



HAL
open science

Drug release and biocompatibility of self-assembled micelles prepared from poly (-caprolactone/glycolide)-poly (ethylene glycol) block copolymers

S.M. Li, Xiangke Su, Rongye Li, Yuandou Wang, Feng Su, Laishun Xi, Yangsheng Chen

► To cite this version:

S.M. Li, Xiangke Su, Rongye Li, Yuandou Wang, Feng Su, et al.. Drug release and biocompatibility of self-assembled micelles prepared from poly (-caprolactone/glycolide)-poly (ethylene glycol) block copolymers. *Polymers for Advanced Technologies*, 2019, 30 (1), pp.40-50. 10.1002/pat.4440. hal-01964885

HAL Id: hal-01964885

<https://hal.umontpellier.fr/hal-01964885v1>

Submitted on 9 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Journal of Applied Polymer Science

Drug release and biocompatibility of self-assembled micelles prepared from poly(ϵ -caprolactone/glycolide)-poly(ethylene glycol) block copolymers --Manuscript Draft--

Full Title:	Drug release and biocompatibility of self-assembled micelles prepared from poly(ϵ -caprolactone/glycolide)-poly(ethylene glycol) block copolymers
Manuscript Number:	
Article Type:	Research Article
Order of Authors:	Suming Li, Dr. Xiangke Sun Rongye Li Yuandou Wang Feng Su Laishun Xi Yangsheng Chen
Manuscript Classifications:	Biodegradable; Biomaterials; Degradation; Drug Delivery Systems; Micelles
Additional Information:	
Question	Response
Please provide the principal investigator's name and affiliation. (Principal investigator MUST be listed as a co-author on the submission; please DO NOT list all other co-authors in this section.)	Feng Su College of Chemical Engineering Qingdao University of Science and Technology China
Please submit a plain text version of your cover letter here. If you also wish to upload a file containing your cover letter, please note it here and upload the file when prompted to upload manuscript files. Please note, if you are submitting a revision of your manuscript, there is an opportunity for you to provide your responses to the reviewers later; please do not add them to the cover letter.	May 22, 2018 Dear Editor: It's my pleasure to submit our revised manuscript entitled "Drug release and biocompatibility of self-assembled micelles prepared from poly(ϵ -caprolactone/glycolide)-poly(ethylene glycol) block copolymers" for publication in publication in Journal of Applied Polymer Science. In this paper, we report on the self-assembly, drug release and biocompatibility of novel P(CL/GA)-PEG copolymers. Spherical micelles were obtained from the various copolymers. The micelle size was larger for copolymers with longer PEG blocks, and the CMC of copolymers increased with decreasing the overall hydrophobic block length. The drug loading and drug release properties were closely related to the copolymer composition. Higher drug loading was obtained for micelles with longer PCL blocks. Drug release was investigated under in vitro conditions, using paclitaxel as a hydrophobic model drug. Faster drug release was obtained for micelles of mPEG2000 initiated copolymers than those of mPEG5000 initiated ones. Higher GA content in the copolymers led to faster drug release. Moreover, drug release rate was enhanced in the presence of lipase from <i>Pseudomonas</i> sp., indicating that drug release is facilitated by copolymer degradation. Furthermore, copolymer micelles present outstanding hemocompatibility and cytocompatibility as evidenced by hemolysis, dynamic clotting time and plasma recalcification time tests, as well as MTT assay and agar diffusion test, thus suggesting that P(CL/GA)-PEG micelles are promising for prolonged release of hydrophobic drugs. Thank you for consideration. Sincerely yours, Suming LI, Ph.D.

1 **Drug release and biocompatibility of self-assembled micelles prepared from**
2 **poly(ϵ -caprolactone/glycolide)-poly(ethylene glycol) block copolymers**
3

4
5
6 Feng Su^{1,2*}, Xiangke Sun¹, Rongye Li¹, Yuandou Wang², Laishun Xi¹, Yangsheng
7
8 Chen^{3*}, Suming Li^{4*}
9

10
11
12 ¹ College of Chemical Engineering, Qingdao University of Science and Technology,
13
14 Qingdao 266042, China
15

16
17 ² Institute of High Performance Polymers, Qingdao University of Science and
18
19 Technology, Qingdao 266042, China
20

21 ³ Qingdao Chiatai Haier Pharmaceutical Co., LTD, Qingdao 266103, China
22

23 ⁴ European Institute of Membranes, UMR 5635, University of Montpellier, CNRS,
24
25 ENSCM, 34095 Montpellier Cedex 5, France
26

27
28
29
30
31
32
33
34
35
36
37
38
39 Corresponding authors: sufengvip@126.com (Feng Su); chenysh@haier.com (Y.
40
41 Chen); suming.li@umontpellier.fr (S. Li)
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2 **ABSTRACT:**

3
4 Poly(ϵ -caprolactone/glycolide)-poly(ethylene glycol) (P(CL/GA)-PEG) diblock
5
6 copolymers were prepared by ring opening polymerization of a mixture of
7
8 ϵ -caprolactone and glycolide using monomethoxy PEG as macro-initiator and
9
10 Sn(Oct)₂ as catalyst. The resulting copolymers were characterized by using ¹H NMR
11
12 and GPC. Self-assembled micelles were prepared from the copolymers using
13
14 nanoprecipitation method. The morphology of micelles was spherical as revealed by
15
16 TEM. The micelle size was larger for copolymers with longer PEG blocks, and the
17
18 CMC of copolymers increased with decreasing the overall hydrophobic block length.
19
20 Drug loading and in vitro drug release studies were performed using paclitaxel as a
21
22 hydrophobic model drug. Higher drug loading was obtained for micelles with longer
23
24 PCL blocks. Faster drug release was obtained for micelles of mPEG2000 initiated
25
26 copolymers than those of mPEG5000 initiated ones. Higher GA content in the
27
28 copolymers led to faster drug release. Moreover, drug release rate was enhanced in the
29
30 presence of a lipase, indicating that drug release is facilitated by copolymer
31
32 degradation. Furthermore, copolymer micelles present outstanding biocompatibility as
33
34 evidenced by hemolysis, dynamic clotting time and plasma recalcification time tests,
35
36 as well as MTT assay and agar diffusion test, thus suggesting that P(CL/GA)-PEG
37
38 micelles are promising for prolonged release of hydrophobic drugs.
39
40
41
42

43 **KEYWORDS:** micelle; poly(ϵ -caprolactone); poly(ethylene glycol); paclitaxel; drug
44
45 release; biocompatibility
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

INTRODUCTION

Paclitaxel, as an anti-microtubule drug, has demonstrated efficient anticancer activity against various tumors¹⁻³. Paclitaxel behaves as promoter of tubulin's assembly and inhibitor of its disassembly, and thus restrain cell mitosis^{4,5}. Unfortunately, clinically used formulation of paclitaxel containing Cremophor EL can accelerate the release of histamine and cause serious adverse events including neutropenia, allergic reaction, neurotoxicity, cardiovascular toxicity, gastrointestinal reaction, liver and gallbladder reaction, etc⁵. Furthermore, conventional cancer chemotherapy using paclitaxel presents no selectivity towards cancer cells and normal cells, thus producing obvious side effects to normal tissues⁶. Therefore, finding a safer and more effective paclitaxel delivery system without Cremophor EL becomes a major issue in the treatment of cancers.

