microparticles with 5% PEG	4.0	50.9	45.1
Microparticles with 10% PEG	9.1	48.3	42.6

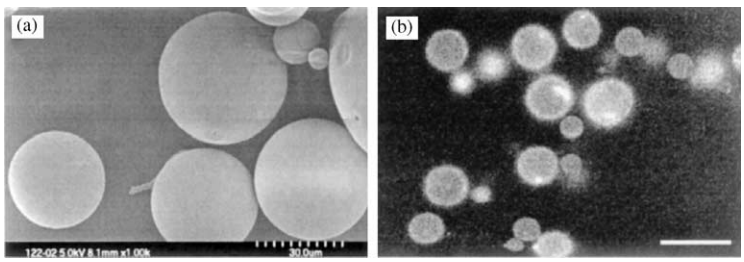


Fig. 1. PLGA microparticles loaded with 0.4 μg/mg ODNs prior to degradation: (a) scanning electron micrograph showing smooth, non-porous surfaces, and (b) confocal micrograph showing the distribution of fluorescein-ODNs within the microparticles. Scale bar is 50 μm in (b).

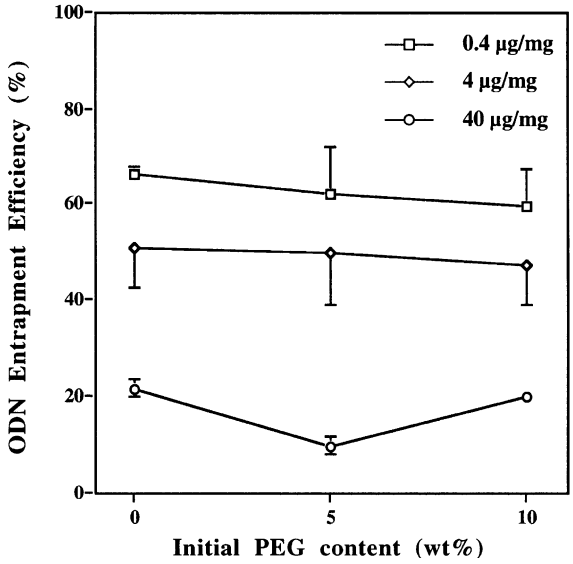


Fig. 2. Entrapment efficiencies of ODNs in PLGA/PEG microparticles with different PEG contents at different loading densities. Error bars represent means ± SD for $n = 4$.

3.2. In vitro odn release kinetics

The release kinetics of the NFκB phosphorothioated decoy ODNs depended on the ODN loading density, PEG content in the PLGA/PEG blend microparticles, and the pH of the incubation medium. For all conditions tested, the release profiles were comprised of three phases: (a) an initial burst occurring during the first 2 days, (b) a linear steady release phase that lasted up to day 18, and (c) a final release phase for the rest of the time frame.

3.2.1. Effects of ODN loading density

The ODN loading density had significant effects on the initial burst and the final release phase (Fig. 3). Higher ODN loading densities resulted in a faster initial burst (phase a) but a slower final release (phase c). Fig. 3a shows the ODN release profiles from PLGA microparticles. By day 2, 23.9 (±1.3)%, 41.3 (±2.3)%, and 56.5 (±8.9)% of loaded ODNs were released for the loading densities of 0.4, 4, and 40 μg/mg, respectively. Nearly 80% percent of ODNs was released at the end of day 28 for all loading densities. Microparticles with 5% PEG (Fig. 3b) and 10% PEG (Fig. 3c) showed similar release patterns for all ODN loading densities.

Lowering the ODN loading density resulted in a decreased initial burst effect, yet the total percentage of released ODNs after 28 days was similar to that of microparticles with the higher ODN loading density. Therefore, ODN release profiles at lower ODN loading densities may be more controllable.

