Syntheses and Biological Activities of Polymers Containing Methacryloyl-2-oxy-1,2,3-propanetricarboxylic Acid or 5-Fluorouracil

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ABSTRACT: A series of novel copolymers, poly(methacryloyl-2-oxy-1,2,3-propanetricarboxylic acid-*co*-*exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic acid) [poly(MTCA-*co*-ETAc)], poly(methacryloyl-2-oxy-1,2,3-propanetricarboxylic acid-*co*-hydrogenethyl-*exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalate) [poly(MTCA-*co*-HEET)], and poly(methacryloyl-2-oxy-1,2,3-propanetricarboxylic acid-*co*-α-ethoxy-*exo*-3,6-epoxy-1,2,3,6-tetrahydrophthaloyl-5-fluorouracil) [poly(MTCA-*co*-EETFU)], were prepared from corresponding monomers by photopolymerizations at 25°C for 48 h. The polymers were identified by FTIR, ^{1}H-NMR, and ^{13}C-NMR spectroscopies. The number-average molecular weights of the fractionated polymers determined by GPC were in the range from 9400 to 14,900 and polydispersity indices were 1.2–1.4. The *in vitro* IC_{50} values of polymers against mouse mammary carcinoma (FM3A), mouse leukemia (P388), and human histiocytic lymphoma (U937) as cancer cell lines and mouse liver cells (AC2F) as a normal cell line were much higher compared to that of 5-fluorouracil (5-FU). The *in vivo* antitumor activities of monomers and polymers against mice bearing sarcoma 180 tumor cell line were better than those of 5-FU. The inhibition of DNA replication and antiangiogenesis activities of MTCA and copolymers were better compared to those of 5-FU. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 94: 57–64, 2004

Key words: polymer prodrug; photopolymerization; antitumor activities; FTIR; antiangiogenesis

INTRODUCTION

It has been reported that the copolymer of divinyl ether and maleic anhydride (DIVEMA) has not only antitumor, antiviral, antibacterial, interferon-inducing, and antifungal activities but also toxic side effects, such as enlarged liver and spleen, for example.[1,2] Many attempts have been made to reduce the toxic side effects and to obtain excellent antitumor activities.[3–8] Polymers containing 5-fluorouracil (5-FU) have been synthesized to obtain higher antitumor activities.[9–14] We previously reported on syntheses and biological activities of polymeric antitumor compounds such as 5-FU–containing acryl derivative polymers,[9–15] tetrahydrophthalic acid derivative polymers (TADPs),[16–23] and methoxyitaconyl polymers,[24] and poly(diallyl ether-*co*-maleic anhydride)[25] and poly(glycinyl maleamic acid) derivatives[26,27] for some years now. Among them, TADPs showed excellent antitumor activities and low cytotoxicities *in vivo*.[16–23]

The aim of this study was to synthesize a series of novel copolymers containing methacryloyl-2-oxy-1,2,3-propanetricarboxylic acid (MTCA) or 5-FU and to evaluate the *in vitro* cytotoxicities, *in vivo* antitumor activities, the inhibitions of DNA replication, and antiangiogenesis activities. Poly(methacryloyl-2-oxy-1,2,3-propanetricarboxylic acid-*co*-*exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic acid) [poly(MTCA-*co*-ETAc)], poly(methacryloyl-2-oxy-1,2,3-propanetricarboxylic acid-*co*-hydrogenethyl-*exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalate) [poly(MTCA-*co*-HEET)], and poly(methacryloyl-2-oxy-1,2,3-propanetricarboxylic acid-*co*-α-ethoxy-*exo*-3,6-epoxy-1,2,3,6-tetrahydrophthaloyl-5-fluorouracil) [poly(MTCA-*co*-EETFU)] were prepared from corresponding monomers by photopolymerizations at 25°C for 48 h using 2,2-dimethoxy-2-phenylacetophenone (DMP) as a photoinitiator. The synthesized copolymers were identified by FTIR, ^{1}H-NMR, and ^{13}C-NMR spectroscopies. The number- and weight-average molecular weights and polydispersity indices were determined by GPC. The *in vitro* cytotoxicities of monomers and polymers were evaluated with mouse mammary carcinoma (FM3A), mouse leukemia (P388), and human histiocytic lymphoma (U937) as cancer cell lines and mouse liver cell (AC2F) as a normal cell line. The *in vivo* antitumor activities of the prepared samples against mice bearing sarcoma 180 tumor cell line were evaluated. The inhibition of DNA

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replication for the synthesized monomers and polymers was evaluated using simian virus 40 (SV40) DNA. The antiangiogeneses of poly(MTCA-*co*-ETA), poly(MTCA-*co*-HEET), and poly(MTCA-*co*-EETFU) were examined by the embryo chorioallantoic membrane (CAM) assay.