In the past decades, aliphatic polyesters such as polylactide (PLA), poly(ϵ -caprolactone) (PCL), poly(glycolide) (PGA) and their copolymers have become the focus of biodegradable materials due to their excellent biodegradability and biocompatibility⁷. In the biomedical field, they are widely used as surgical sutures, controlled drug release devices and tissue engineering scaffolds. These polyesters can degrade by hydrolysis into small molecules which are then eliminated from the body through natural pathways. The physico-chemical properties of polyesters can be tailored by introducing a biocompatible and hydrophilic component such as poly(ethylene glycol) (PEG)^{8,9}. The resulting amphiphilic block copolymers including PLA-PEG, PCL-PEG, poly(lactide-co-glycolide)-PEG (PLGA-PEG) and poly(ϵ -caprolactone/glycolide)-PEG (P(CL/GA)-PEG) have received more and more attention in drug delivery systems¹⁰⁻¹³. In fact, they can self-assemble to form micelles with a core-shell structure in aqueous solution: a hydrophobic core able to encapsulate hydrophobic drugs, and an outer hydrophilic shell ensuring biocompatibility and long circulation in blood. Moreover, the size of micelles (< 200 nm) is small enough to avoid being swallowed by the immune system or filtering out by the liver and spleen¹⁴⁻¹⁶.

1 Biocompatibility is a key factor for clinical applications of biomaterials. It refers to
2 the complex biological, chemical and physical interactions between material and
3 human body, and the extent of endurance of receptors to these reactions. In the case of
4 drug carriers, they should not cause any undesirable local or systemic effects in the
5 recipient or host of therapy ¹⁷. Although the biocompatibility of PCL, PGA and PEG
6 polymers has been well documented, very few data are available on the
7 biocompatibility of copolymer micelles in aqueous environment *in vitro*.
8
9

10
11 In our previous work, we comparatively studied the self-assembly, drug loading
12 and drug release properties of PCL-PEG and P(CL/GA)-PEG micelles as novel carrier
13 of paclitaxel ¹⁸. The morphology of micelles is mainly dependent on the
14 hydrophilic/hydrophobic balance of copolymers and the regularity of hydrophobic
15 chain structure. Worm-like or filomicelles were obtained for PCL-PEG copolymers in
16 all cases. In contrast, spherical micelles were obtained with addition of a small
17 amount of GA component which disrupts the chain structure. Drug release proceeded
18 very slowly in all cases. P(CL/GA)-PEG micelles presented slightly faster release
19 than PCL-PEG micelles, but the overall release rate didn't exceed 10% in 30 days.
20 Therefore, it is of major importance to enhance the drug release from P(CL/GA)-PEG
21 micelles by increasing the proportion of GA component.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36

37 In the present work, a series of P(CL/GA)-PEG diblock copolymers with various
38 EO/(CL/GA) molar ratios were synthesized by ring opening polymerization of a
39 mixture of ϵ -caprolactone and glycolide in the presence of monomethoxy
40 poly(ethylene glycol) (mPEG) as macroinitiator. P(CL/GA)-PEG micelles were
41 prepared by co-solvent evaporation method. Drug loading was performed using
42 paclitaxel as model drug, and drug release was studied under *in vitro* conditions in a
43 pH 7.4 phosphate buffered saline (PBS). The biocompatibility of P(CL/GA)-PEG
44 micelles was evaluated from the aspects of hemocompatibility and cytocompatibility
45 in order to assess the micelles' potential as drug delivery systems.
46
47
48
49
50
51
52
53
54
55
56
57

58 **EXPERIMENTAL**

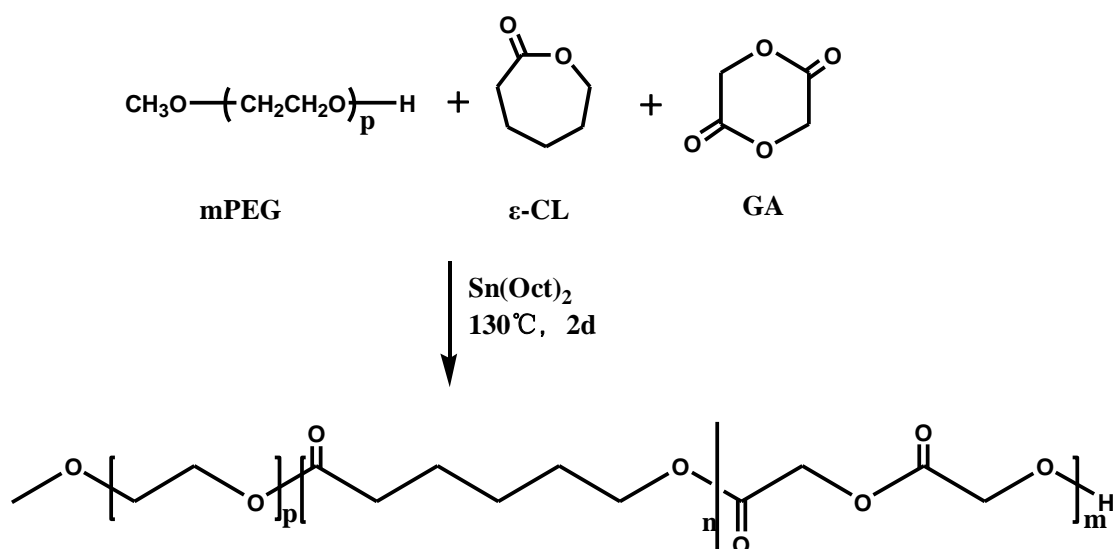
59 **Materials**

60
61
62
63
64
65

1 ϵ -Caprolactone was obtained from J&K (Beijing, China), and purified by distillation.
2 Glycolide was purchased from J&K and purified by crystallization from ethyl acetate.
3 Monomethoxy PEG (mPEG) with molar masses of 2000 or 5000 was supplied by
4 Sigma-Aldrich (Shanghai, China). Stannous octoate ($\text{Sn}(\text{Oct})_2$) and paclitaxel were
5 supplied by Aladdin (Shanghai, China). All organic solvents were of analytic grade.
6
7
8
9

10 Synthesis of P(CL/GA)-PEG Copolymers

11 P(CL/GA)-PEG copolymers were synthesized by ring-opening polymerization of
12 ϵ -caprolactone and glycolide initiated by mPEG in the presence of $\text{Sn}(\text{Oct})_2$ as
13 catalyst as previously reported (Scheme 1) ¹⁹. Briefly, predetermined amounts of
14 mPEG, ϵ -caprolactone, glycolide and $\text{Sn}(\text{Oct})_2$ were added into a polymerization tube.
15 The mixture was degased, and then sealed under vacuum. Polymerization then
16 proceeded at 130°C for 2 d. The resulting product was recovered by dissolution in
17 dichloromethane and precipitation with diethyl ether, followed by vacuum drying up
18 to constant weight.
19
20
21
22
23
24
25
26
27
28
29



Scheme 1. Synthesis route of P(CL/GA)-PEG copolymers

66 Preparation of P(CL/GA)-PEG micelles

67 Micelles were prepared using a solvent evaporation process as previously described ²⁰.
68 Briefly, 20 mg copolymer was dissolved in 400 μL chloroform, and then 20 mL
69
70
71
72
73
74
75

