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Syntheses and Evaluations of Antitumor and Antiangiogenic Phthalate Polymers Containing 5-Fluorouracil and Carboxylates

Sun-Mi Lee[1], Sangwook Jung, Chang-Sik Ha, and Il doo Chung*

Depart of Polymer Science & Engineering, Pusan National University, Busan 609-735, Korea
[1]Division of Life Science, Korea Institute of Science and Technology, Seoul 130-650, Korea

Won-Ki Lee

Division of Chemical Engineering, Pukyong National University, Busan 608-739, Korea

Yong-Ho Park

School of Materials Science and Engineering, Pusan National University, Busan 609-735, Korea

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Abstract: New antitumor active polymers, 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*-hydrogen ethyl-*exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalate) [poly(MTCA-*co*-HEET)], and poly (methacryloyl-2-oxy-1,2,3-propanetricarboxylic acid-*co*-a-ethoxy-*exo*-3,6-epoxy-1,2,3,6-tetrahydrophthaloyl-5-fluorouracil) [poly(MTCA-*co*-EETFU)] were synthesized and characterized. Their antitumor activity, inhibition of DNA replication and antiangiogenesis were examined. The structures of the polymers were identified by FT-IR, ^{1}H and ^{13}C-NMR spectroscopy. The number average molecular weights of the fractionated polymers determined by GPC ranged from 9,400 to 14,900, and polydispersity indices were less than 1.7. The *in vitro* cytotoxicity of these polymers was determined and their antitumor activity was evaluated. The IC_{50} values (the drug concentration at inhibition of 50% tumor growth) indicated that the synthesized polymers were much better inhibitors of cancer cells and showed lower cytotoxicity than the free 5-FU. The *in vivo* antitumor activity of the conjugates was examined using mice bearing the sarcoma 180 tumor cell line. The life spans (T/C) of the mice treated with the conjugates were higher than those treated with the free 5-FU. In addition, the synthesized conjugates showed excellent antiangiogenic activity based on an embryo chorioallantoic membrane assay.

Keywords: antitumor drug, *in vitro* cytotoxicity, DNA replication, antiangiogenesis.

Introduction

It has been reported that the copolymer of divinyl ether and maleic anhydride (DIVEMA) has not only an antitumor, antiviral, antibacterial, interferon-inducing and antifungal activities but also toxic side effects such as enlarged liver and spleen, etc.[1,2] Researchers have used polymer conjugated with low molecular weight drugs to reduce their cytotoxicities with help of slow release.[3-8] We have also reported on syntheses and biological activities of polymeric antitumor compounds such as 5-FU containing acryl derivative polymers,[9-11] tetrahydrophthalic acid derivative polymers (TADP)s,[12-16] poly(diallyl ether-*co*-maleic anhydride),[17] poly(glycinyl maleamic acid) derivatives,[18,19] and methoxyitaconyl-5-fluorouracil[20] for many years. Among them, (TADP)s showed excellent antitumor activities *in vivo* and low cytotoxicities.

The aim of this study was to synthesize new copolymers containing methacryloyl-2-oxy-1,2,3-propanetricarboxylic acid (MTCA) or 5-FU and to evaluate the *in vitro* cytotoxicities and *in vivo* antitumor activities, the inhibitions of DNA replication, and antiangiogenesis activities. Poly(MTCA-*co*-ETA), poly(MTCA-*co*-HEET), and 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. Poly(MTCA-*co*-ETAc) was obtained from was hydrolysis of poly(MTCA-*co*-ETA) with 0.01 N NaOH aqueous solution. The synthesized copolymers were identified by FT-IR, ^{1}H and ^{13}C-NMR spectroscopies. The number and weight average molecular weights and polydispersity indices were determined by GPC. The *in*

*Corresponding Author. E-mail: idchung@pusan.ac.kr

vitro cytotoxicities of copolymers 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 replication for the synthesized monomers and polymers was evaluated using simian virus 40 (SV40) DNA. The antiangiogeneses of poly(MTCA-*co*-ETAc), 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), 2,2-dimethoxy-2-phenylacetophenone (DMP), and 5-fluorouracil (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. Methyl ethyl ketone (MEK), chloroform, dimethyl sulfoxide-d_6 (DMSO-d_6), thionyl chloride, di-methoxyethane (DME), and all other chemicals were reagent grades and used without further purification. The monomers, methacryloyl-2-oxy-1,2,3-propanetricarboxylic acid (MTCA), hydrogen ethyl-*exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalate (HEET) and α-ethoxy-*exo*-3,6-epoxy-1,2,3,6-tetrahydrophthaloyl-5-fluorouracil (EETFU) were prepared by our previous papers.[16,21]

P388, FM3A, and U937 as cancer cell lines and AC2F as a normal cell line were used for *in vitro* evaluation. 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 by published method.[22] Fertilized chick eggs were obtained from Hanshin farm (Kimhae, Korea). Fat emulsion (10%) was purchased from Green Cross Pharm. Co. (Seoul, Korea). Thermanox coverslips were purchased from Nunc Inc. (Naperville, IL, U.S.A).