EXPERIMENTAL

Materials

Citric acid (CA), methacrylic anhydride (MAAH), *exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride (ETA), DMP, and 5-FU (Aldrich Co., Milwaukee, WI) were used without further purification. Triethylamine (TEA; Junsei Co., Tokyo, Japan) was refluxed with acetic anhydride and KOH, and finally distilled. MEK, chloroform, DMSO-d_6, thionyl chloride, dimethoxyethane (DME), and all other chemicals were reagent grade and were used without further purification.

P388, FM3A, and U937 as cancer cell lines and AC2F as a normal cell line were used. Balb/C mouse and sarcoma 180 cell line were purchased from the Center of Genetic Engineering (Korea Institute of Science and Technology). SV40 Tag and SV40 origin-containing circular duplex DNA (pUC-ori$^+$) were prepared according to a previously published method.[28] Fertilized chick eggs were obtained from the Han-shin Farm (Kimhae, Korea). Fat emulsion (10%) was purchased from Green Cross Pharmaceutical Co. (Seoul, Korea). Thermanox coverslips were purchased from Nunc Inc. (Naperville, IL).

Measurements

^{1}H-NMR and ^{13}C-NMR spectra were recorded on an FT-300 MHz Varian Gemini 2000 spectrophotometer (Varian Associates, Palo Alto, CA). IR spectra were obtained with a Jasco FT/IR-5300 spectrophotometer (Jasco, Tokyo, Japan) by use of a KBr pellet for analysis. Elemental analysis was performed on a Carlo Erba Model EA1180 elemental analyzer (Carlo Erba Instruments, Milan, Italy). The number- and weight-average molecular weights were determined by gel permeation chromatography (GPC; Waters 410 instrument; Waters Chromatography Division/Millipore, Milford, MA). Photopolymerization was carried out under the irradiation of UV light (λ_{max} = 313 nm) in a photochemical chamber.

Syntheses of monomers

Synthesis of metharyloyl-2-oxy-1,2,3-propanetricarboxylic acid (MTCA)

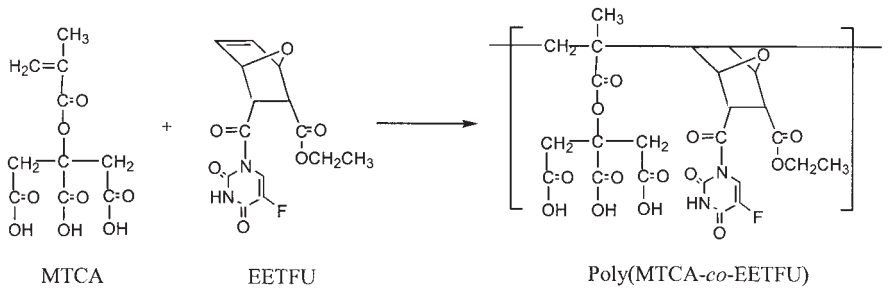
The monomer MTCA was prepared as described in the literature.[29] The general reaction procedure is as follows. To a 500-mL three-neck flask, equipped with a dropping funnel and a mechanical stirrer, was added 30 g (15.6 mmol) of CA, 200 mL of the mixed solvent of MEK and acetic acid (1 : 1, v/v), one drop of sulfuric acid, and 0.5 g of sulfur as an inhibitor, and the mixture was stirred at 80°C for 2 h. MAAH (27.8 mL, 18.6 mmol) was slowly added through the dropping funnel and the mixture was again stirred for 30 min. The obtained contents were poured into a large amount of chloroform to precipitate the monomer. The white product was collected by filtration, dried, and dissolved in about 4× *n*-hexane. After removing the insoluble product, the *n*-hexane solution was reprecipitated in excess chloroform to obtain pure MTCA. The resulting precipitate was dried under reduced pressure. The yield and melting point of MTCA were 32% and 183–184°C, respectively. In the ^{1}H-NMR spectrum of MTCA (DMSO-d_6), the peaks of methyl, methylene, and acid protons appeared at 1.8, 3.3, and 12.4 ppm, respectively. The peaks at 5.6 and 5.9 ppm were assigned to vinyl protons.