1 distilled water was added. The solution was stirred vigorously at room temperature for
2 3.5 h, and allowed to stay 24 h for solvent evaporation. The resulting micellar solution
3 was filtered through 0.45 μm pore-sized syringe filter. The concentration of the final
4 micellar solution was 1.0 mg/mL.
5
6
7

8 9 **Characterization**

10 Proton nuclear magnetic resonance (^1H NMR) was employed to determine the
11 composition of copolymers. The spectra were recorded on Bruker AVANCE III 500
12 spectrometer operating at 500 MHz, using CDCl_3 as a solvent. Chemical shifts (δ)
13 were given in ppm using tetramethylsilane as an internal reference.
14
15

16 The molecular weight and molecular weight distribution of copolymers were
17 determined by gel permeation chromatography (GPC) carried out on a Shimadzu
18 apparatus equipped with a Waters 410 refractometer. Tetrahydrofuran was used as
19 mobile phase at a flow rate of 1.0 mL/min. 60 μL copolymer solution at a
20 concentration of 1.0 mg/mL was injected for analysis. Polystyrene standards were
21 used for calibration.
22
23

24 Dynamic light scattering (DLS) was used to measure the size and size distribution of
25 micelles, using a Nano-ZS90 nanosizer (Malvern, UK) equipped with a digital time
26 correlator. The concentration of micellar solutions was 1.0 mg/mL, and measurements
27 were made at 25 $^\circ\text{C}$ with a scattering angle of 90 $^\circ$.
28
29

30 The critical micelle concentrations (CMC) of P(CL/GA)-PEG micelles were
31 determined by spectrofluorimeter (Hitachi F-7000), using pyrene as fluorescence
32 probe ²¹. Briefly, 1 mL pyrene solution (2×10^{-5} M in benzene) was added to 10 mL
33 volumetric flask, and the solvent was evaporated. Different volumes of micelle
34 solutions were added to the flask. Then distilled water was added to a total volume of
35 10 mL. The resulting micellar concentrations ranged from 9.7×10^{-4} to 0.5 mg/mL,
36 and the final concentration of pyrene was 2.0×10^{-6} M. After equilibrium at room
37 temperature for 24 h, the fluorescence excitation spectra of the micellar solutions
38 were recorded from 350 to 450 nm at an excitation wavelength of 334 nm. The
39 excitation and emission slit widths were 5 nm and 2.5 nm, respectively, and the
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 scanning speed was 500 nm/min. The excitation ratios of I₃₇₅/I₃₉₅ were used for the
2 determination of the CMC value.
3

4 The morphology of micelles was examined by using transmission electron
5 microscopy (TEM). Measurements were realized on JEM-1200EX microscope,
6 operating at a high voltage of 80 kV. The carbon coated copper grid was immersed in
7 a micellar solution of 1.0 mg/mL for 1 min, and then taken out. One drop of 3%
8 phosphotungstic acid (PTA) solution was placed on the copper grid for negative
9 staining, followed by air drying at room temperature before measurements.
10

11 **In vitro drug release**

12 Paclitaxel loading in micelles was performed by using solvent evaporation method ²².
13 Briefly, 2 mg paclitaxel was dissolved in 1 mL methanol, and then dropped into 10
14 mL pre-formed micelle solution under constant stirring at room temperature. The
15 solution was stirred overnight to allow paclitaxel encapsulation. Unloaded paclitaxel
16 was removed by centrifugation at 5,000 rpm for 10 min.
17

18 High performance liquid chromatography (HPLC) was used to determine the amount
19 of paclitaxel in drug loading and drug release experiments. A WATERS-e2695
20 apparatus equipped with a C18 column (4.6 × 250 mm, pore size 5 μm,
21 WATERS-e2695, America) was used, and the detection wavelength of UV detector
22 was set at 227 nm. A mixture of HPLC grade acetonitrile-water (v/v, 55/45) was used
23 as mobile phase at a flow rate of 1.2 mL/min. The drug loading content (LC) was
24 defined as the ratio of the amount of loaded drug to the total amount of drug loaded
25 micelles, and the loading efficiency (LE) as the ratio of the amount of loaded drug to
26 the amount of initially introduced drug ³.
27

28 Drug release experiments were realized under *in vitro* conditions. 10 mL drug loaded
29 micelle solution was introduced into a dialysis membrane (MWCO=3500), and
30 immersed in 10 mL PBS at pH 7.4. Drug release was then conducted at 37°C with
31 constant shaking. At predetermined time intervals, the release medium was totally
32 withdrawn for analysis, and the same amount of fresh PBS was added.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Hemocompatibility evaluation

Hemolysis tests were realized in accordance with ISO 10993-4:2009 and ASTM F756-00^{23, 24}, using fresh acid citrate dextrose (ACD) anticoagulated rabbit whole blood (blood/3.8wt% citrate acid = 9:1, V/V). 10 mL micellar solution at 1 mg/mL was used as test group, 10 mL distilled water and 0.9% saline water as positive and negative controls. All tubes were placed in a water bath thermostated at 37°C for 0.5 h, and 0.2 mL diluted blood (blood/saline=4:5, V/V) was then added. After 1 h, all samples were centrifuged at 3000 rpm for 5 min at room temperature, and the OD value of the supernatants was measured at 545 nm by using UV-visible spectrophotometer (T6-1650F, China). All experiments were repeated three times (n = 3). The hemolysis ratio (HR) was calculated according to following formula²⁵:

$$HR(\%) = \left[\frac{(OD_{\text{test}} - OD_{\text{negative}})}{(OD_{\text{positive}} - OD_{\text{negative}})} \right] \times 100$$

Where OD_{test} , OD_{negative} , and OD_{positive} are the absorbance values of the micellar solution, negative and positive controls, respectively.

The dynamic clotting time was determined according to the literature²⁶. 1 mg/mL micellar solution was prepared in 0.2 M CaCl_2 solution, and 10 μL the above solutions were used as test group. Siliconized and non-siliconized glass tubes containing 10 μL 0.2 M CaCl_2 solution were used as negative and positive controls, respectively. All tubes were thermostated in a 37°C water bath for 5 min. Then 80 μL ACD blood was added into tubes. After 0, 10, 20, 40, 60, 80, 100, 120 min, 20 mL deionized water was gently added. The OD values of the supernatants were measured at 490 nm using a microplate reader. The relative clotting time for each micellar solution was obtained from the OD *versus* time plots. All experiments were repeated five times (n = 5).