Measurements. ^{1}H and ^{13}C-NMR spectra were recorded on a FT-300 MHz Varian Gemini 2000 spectrometer. IR spectra were obtained with a Jasco FT/IR-5300 spectrophotometer by using KBr pellet for analysis. Elemental analysis was performed on a Carlo Erba Model EA1180 elemental analyzer. 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). Photopolymerization was carried out under the irradiation of UV light (λ_{max} = 313 nm) in a photochemical chamber.

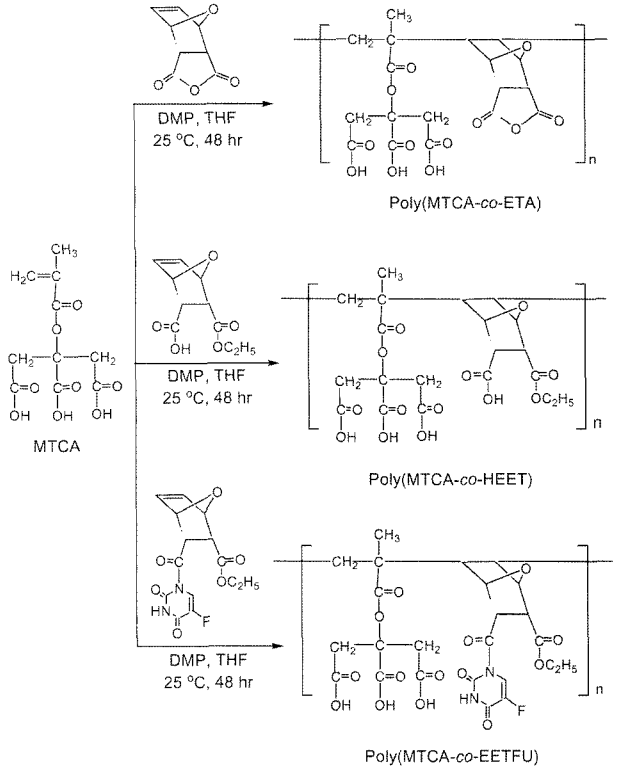

Figure 1. Synthetic routes of the novel copolymers by photopolymerizations.

Syntheses of Copolymers. The copolymers were synthesized by photopolymerization as shown in Figure 1.

Poly(methacryloyl-2-oxy-1,2,3-propanetricarboxylic acid-*co*-*exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic acid) [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 U.V. lamp of 313 nm in a photochemical chamber at 25 °C for 48 h. The obtained polymer solution was slowly dropped in 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 to a constant weight (Conversion, 97%). The obtained poly (MTCA-*co*-ETA) was hydrolyzed with 0.01 N NaOH aqueous solution to obtain poly(MTCA-*co*-ETAc) as shown in Figure 2.

In ^{1}H-NMR spectrum (DMSO-d_6) of poly(MTCA-*co*-ETAc), the peaks of methyl, methylene, and acid protons of MTCA

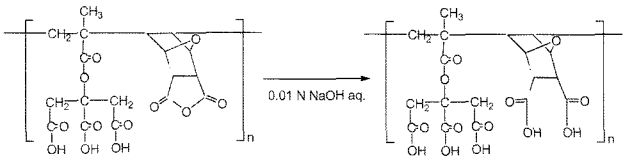

Figure 2. Hydrolysis of poly(MTCA-*co*-ETA).

moiety appeared at 1.95, 1.1, and 12.5 ppm and the methine and acid protons in ETAc moiety showed the peaks at 3.0 and 12.5 ppm, respectively.

Poly(methacryloyl-2-oxy-1,2,3-propanetricarboxylic acid-*co*-hydrogen ethyl-*exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalate) [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 put 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 (Conversion, 65 %).

In ^{1}H-NMR spectrum (DMSO-d_6) of poly(MTCA-*co*-HEET), the methyl and methylene protons of MTCA moiety exhibited the peaks at 1.95 and 1.1 ppm and the peaks of methine and acid protons of HEET moiety showed at 3.0 and 13.0 ppm, respectively.