Synthesis of EETFU[23]

Hydrogen ethyl-exo-*3,6-epoxy-1,2,3,6-tetrahydrophthalate (HEET).* ETA (30 g, 1.8 mmol) and ethanol (16 mL, 2.7 mmol) were refluxed at 40–45°C until the solution was clear. The obtained solution was cooled in an ice bath and the solution was poured into the mixed solvent of diethyl ether and petroleum ether (1 : 1, v/v) to obtain a white precipitate. The obtained precipitate was filtered and dried under vacuum to obtain pure HEET. The melting point and yield were 100–103°C and 53%, respectively. The structure of HEET was identified by FTIR spectroscopy.

α-Ethoxy-exo-*3,6-epoxy-1,2,3,6-tetrahydrophthaloyl chloride (EETC).* The solution of HEET (9.6 g, 0.45 mmol) and thionyl chloride (6 mL) was refluxed at 60°C until HCl gas disappeared. Unreacted thionyl chloride was removed by vacuum distillation. The product was fractionated to yield the half-chloride (yield: 58%), bp 80°C/3 mmHg. The structure of EETC was confirmed by FTIR spectroscopy.

α-Ethoxy-exo-*3,6-epoxy-1,2,3,6-tetrahydrophthaloyl-5-fluorouracil (EETFU).* A solution of 5-FU (2.0 g, 15.4 mmol) and TEA (2.52 mL, 18.4 mmol) in 140 mL of DME was refluxed for 2 h, with precautions being taken to exclude moisture from the atmosphere, and then the clear solution was cooled to 0°C in an ice bath. The prepared EETC solution (5.2 g, 23 mmol) in 20 mL of dry DME was slowly added dropwise with vigorous stirring at 0°C for 2 h. The triethylamine hydrochloride salt was filtered out and the filtrate was concentrated on a rotary evaporator. The concentrated solution was added to a large amount of *n*-hexane. The pale yellow precipitate was collected by filtration, washed successively with diethyl ether and petroleum ether, and dried under vacuum. The precipitate was



Scheme 1 Preparation of poly(MTCA-*co*-EETFU).

again dissolved in MEK. After removing the insoluble product, the clear solution was reprecipitated in *n*-hexane and the precipitate was dried to a constant weight under vacuum to obtain pure EETFU (yield: 20%). The melting point was 250–253°C.

ANAL. calcd for $C_{14}H_{13}O_6N_2F$: C, 51.82%; H, 4.25%; N, 7.93%. Found: C, 51.80%; H, 4.45%; N, 7.35%. In the ^{1}H-NMR spectrum of EETFU (DMSO-d_6), the peaks of methyl and vinyl protons in EETFU appeared at 1.2 and 6.7 ppm, and olefinic and N—H protons of 5-FU indicated at 7.7 and 11.5 ppm, respectively.

Syntheses of copolymers

Poly(MTCA-*co*-ETAc

A solution of MTCA (19.8 g, 6.6 mmol), ETA (10.98 g, 6.6 mmol), and DMP (0.066 g, 0.26 mmol), dissolved in 15 mL of dry THF, was introduced into a dry polymerization tube. The tube was sealed after flushing twice with purified N_2 gas. The tube was irradiated by a UV lamp of 313 nm in a photochemical chamber at 25°C for 48 h. The obtained polymer solution was slowly dropped into 150 mL of *n*-hexane to precipitate the polymer. The precipitated polymer was collected by filtration and washed several times with *n*-hexane, and the obtained polymer was dried under vacuum until it reached a constant weight. The conversion was 97%. The obtained poly(metharyloyl-2-oxy-1,2,3-propanetricarboxylic acid-*co*-*exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride) [poly(MTCA-*co*-ETA)] was hydrolyzed with 0.01*N* NaOH aqueous solution to obtain poly(MTCA-*co*-ETAc).

Poly(MTCA-*co*-HEET)

A solution of MTCA (18 g, 6 mmol), HEET (5.8 g, 6 mmol), and DMP (0.066 g, 0.26 mmol), dissolved in 15 mL of dry THF, was introduced into a dry polymerization tube. The preparation process of poly(MTCA-*co*-HEET) was the same as that described for the copolymerization of MTCA and ETA except for monomer pairs. The copolymerization conversion was 65%.

Poly(MTCA-*co*-EETFU)

The copolymer poly(MTCA-*co*-EETFU) was prepared as shown in Scheme 1. The photopolymerization procedure of MTCA and EETFU was the same as that described for the copolymerization of MTCA and ETA except for monomer pairs. The conversion was 72%.