Plasma recalcification time (PRT) was measured by using a literature method²⁵. Platelet poor plasma (PPP) was acquired by centrifugation of fresh ACD anticoagulated whole blood at 3000 rpm for 10 min. Micellar solutions at 1 mg/mL were prepared in 0.025 M CaCl_2 solution, and 100 μL of the above solutions were used as test group. 100 μL of 0.025 M CaCl_2 solution were added into siliconized and

1 non-siliconized glass tubes, and used as negative and positive controls, respectively.
2 All tubes were thermostated in a 37 °C water bath for 2 min, and then 100 µL PPP was
3 added. The tube was tilted every 2 s to observe the clotting condition of PPP after 50 s.
4 The time of clotting was recorded at the moment when plasma solution could no
5 longer flow. All experiments were repeated three times (n = 3).
6
7
8
9

10 **Cytocompatibility evaluation**

11 Micellar solutions at 1 mg/mL were prepared in growth medium (DMEM containing
12 10% fetal bovine serum), and diluted to various concentrations of 0.05, 0.1, 0.2, 0.5
13 mg/mL. MTT assay was employed to evaluate the cytotoxicity of micellar solutions in
14 vitro^{27, 28}, using L-929 cells in logarithmic growth phase. Cell suspensions were
15 prepared with growth medium to a concentration of 1×10^4 cells / mL, and 100 µL
16 cell suspension was seeded in 96-well plates (Corning Costar, USA) placed in 5%
17 CO₂ incubator (NU-4850; NuAire, USA) at 37 °C under humidified environment.
18 After 24 h incubation, the medium was removed and replaced by 100 µL micellar
19 solution. 100 µL fresh medium was used as the negative control, and 100 µL 6.4%
20 phenol aqueous solution as the positive control. After incubation for 1, 2 and 3 days,
21 20 µL MTT solution at 5 mg / mL was added. The medium in the well was removed
22 after 4 h incubation, and then 150 µL DMSO was added. After 10 min shaking, the
23 optical density (OD) was measured at 490 nm by using microplate reader (Elx800,
24 BioTek, USA). All samples were measured three times (n=3). The relative growth
25 rate (RGR) was determined by using the following equation:
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44

$$45 \quad RGR(\%) = (OD_{test\ group} / OD_{negative\ control}) \times 100$$

46
47 Agar diffusion test was realized according to the literature method²⁹, using L-929
48 cells in logarithmic growth phase. The cells were diluted with growth medium
49 (DMEM containing 10% fetal bovine serum) to a concentration of 2.5×10^4 cells /
50 mL. 10 ml cell suspension was placed in a Petri dish and cultured in 5% CO₂
51 incubator at 37 °C. After 24 h, the culture medium was discarded, and the dish washed
52 with PBS. 12 mL of 1.5% fresh agar medium were then added. When the agar
53 medium was completely coagulated, 10 ml neutral red solution was added. The
54
55
56
57
58
59
60
61
62
63
64
65

1 neutral red solution was removed after 15-20 min. 100 μ L micellar solution was
2
3 dropped into a piece of filter paper (diameter 5 mm) which was carefully placed on a
4
5 dish. After exposure at 37 $^{\circ}$ C in a 5% CO₂ humidified atmosphere for 24 h, the toxicity
6
7 was assessed by inverted microscopy from the width of the decolorization zone
8
9 around the test solution and sloughing or lysis within the zone. 100 μ L fresh medium
10
11 and 6.4% phenol aqueous solution were used as the negative and positive controls,
12
13 respectively.

14 15 16 **Statistics analysis**

17
18 Statistics analysis was performed using SPSS 10.0 software. Descriptive data were
19
20 expressed as the arithmetic mean value plus or minus the standard deviation. All
21
22 quantitative results were obtained from at least triplicate samples. A value of $p <$
23
24 0.001 was considered to be extremely significant, $p < 0.01$ very significant, $p < 0.05$
25
26 statistically significant, and $p > 0.05$ not significant.

27 28 29 30 **Results and discussion**

31 32 **Characterization of Copolymers**

33
34 A series of 6 P(CL/GA)-PEG diblock copolymers were obtained by ring-opening
35
36 polymerization of a mixture of CL and GA monomers, using monomethoxy PEG with
37
38 Mn of 2000 or 5000 as macroinitiator and Sn(Oct)₂ as catalyst. The initial CL/EO
39
40 feed ratio was 0.5, and the CL/GA ratio was 1, 2 or 5. The various copolymers were
41
42 named as P(CL/GA)-PEG2K X or P(CL/GA)-PEG5K X, where 2K and 5K represent
43
44 the molecular weight of the PEG block and X the CL/GA ratio.

45
46 The composition of the copolymers was determined by ¹H NMR ³⁰. Fig. 1 shows the
47
48 ¹H NMR spectrum of P(CL/GA)-PEG2K 5. The signal at 3.63 ppm (b) is attributed to
49
50 the methylene groups of PEG block, whereas the signals at 4.07 (c), 2.31 (f), 1.65 (d)
51
52 and 1.38 ppm (e) are assigned to the methylene groups of PCL moieties. The signal in
53
54 the 4.6-4.8 ppm zone (g) belongs to the methylene group of PGA block. Besides, two
55
56 smaller downfield signals are detected beside those at 4.07 (c) and 2.31 (f). These new
57
58 signals are attributed to the caprolactone units which are linked to glycolidyl units in
59
60
61
62
63
64
65

the P(CL/GA) block. These findings demonstrate that P(CL/GA)-PEG diblock copolymers were successfully obtained³¹.

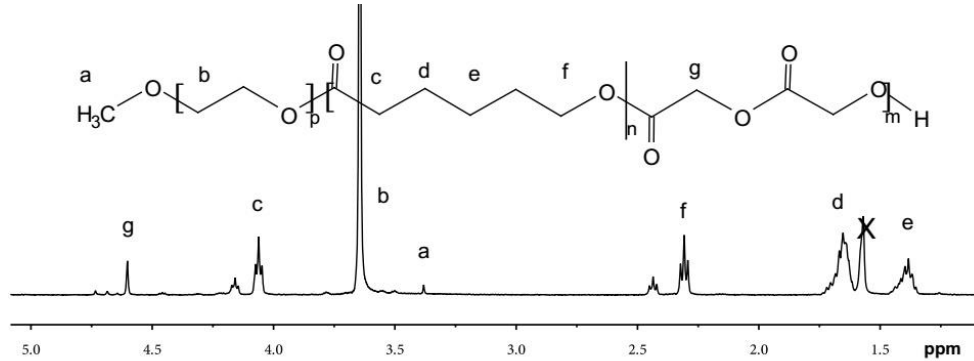


Figure 1. ¹H NMR spectrum of P(CL/GA)-PEG diblock copolymers

The CL/EO and CL/GA molar ratios of the copolymers were calculated from the integrations of signals at 3.63 ppm for PEG, at 2.31 ppm for PCL and at 4.6-4.8 ppm for PGA. The degree of polymerization (DP) of three components, as well as the number average molecular weight (M_n) of the copolymers were determined using the following equations:

$$DP_{\text{PEG}} = M_{n\text{PEG}}/44 \quad (1)$$

$$DP_{\text{PCL}} = DP_{\text{PEG}} \times (\text{CL/EO}) \quad (2)$$

$$DP_{\text{PGA}} = DP_{\text{PCL}}/(\text{CL/GA}) \quad (3)$$

$$M_n = M_{n\text{PEG}} + DP_{\text{PCL}} \times 114 + DP_{\text{PGA}} \times 116 \quad (4)$$

Table 1 summarizes the molecular characteristics of the various P(CL/GA)-PEG copolymers. The DP_{PEG} is 45 or 114 for mPEG2000 and mPEG5000, respectively. The DP_{PCL} ranges from 24 to 26 for mPEG2000 initiated copolymers, and from 52 to 62 for mPEG5000 initiated copolymers. Moreover, the DP_{PGA} ranges from 5 to 20 for mPEG2000 initiated copolymers, and from 12 to 65 for mPEG5000 initiated copolymers. In all cases, the compositions of the resulting copolymers are close to those of the feeds, which is consistent with a good conversion of monomers. In fact, all copolymers were obtained with high yields above 80%. Concerning the M_n , it ranges from 5540 to 7060 for mPEG2000 initiated copolymers, and from 13460 to

18450 for mPEG5000 initiated copolymers.