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)]. The photopolymerization procedure of MTCA and EETFU was the same as that described for the copolymerization of MTCA and ETA except for monomer pairs (Conversion, 72%).

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.

***In Vitro* Cytotoxicity Test.** The *in vitro* cytotoxicities of the 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.[23] The samples which have 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 which 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 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 concentration of samples that reduced the absorbance of the treated cells by 50%.

***In Vivo* 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 of 20 ± 1.5 g in weight 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 activities of 5-FU also were 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.[22] In brief, reaction mixtures (40 μL) included 40 mM creatine phosphate-di-tris salt (pH 7.7), 1 μg of creatine kinase, 7 mM $MgCl_2$, 0.5 μg of bovine serum albumin, 0.5 mM dithiothreitol (DTT), 4 mM adenosine 5'-triphosphate (ATP) (pH 7.5), 334 μM uridine 5'-triphosphate (UTP), guanosine 5'-triphosphate (GTP), cytidine 5'-triphosphate (CTP), 100 μM deoxyadenosine 5'-triphosphate (dATP), deoxyguanosine 5'-triphosphate (dGTP), deoxycytidine 5'-triphosphate (dCTP), 25 μM [^{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 mM ethylenediaminetetraacetic acid (EDTA) (pH 8.0), 2 % sodium dodecyl sulfate (SDS), 1 mg/mL *E.coli* tRNA and 20 mM 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.[24] Embryos with chorioallantois of 3-5 mm in diameter were employed for the assay of antiangiogenesis activity. Five microliters of an aqueous, salt-free solution of each sample was applied to sterile Thermanox 15 mm disks and allowed to dry under laminar flow conditions.[25] 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

Table I. Solubility of the Synthesized Monomers and Polymers

Sample	Solvent					
	Water	Acetone	MEK	DMSO	DMF	Ether
MTCA	PS[b]	IS[c]	PS	PS	S[a]	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]Soluble. [b]Poorly soluble. [c]Insoluble.

Table II. Average Molecular Weights and Polydispersities of Polymers

Sample	M_n[a]	M_w[a]	M_w/M_n
Poly(MTCA-*co*-ETAc)	14,400	21,600	1.5
Poly(MTCA-*co*-HEET)	14,900	22,400	1.5
Poly(MTCA-*co*-EETFU)	9,400	16,000	1.7

[a]Determined by GPC in DMF.

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 3 mm or larger in diameter, the response was scored as a positive according to the method of Crum *et al.*.[26] 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 microphotograped.

Results and Discussion

Solubility of the Prepared Monomers and Polymers. The solubilities of the monomers and copolymers were listed in Table I. Monomers and polymers were soluble in DMF and poorly soluble in water, acetone, MEK, and DMSO, except for good soluble of poly(MTCA-*co*-ETAc) in water. But they were insoluble in diethyl ether except EETFU.

Average Molecular Weights. The average molecular weights of the synthesized polymers were listed in Table II. To obtain polymers with narrow molecular weight distribution, they were fractionated three times using MEK as a poorly soluble solvent and the mixed solvent of DMF and ether (3:7 v/v) as a non-solvent. The number and weight average molecular weights and polydispersity indices determined with GPC were as follows: M_n = 14,400, M_w = 21,600, M_w/M_n = 1.5 for poly(MTCA-*co*-ETAc), M_n = 14,900, M_w = 22,400, M_w/M_n = 1.5 for poly(MTCA-*co*-HEET), and M_n = 9,400, M_w = 16,000, M_w/M_n = 1.7 for poly(MTCA-*co*-EETFU). Ottenbrite *et al.* has reported that good antitumor activity can be obtained in the range of average molecular weights from 10,000 to 30,000 depending on polymers.[27] The average molecular weights of the synthesized polymers were in a reasonable range of medium molecular weight by the literature, which means the synthesized polymers may exhibit the antitumor activity.

***In Vitro* Cytotoxicities.** The *in vitro* cytotoxicities of the synthesized polymers were evaluated against three cancer cell lines such as 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. For cancer cell lines, 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 decreased 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 as compared to those of 5-FU, which was known as a low molecular weight antitumor agent. However, the cytotoxicities of synthesized polymers against normal cell line were much weaker as compared with those of 5-FU. Poly(MTCA-*co*-EETFU) showed higher antitumor activity and lower cytotoxicity as compared with the other copolymers.