3.2.2. Effects of PEG content

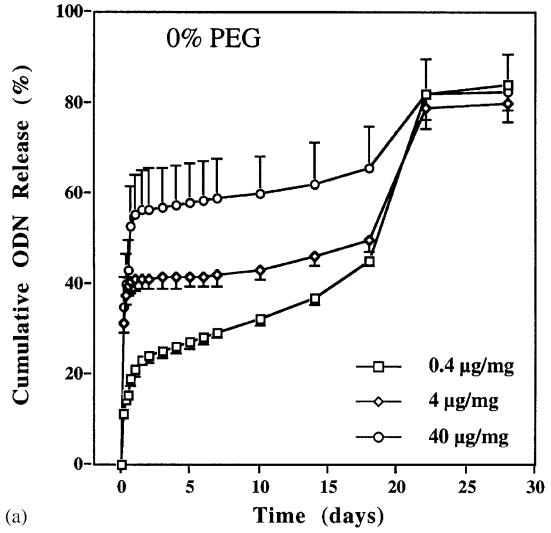
For microparticles loaded with 4 μg/mg ODN and incubated with pH 7.4 PBS, the PEG content did not affect the ODN release kinetics (Fig. 4a). However, when the microparticles were incubated with buffers of pH 5 (Fig. 4b) and pH 10 (Fig. 4c), PEG affected the release kinetics dramatically, especially during the initial burst phase. In the pH 5 buffer, about 15.1 (±0.9)% and 55.2 (±3.6)% of ODNs were released after 2 days for initial PEG contents of 0% and 10%, respectively (Fig. 4b). The corresponding percentages of release after 28 days were 38.8 (±1.4)% and 83.5 (±4.6)%. In the pH 10 buffer, about 26.5 (±5.0)% and 69.4 (±5.2)% of ODNs were released after 2 days for initial PEG contents of 0% and 10%, respectively (Fig. 4c). The corresponding cumulative release after 28 days were and 49.8 (±3.7)% and 85.3 (±5.2)%.

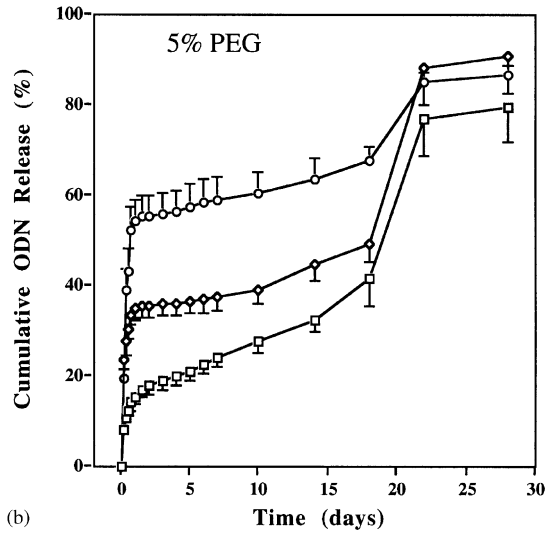
Under non-physiologic pH conditions, significant amounts of entrapped ODNs remain in the PLGA carrier after 4 weeks. This may be due to the aggregation of PLGA microparticles, as reported previously [28]. The results of the current study show that a possible method to address this problem is via the incorporation of PEG into the PLGA microparticles. In addition to the acidic PLGA degradation products, tumor sites may contain necrotic areas that may be acidic. Therefore, PLGA/PEG microparticles might provide a better ODN delivery vehicle than PLGA microparticles in some local delivery oncology applications.

3.2.3. Effects of buffer pH

When PLGA microparticles were incubated with different pH buffers, the release kinetics were very different from each other (Fig. 5a). About 15.1 (±0.9)%, 41.2 (±2.3)%, and 26.5 (±5.0)% of ODNs were released after 2 days in pH buffers 5, 7.4, and 10, respectively. During the steady release phase from day 3 to 18, the release rate at pH 5 (18.6 ± 2.0 ng/mg/day) was significantly ($p < 0.05$) higher than that at pH 7.4 (11.4 ± 0.4 ng/mg/day) and pH 10 (12.9 ± 3.2 ng/mg/day). The amounts of total released ODNs after day 28 at both pH 5 (795 ng/mg) and 10 (1,020 ng/mg) conditions were much lower than that of ODNs released at pH 7.4 (1,632 ng/mg).