The molecular weights and dispersity ($D=M_w/M_n$) of P(CL/GA)-PEG copolymers were also determined by GPC. As shown in **Table 1**, the $M_{n(GPC)}$ ranges from 6250 to 8180 for mPEG2000 initiated copolymers, and from 15310 to 19620 for mPEG5000 initiated copolymers. In fact, the $M_{n(GPC)}$ values were obtained with respect to polystyrene standards, and are generally higher than the absolute molecular weights determined by NMR. The dispersity ($D= M_w/M_n$) values of all copolymers range from 1.3 to 1.5, indicating a narrower distribution of molecular weights.

Table 1. Structural characteristics of P(CL/GA)-PEG block copolymers

Copolymer	M_{nPEG}	CL/EO ^a	CL/GA ^a	DP _{PEG}	DP _{PCL}	DP _{PGA}	$M_{n(NMR)}$	$M_{n(GPC)}$	D
P(CL/GA)-PEG2K 1	2000	0.53 (0.5)	1.2 (1)	45	24	20	7060	8180	1.41
P(CL/GA)-PEG2K 2	2000	0.56 (0.5)	1.8 (2)	45	25	14	6480	7200	1.45
P(CL/GA)-PEG2K 5	2000	0.58 (0.5)	5.7 (5)	45	26	5	5540	6250	1.39
P(CL/GA)-PEG5K 1	5000	0.46 (0.5)	0.8 (1)	114	52	65	18450	19620	1.36
P(CL/GA)-PEG5K 2	5000	0.51 (0.5)	1.5 (2)	114	58	39	16140	17260	1.51
P(CL/GA)-PEG5K 5	5000	0.54 (0.5)	5.2 (5)	114	62	12	13460	15310	1.40

^a Data in parentheses correspond to the feed ratio.

Self-assembly of P(CL/GA)-PEG copolymers

Micelles of P(CL/GA)-PEG copolymers were prepared using a solvent evaporation process. The morphology of micelles was examined by means of TEM. As shown in Fig. 2, micelles of P(CL/GA)-PEG2K 5 exhibit a spherical shape with a diameter of about 25 nm. Similar spherical shapes are observed for other copolymers. The average particle size and polydispersity index (PDI) were determined by means of DLS. As shown in Table 2, the micelle diameter is 136.8, 121.3 and 104.7 nm for P(CL/GA)-PEG2K 1, P(CL/GA)-PEG2K 2 and P(CL/GA)-PEG2K 5, respectively. This finding shows that the micelle size decreases with decreasing hydrophobic block length ($DP_{PCL}+DP_{PGA}$). Meanwhile, a diameter of 158.6, 147.2, and 132.8 nm is obtained for P(CL/GA)-PEG5K 1, P(CL/GA)-PEG5K 2 and P(CL/GA)-PEG5K 5, respectively. These findings suggest that the micelle size increases with increasing length of hydrophilic PEG segments in the copolymer, which is consistent with literature data³². The PDI of micelle size is in the range from 0.251 to 0.272, in

agreement with narrow size distribution. It is also of interest to note that the diameter obtained from TEM is much lower than that from DLS, which can be attributed to the dehydration and shrinkage of micelles during TEM measurements³³.

Table 2. Characterization of P(CL/GA)-PEG diblock copolymer micelles

copolymer	Size (nm) ^a	PDI ^a	CMC(mg/mL)	LE(%) ^a	LC(%) ^a
P(CL/GA)-PEG2K 1	136.8±12.7	0.253±0.02	0.0024	52.8±4.3	8.8±0.6
P(CL/GA)-PEG2K 2	121.3±10.2	0.256±0.03	0.0042	58.8±5.7	9.8±0.8
P(CL/GA)-PEG2K 5	104.7±11.4	0.251±0.05	0.0048	68.4±6.4	11.4±0.9
P(CL/GA)-PEG5K 1	158.6±14.6	0.272±0.04	0.0036	55.2±8.6	9.2±1.2
P(CL/GA)-PEG5K 2	147.2±12.4	0.264±0.07	0.0040	63.6±7.6	10.6±0.7
P(CL/GA)-PEG5K 5	132.8±10.8	0.258±0.08	0.0068	76.8±10.2	12.8±1.4

^a Data represent mean value ±S.D., n = 3.

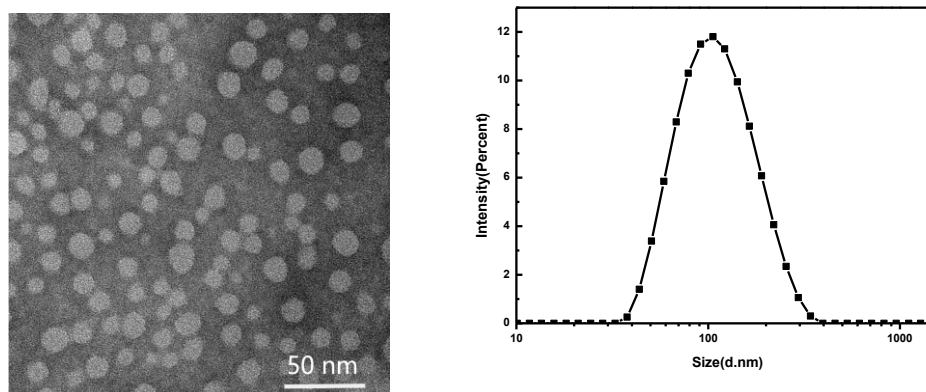
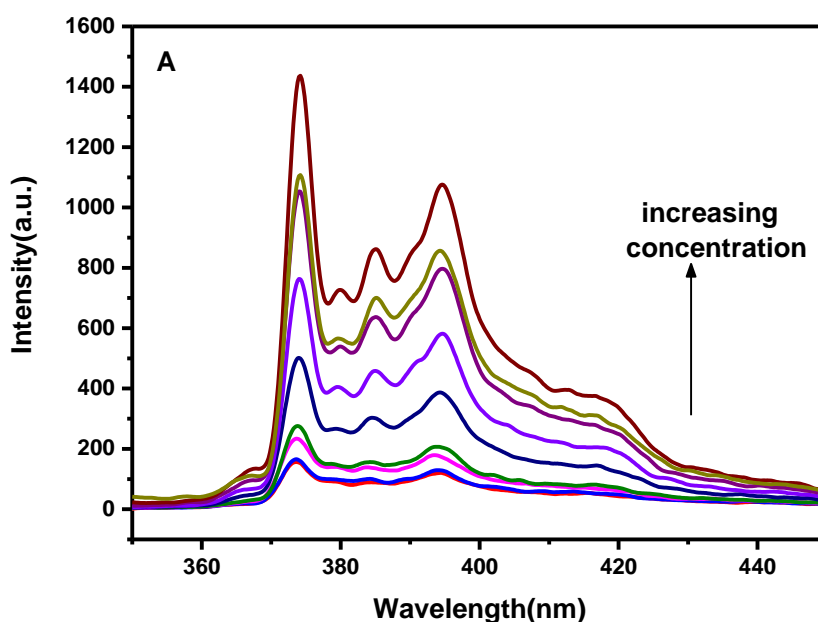


Figure 2. TEM image (left) and DLS graph (right) of P(CL/GA)-PEG2K 5 micelles.