Table III. *In Vitro* Cytotoxicity of the Samples

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

***In Vivo* Antitumor Activities.** The *in vivo* antitumor activities of monomers and polymers against mice bearing sarcoma 180 tumor cell line were listed in Table IV together

Table IV. *In Vivo* Antitumor Activity of the Samples

Samples	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 130 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 130 days.

with those of 5-FU 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 in order to evaluate the toxic side effect of the samples within short period. The *in vivo* antitumor activity of the samples at 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.[9] This result was ascribed to the more carboxylic acid groups in a repeat unit of the synthesized polymers as compared with those of DIVEMA which has two carboxylic groups. The life spans (T/C) of mice treated with the samples were longer as compared with those of 5-FU and the control group at dosage of 0.8 mg/kg which has not exhibited higher toxic side effect than 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 g/mL) and EETFU (IC_{50} = 0.01 g/mL) against a normal cell line. The order for the copolymers may be attributed to the degree of ease of counter-charge interactions between –COOH and $–COO^-$ per repeat unit in the polymer, and –OH, –NH–CO–, etc, 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 better endocytosis of the polymers proceeds. 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 as 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 was known as an inhibitor for the 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*

Table V. Antiangiogenic Activities and *in vitro* Inhibition of SV40 DNA Replication

Sample	Antiembryogenesis[a]	Antiangiogenesis[b]	Inhibition of SV40 DNA Replication (%)
	Inhibition (%)	Inhibition (%)	
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.

vitro of the samples was 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 were greater than those of control. The inhibition of SV40 DNA replication of MTCA was more efficient as compared with that of the synthesized polymers. However the inhibition values for the DNA replication of the synthesized copolymers were not much different.

Antiangiogenic Activities of Monomers and Polymers. In 1996, Dr. J. Folkman reported that the inhibition of angiogenesis may lead to inhibition of tumor growth and metastasis.[28] Because the antiangiogenic compounds prevent the formation of new blood vessels which 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 > control. Poly(MTCA-*co*-EETFU) showed much higher antiangiogenesis as compared to the other samples.

The microphotographs of monomers and polymers by the CAM assay are shown in Figure 3. The blood vessels of 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; this result agreed well with the *in vivo* antitumor activity (T/C) of poly(MTCA-*co*-EETFU) at 0.8 mg/kg.

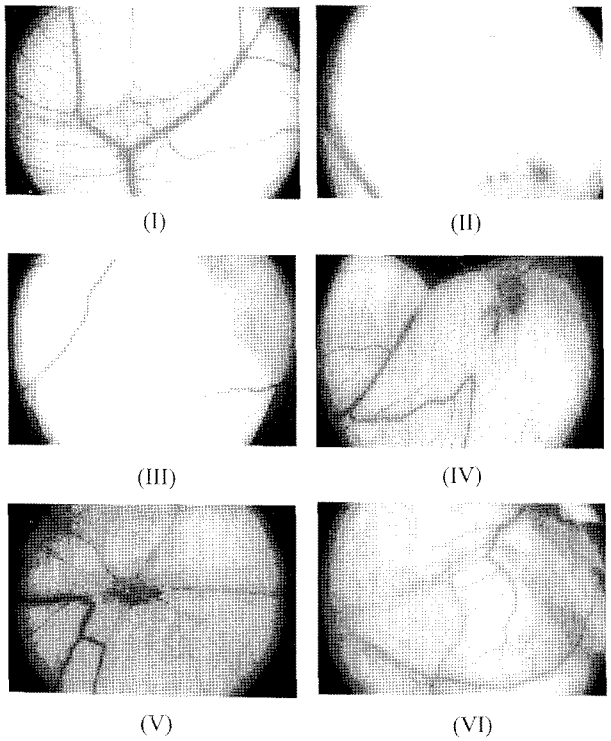

Figure 3. Optical microphotographs of control (I), MTCA (II), EETFU (III), poly(MTCA-*co*-ETAc) (IV), poly(MTCA-*co*-HEET) (V), and poly(MTCA-*co*-EETFU) (VI) on embryonic angiogenesis in CAM (×100).

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.

Conclusions

The new 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 *in vitro* and *in vivo* studies show that the synthesized polymers have much improved antitumor activities than free 5-FU. The inhibition of SV40 DNA replication for the synthesized samples was much greater as compared with control. The synthesized monomers and copolymers showed antiangiogenesis in the embryo chorioallantoic membrane (CAM) assay. The research we have demonstrated describes novel structure that may have potential applications in drug delivery system for antitumor drugs. Future research will focus on the kinetic studies on the release of 5-FU as well as the additional antiangiogenesis effects such as inhibition effect of HUVEC cell proliferation without cytotoxicity, matrigel assay, and so on.

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