When PLGA microparticles with 10% PEG were incubated with different pH buffers, the release kinetics were very different in both the initial burst phase and the steady release phase (Fig. 5b). About 55.2 (±3.6)%, 35.3 (±3.0)%, and 69.4 (±5.2)% of ODNs were released after 2 days in pH buffers 5, 7.4, and 10, respectively. During the steady release phase from day 3 to 18, the release rate at pH 5 (17.3 ± 5.1 ng/mg/day) was significantly ($p < 0.05$) higher than that at pH 7.4 (9.3 ± 1.0 ng/mg/day) and pH 10 (7.6 ± 0.2 ng/mg/day). The initial burst rates were greatly enhanced at both pH 5 and pH 10 conditions compared to pH 7.4 condition ($p < 0.01$). By the end of day 28, the amounts of total released ODNs at pH 5 (1,585 ng/mg), pH 7.4 (1,516 ng/mg), and pH 10 (1,644 ng/mg) were similar.





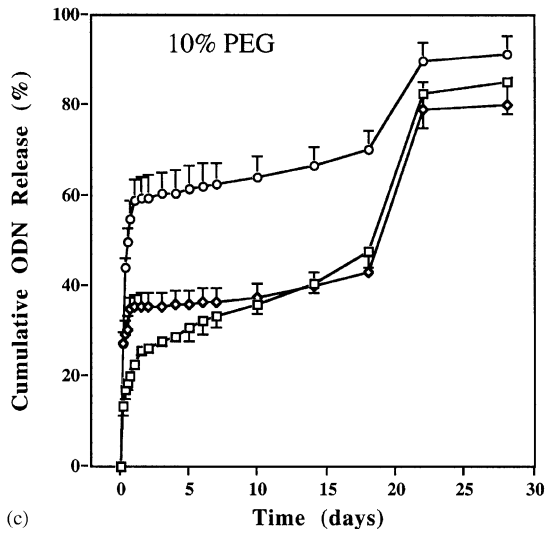
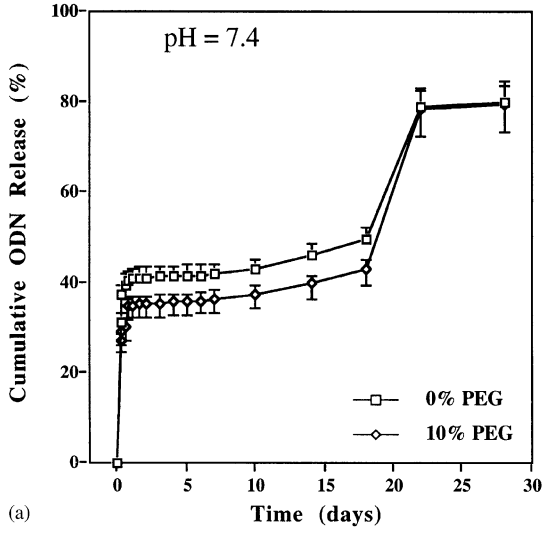
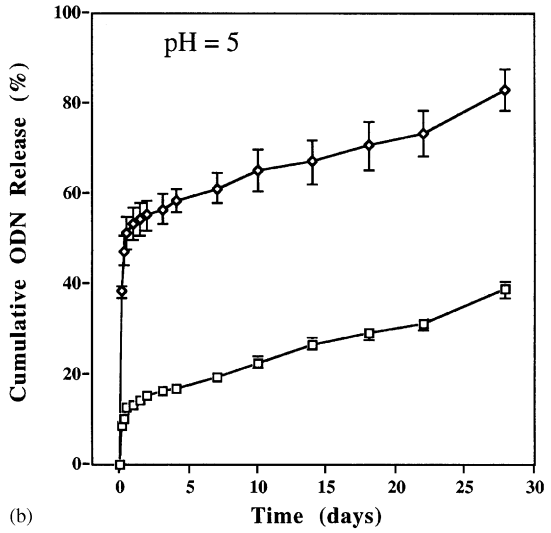


Fig. 3. Cumulative release kinetics of ODNs from PLGA/PEG microparticles at different loading densities in pH 7.4 PBS with (a) 0% PEG, (b) 5% PEG and (c) 10% PEG. Error bars represent means ± SD for $n = 4$.