As a key parameter that characterizes the stability of micelles, the CMC of diblock copolymers was evaluated by using fluorescent probe method. Fig. 3(A) shows the emission spectra of pyrene at different micellar concentrations. An increase in the fluorescence intensity and a red shift are observed with increasing concentration. Fig. 3(B) shows the intensity ratio (I_{375}/I_{395}) vs. log C plots of P(CL/GA)-PEG2K 1. The CMC value was obtained from the cross-over point of the regression lines. As shown in Table 2, all copolymers present very low CMC values (2.4-6.8 $\mu\text{g}/\text{mL}$), which should be beneficial for micellar stability after intravenous administration and dilution

1 in bloodstream ³⁴. These values are comparable to those of other copolymers that
2 generally present very low CMC values ranging from 1 mg/mL to 10 mg/mL ³⁵, and
3 are much lower than typical CMC values of low molecular weight surfactants ³⁶. It is
4 also noteworthy that copolymers with longer hydrophobic blocks exhibit lower CMC
5 values for both mPEG2000 and mPEG5000 initiated copolymers, in agreement with
6 higher micelle stability as reported in literature ³⁴. Jelonek et al. reported that the
7 CMC of PLA-PEG diblock copolymers decreases as the length of hydrophobic PLA
8 segment increases. In fact, copolymers with longer hydrophobic blocks present a
9 lower CMC as they can more easily self-assemble to form micelles ³⁵.



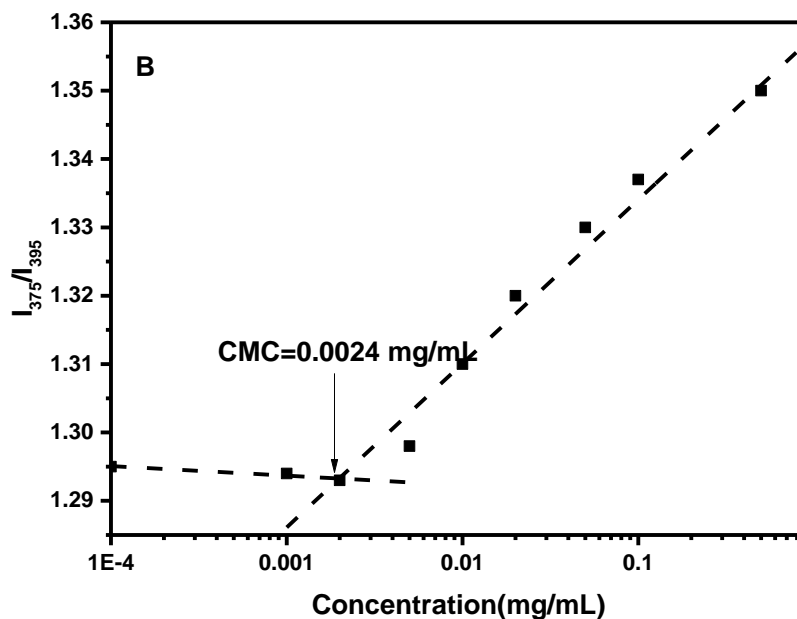


Figure 3. (A) Fluorescence emission spectra of pyrene with increasing concentrations of P(CL/GA)-PEG2K 1. (B) Plots of the intensity ratios (I_{375}/I_{395}) versus the concentration of P(CL/GA)-PEG2K 1 copolymer.

Paclitaxel release from P(CL/GA)-PEG micelles

Paclitaxel loading in micelles was realized by employing solvent evaporation method. Drug loading content (LC) and loading efficiency (LE) data of mPEG2000 and mPEG5000 initiated copolymers are summarized in Table 2. The loading content is 8.8%, 9.8%, and 11.4% for P(CL/GA)-PEG2K 1, P(CL/GA)-PEG2K 2, and P(CL/GA)-PEG2K 5, respectively. These findings could appear surprising as copolymers with longer hydrophobic blocks should lead to higher drug loading according to literature ³⁴, and P(CL/GA)-PEG2K 1 has the longest overall hydrophobic block among the three mPEG2000 initiated copolymers. It is thus assumed that micelles with longer PCL block length lead to higher drug loading. Similar findings were reported for micelles prepared from PEG-b-P(CL-co-TMC) diblock copolymers ³⁷. Latere et al. found that higher PCL content in the copolymers tends to enhance the solubility of furosemide. The drug loading properties of mPEG5000 initiated copolymers are slightly better than those of mPEG2000-initiated

1 ones, which is in accordance with the literature data ²². The loading content is 9.2%,
2 10.6%, and 12.8% for P(CL/GA)-PEG5K 1, P(CL/GA)-PEG5K 2, and
3 P(CL/GA)-PEG5K 5, respectively.
4
5

6 Fig. 4 and 5 present the release profiles of paclitaxel from the various micellar
7 systems in pH =7.4 PBS at 37°C. The drug release proceeds slowly for all copolymer
8 micelles without burst release. However, some differences are noticed for the various
9 micellar systems. 25.3%, 23.2% and 20.8% of paclitaxel release are obtained for
10 P(CL/GA)-PEG2K 1, P(CL/GA)-PEG2K 2, P(CL/GA)-PEG2K 5 in 30 days,
11 respectively. This indicates that higher PGA content in the copolymers leads to faster
12 paclitaxel release. In fact, faster degradation of micelles leads to faster drug release
13 [38]. With the increase of PGA component, the disruption of the chain structure and
14 chain cleavage would be enhanced as in the case of PLGA copolymers ^{38, 39}, thus
15 improving the drug release rate. Similarly, 16.8%, 14.8% and 13.2% of released
16 paclitaxel were obtained for micelles of P(CL/GA)-PEG5K 1, P(CL/GA)-PEG5K 2
17 and P(CL/GA)-PEG5K 5 after 30 days, respectively. Thus paclitaxel release is faster
18 for mPEG2000 initiated copolymers than for mPEG5000 initiated ones. This finding
19 could be attributed to the larger micelle size, and to the larger hydrophobic core of
20 mPEG5000 initiated copolymer micelles with longer hydrophobic blocks. Both
21 disfavors drug diffusion. Similar findings have been reported for PLA-PEG
22 copolymer micelles ³⁴.
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40

41 Drug release was also studied in pH=7.4 PBS containing lipase from *Pseudomonas* sp.
42 to evaluate the effect of enzymatic degradation on drug release rate. This enzyme is
43 known to accelerate the degradation of PCL based polymers ⁹, although it is not
44 present in the body. Faster paclitaxel release is observed in the presence of enzymes in
45 all cases. For example, 25.3% of drug release were detected for P(CL/GA)-PEG2K 1,
46 in contrast to 32.0% of drug release for P(CL/GA)-PEG2K 1E (E referring to
47 enzymes). Similarly, 16.8% of released paclitaxel were obtained for micelles of
48 P(CL/GA)-PEG5K 1, whereas 23.8% were found for P(CL/GA)-PEG5K 1E.
49 Therefore, the presence of lipase from *Pseudomonas* sp. enhanced the drug release
50 rate P(CL/GA)-PEG micelles.
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