Determination of average molecular weights

The average molecular weights and polydispersity (PD = M_w/M_n) were determined by gel permeation chromatography (GPC) using a Waters GPC 410 instrument with a refractive index detector and four μ-Styragel columns with pore sizes of 10^5, 10^4, 10^3, and 500 Å, connected in series. The standard used was polystyrene and the eluent was DMF at a flow rate of 1 mL/min (40°C).

In vitro cytotoxicity test[20–23]

The *in vitro* cytotoxicities of monomers and the synthesized polymers were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay method.[30] The samples that had poor solubility in water were dissolved in dimethyl sulfoxide (DMSO) of minimum quantity and the obtained solution was diluted with phosphate-buffered saline just before use. The prepared sample solution was added to the P388, FM3A, and U937 cancer cell lines and the AC2F normal cell line (1.5×10^4 cells/mL) in 96-well microtiter plates and cultured at 37°C for 3 days. The cultured cell lines were mixed with 20 μL of MTT solution and incubated at 37°C for 4 h. The supernatant was removed from each well and 100 μL of 100% DMSO was added to solubilize the formazan crystals that were formed by the cellular reduction of MTT. After mixing by a mechanical plate mixer, absorbance spectra were measured on an ELISA Processor II microplate reader (ProLab Diagnostics, Edmonton, Alberta, Canada) at the wavelength of 570 nm. The percentage cytotoxicity was determined by comparing results from treated and untreated cell lines. The 50% cytotoxic dose (IC_{50}) was defined as the con-

centration of samples that reduced the absorbance of the treated cells by 50%.

In vitro antitumor activities test

To evaluate the *in vivo* antitumor activity of the synthesized samples, mice bearing sarcoma 180 tumor cells were used. Ten Balb/C mice (weight: 20 ± 1.5 g) for each group were first intraperitoneally (i.p.) implanted with sarcoma 180 cells (2×10^5 cells/mL). The mice were then treated with a saline of sample on days 1–4. Three different dosages such as 0.8, 80, and 800 mg/kg were tested. For comparison, the antitumor activity of 5-FU was also tested by the same method. A control group was divided into two groups. One group was treated with sarcoma 180 cells along with the same volume of saline and the other group was treated with only sarcoma 180 cells. The ratio (T/C) obtained by survival time of mice treated with polymer (T) to that of mice in control groups (C) was used as the index of the antitumor activity.

In vitro inhibition of SV40 DNA replication

Replication reactions were carried out as described previously.[28] In brief, reaction mixtures (40 μL) included 40 m*M* creatine phosphate-ditris salt (pH 7.7), 1 μg of creatine kinase, 7 m*M* $MgCl_2$, 0.5 μg of bovine serum albumin, 0.5 m*M* dithiothreitol (DTT), 4 m*M* adenosine 5′-triphosphate (ATP) (pH 7.5), 334 μ*M* uridine 5′-triphosphate (UTP), guanosine 5′-triphosphate (GTP), cytidine 5′-triphosphate (CTP), 100 μ*M* deoxyadenosine 5′-triphosphate (dATP), deoxyguanosine 5′-triphosphate (dGTP), deoxycytidine 5′-triphosphate (dCTP), 25 μ*M* [^{3}H]dTTP (1000 cpm/pmol), 0.5 μg of SV40 Tag, 0.25 μg of SV40 origin-containing DNA (pUC-ori$^+$), and the indicated amounts of human replication protein A (RPA). The reaction ran at 37°C for 2 h, after which the acid-insoluble radioactivity was measured. Replication products were analyzed with [α-^{32}P]dATP (30,000 cpm/pmol) instead of [^{3}H]dTTP in the replication reactions just described. After incubation, the reactions were stopped by the addition of 40 μL of a solution containing 40 m*M* ethylenediaminetetraacetic acid (EDTA) (pH 8.0), 2% sodium dodecyl sulfate (SDS), 1 mg/mL *E. coli* tRNA and 20 m*M* Tris–Cl (pH 7.8). One tenth of the reaction mixture was used to measure the acid-insoluble radioactivity. Replication products in the remaining reaction mixture were analyzed by electrophoretical separation of the isolated DNA in a 1.0% agarose gel overnight at 42 V. The gel was subsequently dried and exposed to X-ray film.