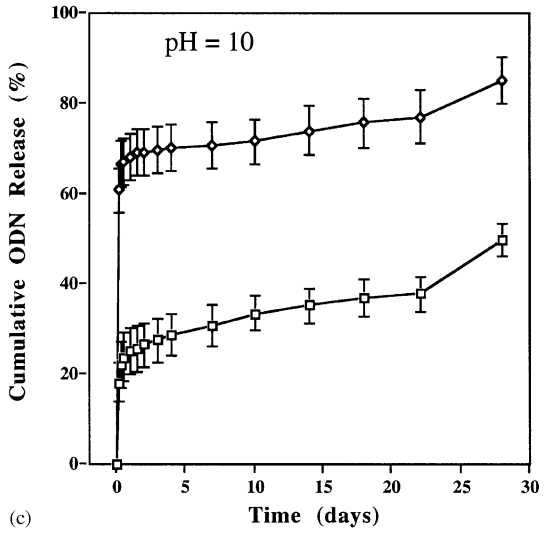


Fig. 4. Cumulative release kinetics of ODNs from PLGA/PEG microparticles with different PEG contents in (a) pH 7.4 PBS, (b) pH 5 buffer, and (c) pH 10 buffer. The ODN loading density was 4 μg/mg for all microparticles. Error bars represent means ± SD for $n = 4$.

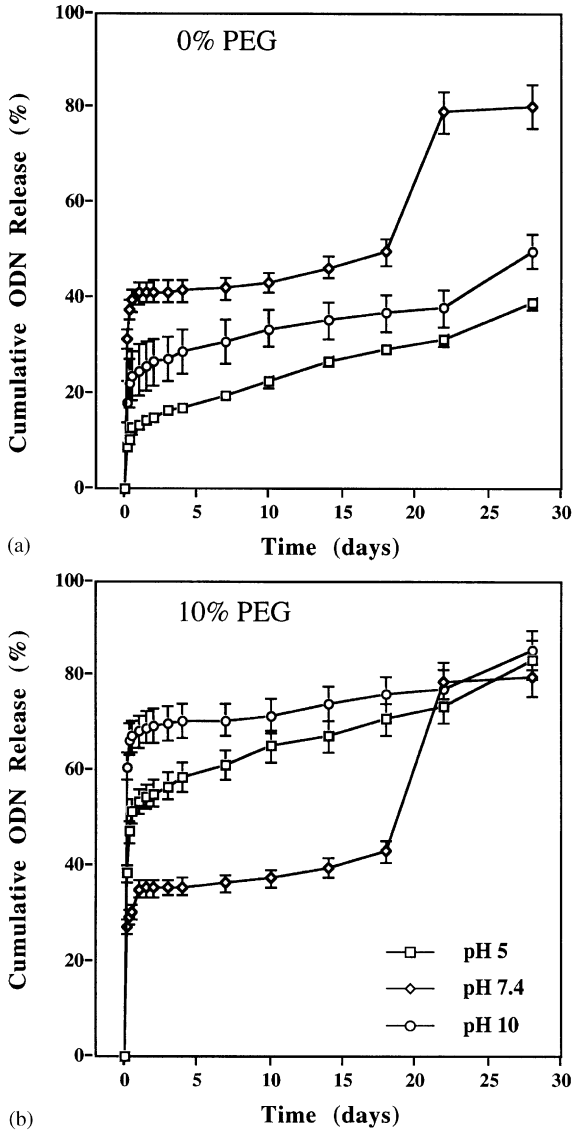


Fig. 5. Cumulative release kinetics of ODNs from PLGA/PEG microparticles with (a) 0% PEG and (b) 10% PEG in different pH buffers. The ODN loading density was 4 μg/mg. Error bars represent means ± SD for $n = 4$.