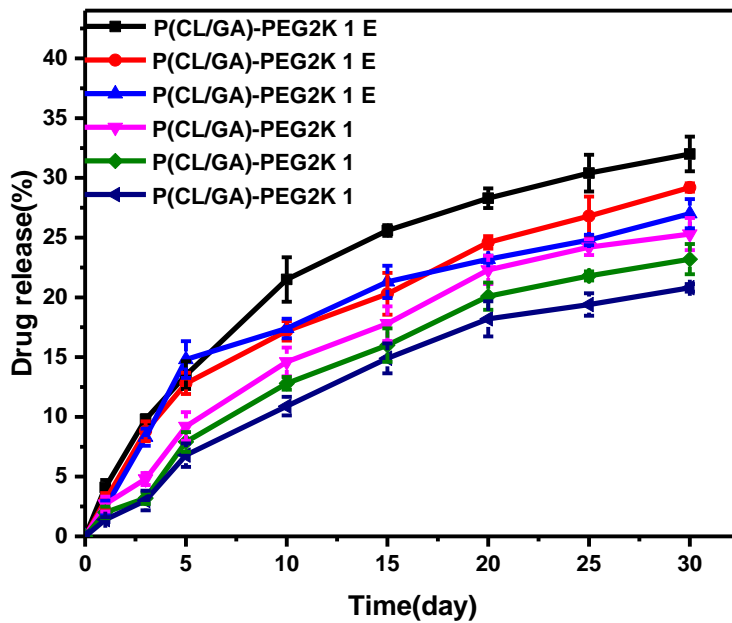


Figure 4. In vitro release profiles of paclitaxel in pH =7.4 PBS and pH =7.4 PBS with lipase from mPEG2000-initiated copolymer micelles (S.D. shown as error bars, n=3)

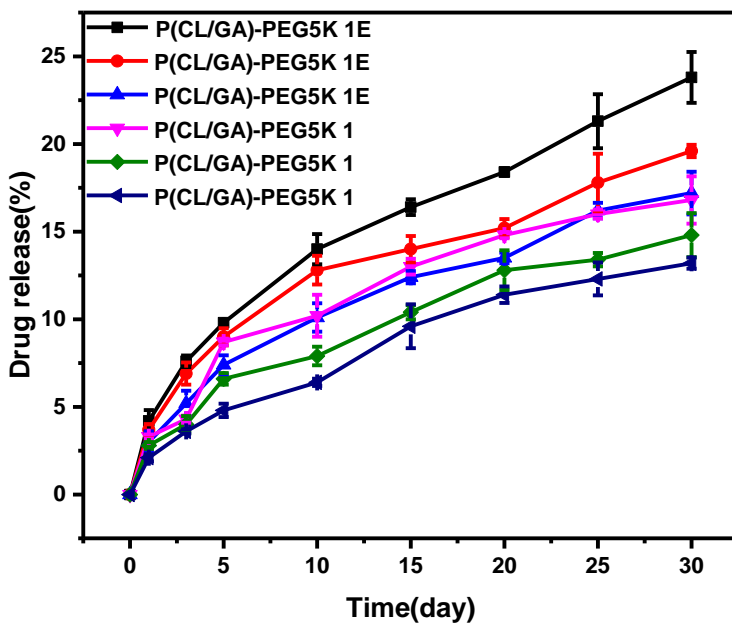


Figure 5. In vitro release profiles of paclitaxel in pH =7.4 PBS and pH =7.4 PBS with lipase from mPEG5000-initiated copolymer micelles (S.D. shown as error bars, n=3).

1 It is also of interest to note that in our previous work, paclitaxel release rate is below
2 10% for micelles prepared from PCL-PEG and P(CL/GA)-PEG with CL/GA ratio of
3
4 10¹⁸. This further confirms that drug release can be improved by enhancing
5
6 copolymer degradation by incorporation of faster degrading PGA component.
7
8
9

10 **Hemocompatibility evaluation**

11 **Hemolysis test**

12 Hemolysis, which demonstrates the disturbed integrity of the membrane structure of
13 red blood cells (RBC), is determined by the release of intracellular hemoglobin.
14 Hemolysis test is widely used in vitro to evaluate the biocompatibility of biomaterials
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

The HR of the different micellar solutions was obtained from the OD values as shown in Table 3. The HR values of all micelles are lower than 5%. These findings suggest that all micelles have little effect to the erythrocytes, indicating the outstanding anti-hemolysis properties of micelles.

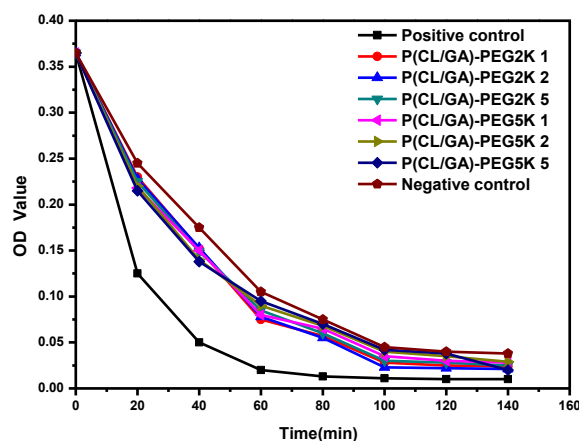
Table 3. Hemolysis ratios of different copolymer micelles

Copolymer	OD Value	Hemolysis ratio (%)
P(CL/GA)-PEG2K 1	0.048±0.005	2.9±0.8
P(CL/GA)-PEG2K 2	0.052±0.004	3.3±0.7
P(CL/GA)-PEG2K 5	0.042±0.007	2.2±0.8
P(CL/GA)-PEG5K 1	0.057±0.010	3.9±0.6
P(CL/GA)-PEG5K 2	0.039±0.003	1.8±0.5
P(CL/GA)-PEG5K 5	0.046±0.008	2.6±0.6
Negative control	0.023±0.006	-
Positive control	0.895±0.025	-

61 **Dynamic clotting time**

1 Dynamic clotting time experiments are often employed to assess the activated degree
2 of endogenous coagulation factors. There is a close relationship between endogenous
3 coagulation process and blood coagulation occurrence. Endogenous coagulation
4 occurs when the micelle is in contact with blood, and the degree of blood clotting
5 gradually increases as the contact time increases. The absorbance-time curve reflects
6 the trend of blood clotting and the duration of clotting time ⁴². The higher the
7 absorbance value, the better the anticoagulation properties. It is generally admitted
8 that the initial clotting time is reached when the absorbance is reduced to 0.1, and the
9 blood is completely coagulated when the absorbance drops to 0.01.
10
11
12
13
14
15
16
17
18

19 As shown in Fig. 6, the dynamic clotting time curves of micellar solutions are similar
20 to that of the negative control, showing a slowly descending trend. In contrast, the
21 positive control exhibits a faster decrease of the OD value. The initial clotting time of
22 P(CL/GA)-PEG2K 1, P(CL/GA)-PEG2K 2, P(CL/GA)-PEG2K 5, P(CL/GA)-PEG5K
23 1, P(CL/GA)-PEG5K 2 and P(CL/GA)-PEG5K 5 was 53.4, 58.2, 58.8, 57.3, 59.4 and
24 59.8 min, respectively, which was close to the negative control group (64.2 min). In
25 contrast, much shorter initial clotting time was observed for the positive control (28.2
26 min). These findings suggest that the micelles have good anticoagulation properties
27 and would not cause the activation of endogenous coagulation factor.
28
29
30
31
32
33
34
35
36
37
38
39



50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
Figure 6. Curves of dynamic clotting time of micelles in comparison with the controls

Plasma recalcification time (PRT)

The first stage of blood coagulation happens through the endogenous and exogenous pathways, leading to the formation of prothrombinase. There exists a cascade of reactions between coagulation factors⁴³. Plasma recalcification time (PRT) represents the time required for fibrin clot formation when Ca^{2+} is added into PPP. It is thus an important indicator of micelle induced coagulation activation⁴⁴.