Chorioallantoic membrane (CAM) assay for antiangiogenesis

The fertilized chicken eggs used in this study were kept in humidified incubator at 37°C. After incubation for 3 days, about 1 mL of albumin was aspirated from each eggs with an 18-gauge hypodermic needle through a small hole drilled at the narrow end of the eggs, allowing the small CAM and yolk sac to drop away from the shell membrane. On day 4, the shell covering the air sac was punched out and removed by forceps, and the shell membrane on the floor of the air sac was peeled away.[31] Embryos with chorioallantois of 3–5 mm in diameter were used for the assay of antiangiogenesis activity. An aqueous, salt-free solution of each sample (5 μL) was applied to sterile Thermanox 15-mm disks and allowed to dry under laminar flow conditions.[32] The loaded disks were inverted and applied to the CAM surface of 4.5-day-old embryos through the windows. The air sac ends of the embryo with shells were covered with Scotch tape. Two days later, an appropriate volume of a 10% fat emulsion was injected using a 33-gauge needle into the 6.5-day embryo chorioallantois so that the vascular network of the CAM stood out against the white background of lipid.

The antiangiogenic response was assessed by the measurement of a vascular zone of the CAM beneath the disk. When the CAM showed a vascular zone of diameter ≥ 3 mm, the response was scored as a positive according to the method of Crum et al.[33] Only the frequency was monitored, so it was not indicated whether a higher dose also yielded larger vascular zones. At least 20 eggs were used for each dose of agent. Finally, the chorioallantoic membranes were microphotographed.

RESULTS AND DISCUSSION

Identification of the synthesized polymers

In the ^{1}H-NMR spectrum (DMSO-d_6) of poly(MTCA-*co*-ETAc), the peaks of methyl, methylene, and acid protons of MTCA moiety appeared at 1.95, 1.1, and 12.5 ppm and the methine and acid protons in ETAc moiety showed peaks at 3.0 and 12.5 ppm, respectively. In the ^{1}H-NMR spectrum (DMSO-d_6) of poly(MTCA-*co*-HEET), the methyl and methylene protons of MTCA moiety exhibited peaks at 1.95 and 1.1 ppm and the peaks of methine and acid protons of HEET moiety appeared at 3.0 and 13.0 ppm, respectively. The ^{1}H-NMR spectrum (DMSO-d_6) of poly(MTCA-*co*-EETFU) showed the peaks of methyl, methylene, and acid protons of MTCA moiety at 1.95, 1.1, and 12.7 ppm and methyl and N—H protons of EETFU moiety at 1.2 and 10.7 ppm, respectively.

Solubility of the prepared monomers and polymers

The solubilities of the monomers and copolymers are listed in Table I. Monomers and polymers were soluble in DMF and poorly soluble in water, acetone,

TABLE I
Solubility of the Synthesized Monomers and Polymers

Sample	Solvent[a]					
	Water	Acetone	MEK	DMSO	DMF	Ether
MTCA	PS	IS	PS	PS	S	IS
EETFU	S	S	S	S	S	PS
Poly(MTCA-*co*-ETAc)	S	IS	PS	PS	S	IS
Poly(MTCA-*co*-HEET)	PS	PS	PS	PS	S	IS
Poly(MTCA-*co*-EETFU)	PS	PS	PS	PS	S	IS

[a] S, soluble; PS, poorly soluble; IS, insoluble.

MEK, and DMSO, except for the good solubility of poly(MTCA-*co*-ETAc) in water, although they were insoluble in diethyl ether (except EETFU).

Average molecular weights

The average molecular weights of the fractionated polymers are listed in Table II. The number- and weight-average molecular weights and polydispersity indices determined with GPC are as follows: M_n = 14,400, M_w = 19,600, M_w/M_n = 1.4 for poly(MTCA-*co*-ETA); M_n = 14,900, M_w = 20,800, M_w/M_n = 1.4 for poly(MTCA-*co*-HEET); and M_n = 9400, M_w = 10,900, M_w/M_n = 1.2 for poly(MTCA-*co*-EETFU). Ottenbrite et al.[34] reported that good antitumor activity can be obtained in the range of average molecular weights from 10,000 to 30,000 depending on polymers. The average molecular weights of the synthesized polymers were in a reasonable range to exhibit antitumor activity.

In vitro cytotoxicity

The *in vitro* cytotoxicities of the synthesized polymers were evaluated against three cancer cell lines, P388, FM3A, U937, and one AC2F normal cell line. As shown in Table III, the IC_{50} values of synthesized samples against several tumor cell lines decreased in the following order: 5-FU ≈ EETFU > poly(MTCA-*co*-EETFU) > poly(MTCA-*co*-ETAc) > MTCA > poly(MTCA-*co*-HEET) for FM3A and P388; EETFU > 5-FU > poly(MTCA-*co*-EETFU) > poly(MTCA-*co*-HEET) > poly(MTCA-co-ETAc) > MTCA for U937.