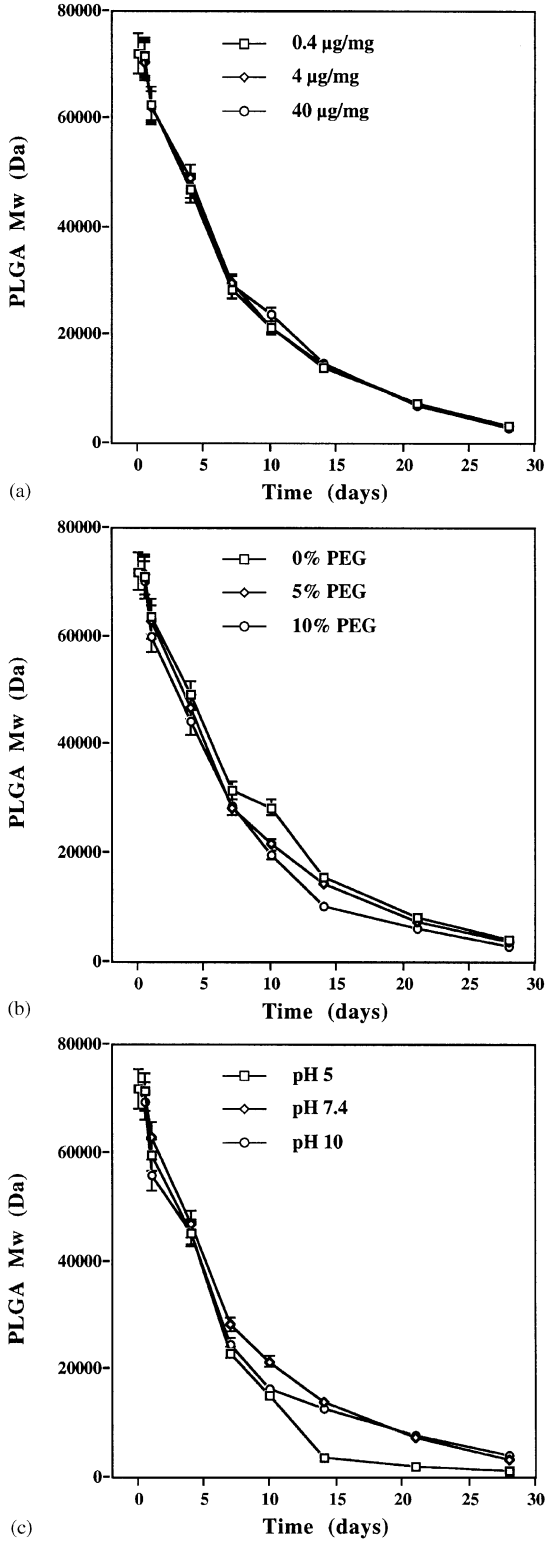


Fig. 6. Decrease of weight average molecular weight (M_w) of PLGA in PLGA/PEG microparticles: (a) microparticles with different ODN loading densities and 5% PEG incubated in pH 7.4 PBS, (b) microparticles with different PEG contents and 0.4 μg/mg ODNs incubated in pH 7.4 PBS, and (c) microparticles with 0.4 μg/mg ODNs and 5% PEG incubated with different pH buffers. Error bars represent means ± SD for $n = 4$.

These results indicate that the ODN release at neutral pH is more sustained and complete compared to the abnormal pH conditions. The incorporation of PEG in the microparticles increases the total release of ODNs under non-physiologic pH conditions and reduces the pH effects on ODN release kinetics.