TABLE II
Average Molecular Weights and Polydispersity of Polymers

Sample	M_n[a]	M_w[a]	M_w/M_n
Poly(MTCA-*co*-ETA)	14,400	19,600	1.4
Poly(MTCA-*co*-HEET)	14,900	20,800	1.4
Poly(MTCA-*co*-EETFU)	9,400	10,900	1.2

[a] The number- (M_n) and weight- (M_w) average molecular weights of polymers were determined by GPC in DMF.

The lower the IC_{50} value of compound means the stronger its *in vitro* antitumor activity. Thus, the *in vitro* antitumor activities of polymers were found to be greater than those of MTCA except for poly(MTCA-*co*-HEET) for FM3A and P388. The cytotoxicities of 5-FU, MTCA, and the prepared polymers against a normal cell line increased in the following order: EETFU > 5-FU > MTCA > poly(MTCA-*co*-ETAc) > poly(MTCA-*co*-HEET) ≈ poly(MTCA-*co*-EETFU). The cytotoxicities of polymers against normal cell line were lower than those of EETFU and 5-FU. The *in vitro* antitumor activities of the synthesized polymers against cancer cell lines were much lower compared to those of 5-FU, which is known as a low molecular weight antitumor agent. However, the cytotoxicities of the synthesized polymers against normal cell line were much weaker compared to those of 5-FU. Poly(MTCA-*co*-EETFU) showed higher antitumor activity and lower cytotoxcity compared to those of the other copolymers.

In vitro antitumor activity

The *in vivo* antitumor activities of monomers and polymers against mice bearing sarcoma 180 tumor cell

TABLE III
Cytotoxicity of the Synthesized Samples Against Cancer Cell Lines

Sample	IC_{50} (μg/mL) for cell line[a]			
	Cancer cell			Normal cell
	FM3A[b]	P388[c]	U937[d]	AC2F[e]
5-FU	0.03	0.04	0.05	0.16
MTCA	84.00	44.00	100.00	10.00
EETFU	0.04	0.04	0.03	0.01
Poly(MTCA-*co*-ETAc)	25.00	17.00	37.50	11.00
Poly(MTCA-*co*-HEET)	100.00	82.00	34.00	15.00
Poly(MTCA-*co*-EETFU)	23.00	15.50	23.00	15.00

[a] The 50% growth inhibition concentration (IC_{50}).
[b] Mouse mammary carcinoma cell.
[c] Mouse leukemia cell.
[d] Human histiocytic lymphoma cell.
[e] Mouse liver cell.

TABLE IV
***In Vivo* Antitumor Activity of the Synthesized Samples**

Sample	Dosage (mg/kg)	Mean survival time (day)[a]	T/C (%)[b]	S/E[c]
Control	—	14.7 ± 2.3	100	0/10
	saline	15.7 ± 0.5	100	0/10
5-FU	800.0	5.9 ± 0.3	39	0/10
	80.0	21.3 ± 2.8	140	0/10
	0.8	20.3 ± 1.8	134	0/10
MTCA	800.0	25.0 ± 1.0	170	0/10
	80.0	34.0 ± 1.0	231	0/10
	0.8	29.5 ± 0.7	201	0/10
EETFU	800.0	9.6 ± 1.1	61	0/10
	80.0	74.2 ± 8.9	463	2/10
	0.8	31.0 ± 6.0	197	0/10
Poly(MTCA-*co*-ETAc)	800.0	27.0 ± 1.7	184	0/10
	80.0	39.5 ± 8.4	269	0/10
	0.8	51.4 ± 1.5	350	0/10
Poly(MTCA-*co*-HEET)	800.0	21.0 ± 0.6	143	0/10
	80.0	30.2 ± 3.1	205	0/10
	0.8	41.1 ± 0.9	280	0/10
Poly(MTCA-*co*-EETFU)	800.0	7.0 ± 1.2	48	0/10
	80.0	43.5 ± 5.9	296	0/10
	0.8	53.4 ± 4.9	363	0/10

[a] Mean survival time of animals dying within experiment period of 75 days.
[b] T/C (%) represents the ratio of the survival time of treated (T) to control (C) animals × 100.
[c] S/E denotes the ratio of the number of survival mice (S) to number of experimental mice (E) after experimental period of 75 days.

line are listed in Table IV and 5-FU was used for comparison. The ratio T/C was used as the index of the antitumor activity:

$$T/C\ (\%) = \frac{\text{Survival time of mice treated with polymer } (T)}{\text{Survival time of mice in a control group } (C)} \times 100$$

The synthesized copolymers showed antitumor activities in the range of 143–363% at all dosages except for poly(MTCA-*co*-EETFU) at 800 mg/kg. We applied the high dosage (800 mg/kg) to mice to evaluate the toxic side effect of the samples within a short period. The *in vivo* antitumor activity of the samples at a dosage of 0.8 mg/kg decreased in the following order: poly(MTCA-*co*-EETFU) > poly(MTCA-*co*-ETAc) > poly(MTCA-*co*-HEET) > MTCA ≈ EETFU > 5-FU.