3.3. In vitro degradation of microparticles

3.3.1. Effects of ODN loading

The M_w of PLGA decreased continuously throughout the experimental time course for microparticles with 5% PEG placed in pH 7.4 PBS (Fig. 6a). By day 14, 19.5 (±0.8)%, 19.8 (±0.3)%, and 20.5 (±0.3)% of the day

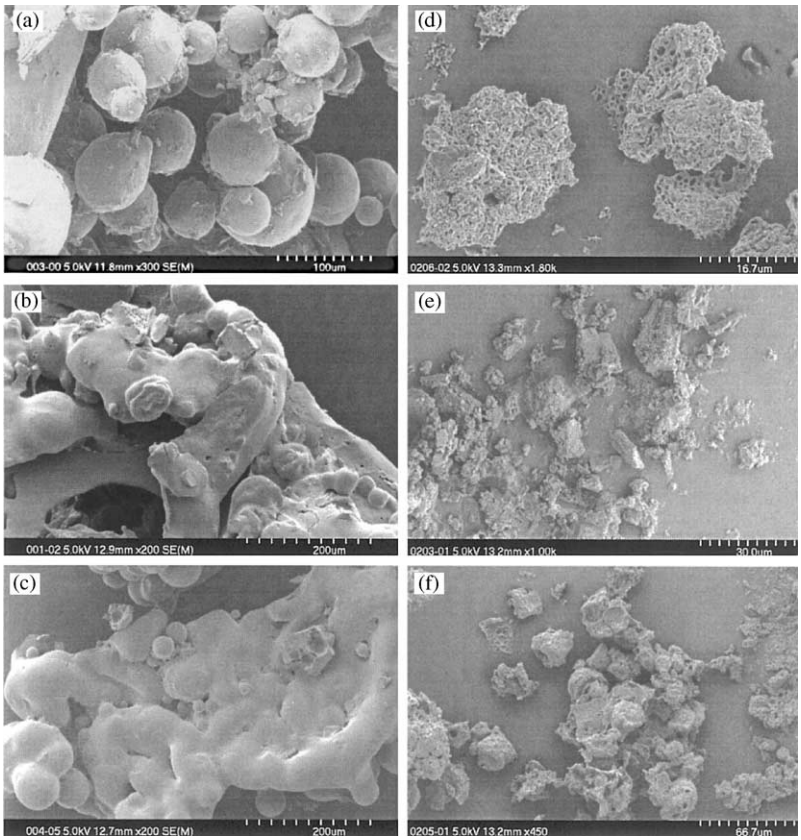


Fig. 7. Scanning electron micrographs of PLGA/PEG microparticles with 0.4 μg/mg ODNs and 5% PEG after (a–c) 14 days and (d–f) 28 days of in vitro degradation in (a, d) pH 7.4, (b, e) pH 5, and (c, f) pH 10 buffers.

zero M_w remained for microparticles with 0.4, 4, and 40 μg/mg ODNs, respectively. The corresponding half-lives of PLGA were 6.33 ± 0.06, 6.22 ± 0.06, and 6.18 ± 0.03 days. The degradation profiles for microparticles at three different ODN loading densities were similar ($p > 0.05$). These findings indicate that there is little effect of ODN loading on PLGA degradation. Therefore, a loading density of 0.4 μg/mg was chosen to study the effects of other factors on PLGA degradation.

3.3.2. Effects of PEG content

The M_w of PLGA decreased continuously throughout the time course for microparticles with varied PEG contents placed in pH 7.4 PBS (Fig. 6b). By day 14, only 21.5 (± 0.5)%, 19.5 (± 0.8)%, and 14.0 (± 0.8)% of the day zero M_w remained for microparticles with 0, 5, and 10 wt% initial PEG, respectively. The corresponding half-lives of PLGA were 6.66 ± 0.03, 6.33 ± 0.06, and 5.92 ± 0.03 days. The degradation profiles for all three PEG contents were similar, except for an increased rate of PLGA degradation ($p < 0.05$) when increasing the PEG content from 0% to 10%. The greater water uptake through the micropores created by the rapid dissolution of the PEG fraction from the microparticles could explain the increased PLGA degradation rate.