The optimum *in vivo* antitumor activities of the synthesized copolymers containing MTCA moiety were found to be greater than those of DIVEMA.[12] This result was ascribed to the greater number of carboxylic acid groups in a repeat unit of the synthesized polymers compared with those of DIVEMA, which has two carboxylic groups.

The life spans (T/C) of mice treated with the samples were longer compared with those of 5-FU and the control group at a dosage of 0.8 mg/kg, which did not exhibit a higher toxic side effect than that at the higher concentration (800 mg/kg). However, T/C values of 5-FU, EETFU, and poly(MTCA-*co*-EETFU) at the 800 mg/kg dose were much lower than those at lower concentrations because of the toxic side effects at high concentration. The results were related to the *in vitro* cytotoxicities of 5-FU (IC_{50}: 0.16 μg/mL) and EETFU (IC_{50}: 0.01 μg/mL) against a normal cell line.

The order for the copolymers may be attributed to the degree of ease of countercharge interactions between –COOH and $–COO^-$ – per repeat unit in the polymer and –OH, –NH—CO–, and so forth, in the units of glycoprotein and oligosaccharide on a specific area of the cell surface, and to the degree of inhibition for the growth of tumor cells.

The more easily the interactions occur, the more efficiently endocytosis of the polymers proceeds. Thus the above order for the poly(MTCA-*co*-ETAc) and poly(MTCA-*co*-HEET) is ascribed to the degree of ease of endocytosis of the polymers. Poly(MTCA-*co*-ETAc) has five COOH groups compared to poly(MTCA-*co*-HEET), which has four COOH groups in a repeat unit.

Poly(MTCA-*co*-EETFU) showed the highest *in vivo* antitumor activity. This result was attributed to the action of the released 5-FU moiety, which is known as an inhibitor for DNA synthesis. More experimental evidence is needed to verify the above explanation.

Inhibition of SV40 DNA replication for monomers and polymers

The inhibition of SV40 DNA replication *in vitro* of the samples is listed in Table V. The greater the values of inhibition are, the more efficiently the polymer inhibits the SV40 DNA replication. As shown in Table V, the inhibition values of the synthesized samples are greater than those of control. The inhibition of SV40 DNA replication of MTCA was more efficient compared with that of the synthesized polymers. However, the inhibition values for the DNA replication of the synthesized copolymers were not much different.

Antiangiogenetic activities of monomers and polymers

In 1996 Folkman[35] reported that the inhibition of angiogenesis may lead to inhibition of tumor growth and metastasis. Because the antiangiogenic compounds prevent the formation of new blood vessels that supply nutrients to tumor cells. As shown in Table V, the antiangiogenesis of the synthesized monomers and polymers decreased in the following order: poly(MTCA-*co*-EETFU) > poly(MTCA-*co*-ETAc) > EETFU > poly(MTCA-*co*-HEET) > MTCA > 5-FU

TABLE V
Antiangiogenic Activities and the *In Vitro* Inhibition of SV40 DNA Replication for the Samples

Sample	Inhibition (%)		Inhibition of SV40 DNA replication (%)
	Antiembryogenesis[a]	Antiangiogenesis[b]	
Control	16.7	30.0	0
5-FU	52.3	50.0	12.7
MTCA	35.0	52.0	69.8
EETFU	50.0	55.6	11.5
Poly(MTCA-*co*-ETAc)	32.0	62.0	35.8
Poly(MTCA-*co*-HEET)	29.0	53.0	32.8
Poly(MTCA-*co*-EETFU)	49.0	72.0	30.2

[a] Number of antiembryogenic eggs/number of total eggs × 100.
[b] Number of antiangiogenic eggs/number of total eggs × 100.

> control. Poly(MTCA-*co*-EETFU) showed much higher antiangiogenesis compared to that of the other samples.