3.3.3. Effects of buffer pH

Heterogeneous bulk degradation of PLGA has been shown to result in the accumulation of acidic degradation products in the specimen center. Degradation profiles of PLGA microparticles with 5% initial PEG content in various pH buffers are shown in Fig. 6c. By day 14, the residual M_w of PLGA placed in pH 5, 7.4 and 10 buffers were 5.1 (± 0.5)%, 19.5 (± 0.8)%, and 17.4 (± 1.0)% of the day zero values, respectively. The corresponding half-lives of PLGA were 4.31 ± 0.06, 6.33 ± 0.06, and 6.73 ± 0.19 days. Acidic conditions (pH 5) dramatically increased the rate of PLGA degradation compared to that at neutral pH ($p < 0.01$). The basic condition (pH 10) did not significantly ($p > 0.05$) change the half-life value but did result in a faster degradation rate after day 7 ($p < 0.05$). These results were consistent with those previous reported indicating the degradation of PLGA was enhanced at both pH 5 and 10 compared to pH 7.4 [44].

3.3.4. Morphology

PLGA/PEG microparticles with 5% PEG were incubated with different pH buffers and examined by SEM after day 14 (Figs. 7a–c) and day 28 (Figs. 7d–f). In neutral pH buffers, the microparticles developed

rough surfaces after 14 days (Fig. 7a) and became irregularly shaped and porous after 28 days (Fig. 7d). Microparticles incubated with pH 5 (Figs. 7b and e) and pH 10 (Figs. 7c and f) buffers degraded faster and showed similar morphology. In addition, microparticle aggregation was observed under both acidic and basic conditions (Figs. 7b and c).

4. Conclusions

The release profiles of NFκB decoy phosphorothioated ODNs from biodegradable PLGA/PEG blend microparticles were investigated. Phosphorothioated ODNs were encapsulated into the microparticles and released in a controlled fashion in vitro for up to 28 days. Many important parameters have been identified that affect both the ODN release kinetics from the PLGA/PEG microparticles and PLGA degradation, including ODN loading density, PEG content, and pH of the incubation medium.

Increasing the ODN loading density from 0.4 to 40 μg/mg resulted in lower entrapment efficiency and faster release. For PLGA microparticles, approximately 80% of the loaded ODNs were released in pH 7.4 buffer after 28 days. However, when the buffer pH was decreased to 5 or increased to 10, the overall release was dramatically decreased to $<50\%$ by day 28. The pH effects were reduced by the incorporation of PEG into the microparticles. The cumulative ODN release after 28 days was found similar for microparticles with 10% PEG regardless of buffer pH.

The PLGA degradation rates were similar for various loading densities studied. Increasing the PEG content from 0% to 10% increased the degradation rate. PLGA degradation was also significantly faster at pH 5 compared to pH 7.4 and 10. Microparticle aggregation was observed during microparticle degradation under both acidic and basic conditions.

ODNs were released from the PLGA/PEG blend microparticles in a tri-phasic fashion. The release profiles were similar to that previously established for protein release from biodegradable microparticles [28,45]. The initial burst effect was due to the desorption of ODNs at the microparticle surface. Polymer hydration and ODN diffusion led to a linear release phase. Subsequent solubilization and release of low molecular weight degradation products from the microparticles resulted in polymer mass loss and a final release phase. The time course of the last release phase correlated well with polymer erosion.

In summary, we have identified the important factors that affect the ODN release kinetics from PLGA/PEG microparticles. Thus, desired local therapeutic concentrations of ODNs might be obtained by varying these factors. ODNs released from such PLGA/PEG delivery system were protected from degradation and shown to inhibit the proliferation of glioblastoma cells up to 50% in three days [32]. Our results suggest that PLGA/PEG microparticles are appropriate as delivery vehicles for controlled release of NFκB decoy phosphorothioated ODNs. Since most antisense ODNs are phosphorothioated ODNs, PLGA/PEG microparticles may also be appropriate as delivery vehicles for controlled release of antisense ODNs.

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