The microphotographs of monomers and polymers by the CAM assay are shown in Figure 1. The formed blood vessels of eggs treated with the synthesized monomers and polymers were fewer than those of the control. This means that the synthesized monomers and polymers had antiangiogenesis. Among the synthesized monomers and polymers, poly(MTCA-*co*-EETFU) showed the highest antiangiogenesis, a result that agreed well with the *in vivo* antitumor activity (T/C) of poly(MTCA-*co*-EETFU) at 0.8 mg/kg.

According to the *in vitro* (Table III), *in vivo* (Table IV), and antiangiogenic (Table V) data, the synthesized monomers and polymers are active in both tumor cells and endothelial cells, although the extent of their activity differs.

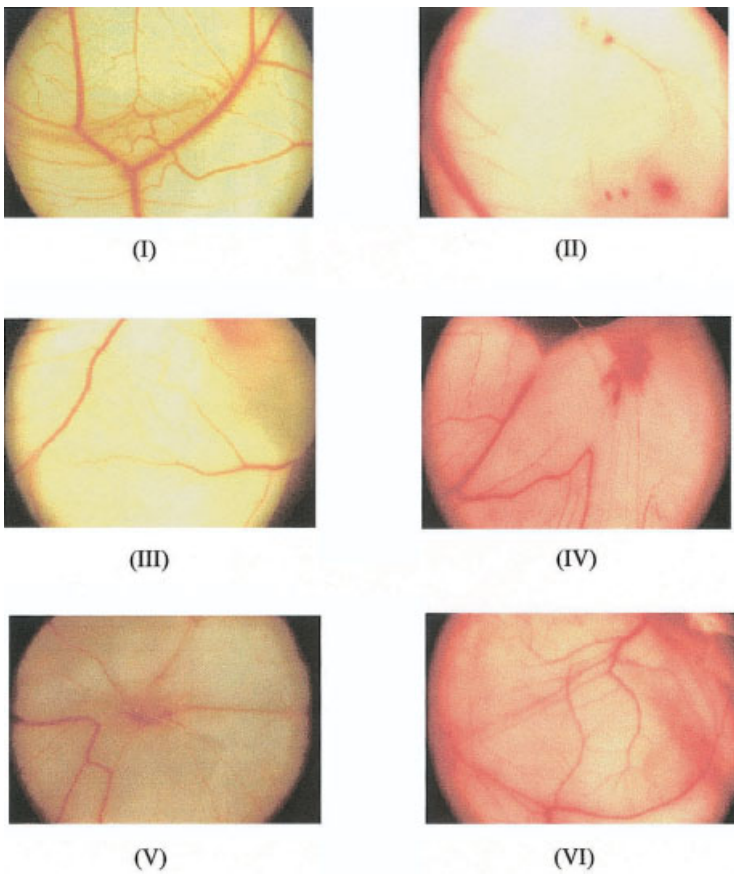


Figure 1 Microphotographs of control (I), MTCA (II), EETFU (III), poly(MTCA-*co*-ETAc) (IV), poly(MTCA-*co*-HEET) (V), and poly(MTCA-*co*-EETFU) (VI) on embryonic angiogenesis in CAM (×100).

CONCLUSIONS

A series of novel polymers, such as poly(MTCA-*co*-ETAc), poly(MTCA-*co*-HEET), and poly(MTCA-*co*-EETFU), were prepared from corresponding monomers by photopolymerizations at 25°C for 48 h using DMP as a photoinitiator. The synthesized monomers and polymers were identified by FTIR, ^{1}H-NMR, and ^{13}C-NMR spectroscopies. The number- and weight-average molecular weights and polydispersity indices determined with GPC were as follows: M_n = 14,400, M_w = 19,600, M_w/M_n = 1.4 for poly(MTCA-*co*-ETA); M_n = 14,900, M_w = 20,800, M_w/M_n = 1.4 for poly(MTCA-*co*-HEET); and M_n = 9400, M_w = 10,900, M_w/M_n = 1.2 for poly(MTCA-*co*-EETFU).

The IC_{50} values of the synthesized polymers against cancer cell lines were much greater than those of 5-FU. The *in vitro* cytotoxicities of 5-FU and the prepared samples against a normal cell line increased in the following order: EETFU > 5-FU > MTCA > poly(MTCA-*co*-ETAc) > poly(MTCA-*co*-HEET) = poly(MTCA-*co*-EETFU). The *in vivo* antitumor activities of the prepared samples against mice bearing sarcoma 180 tumor cell line were better than those of 5-FU. The inhibition of SV40 DNA replication for the synthesized samples was much greater compared with that of control. The synthesized monomers and copolymers showed antiangiogenesis in the embryo chorioallantoic membrane (CAM) assay.

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