The longer the PRT, the better the hemocompatibility of biomaterials. The PRT is 374.7, 417.0, 435.3, 397.8, 428.6 and 452.4 s for P(CL/GA)-PEG2K 1, P(CL/GA)-PEG2K 2, P(CL/GA)-PEG2K 5, P(CL/GA)-PEG5K 1, P(CL/GA)-PEG5K 2 and P(CL/GA)-PEG5K 5, respectively (Fig. 7). These values are slightly smaller than that of the negative control (479.6 s), but significantly larger than that of the positive control (243.3 s), thus showing that the micelles present good hemocompatibility.

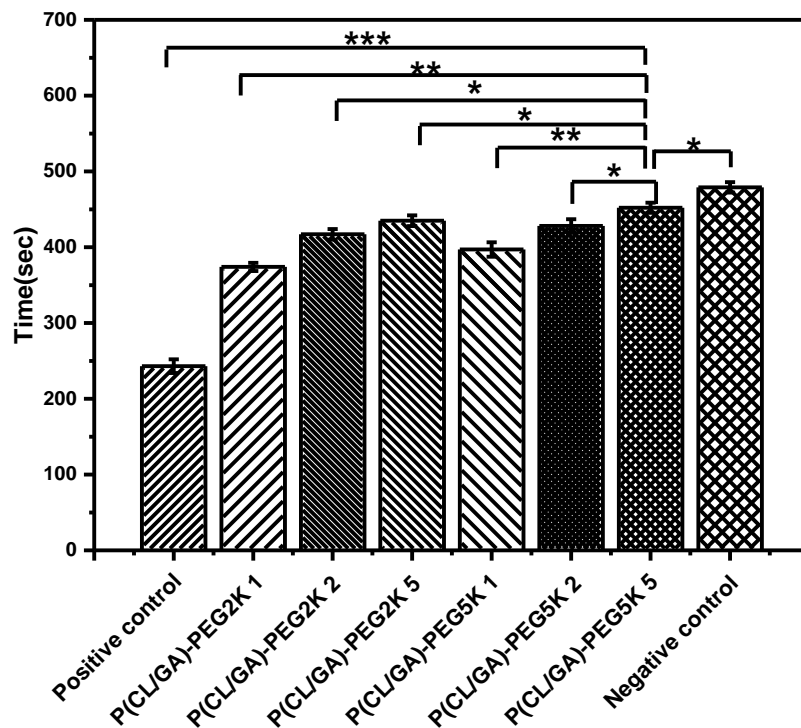


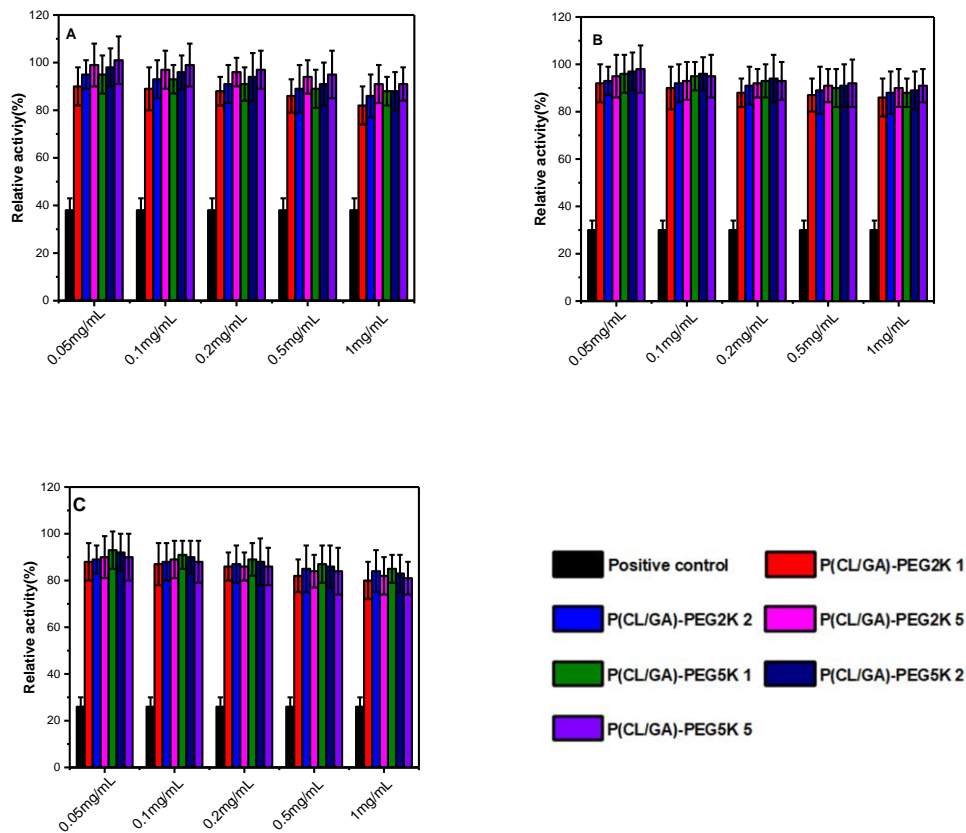
Figure 7. Plasma recalcification time of micelles. ** indicates $p < 0.01$, *** $p < 0.001$, and * $p > 0.05$.

Cytocompatibility evaluation

MTT assay

1 MTT assay is one of the preferred methods to evaluate the cytocompatibility of
 2 biomaterials ⁴⁵. L-929 cell line (mouse fibroblast) was used for MTT test as it is a
 3 commonly used standard cell line in cytocompatibility evaluation.
 4
 5

6 The relative activity of cells in the presence of micellar solutions is shown in Fig. 8. It
 7 appears that the cell viability in the positive control is very low, while the micellar
 8 solutions have little effect on the growth of L-929 cells as well as the negative control
 9 group (100%). The cell viability slightly varies with the incubation time and micellar
 10 concentration. The cell viability slightly decreases ($P > 0.05$) with the increase of
 11 micellar concentration from 0.05 to 1.0 mg/mL, but the incubation time up to 72 h
 12 doesn't influence the viability in all groups ($P > 0.05$). It is worth noting that even with
 13 the highest concentration of 1 mg/mL and at the longest time of 72 h, the relative
 14 activity is above 80% for all micellar solutions. All these data show that
 15 P(CL/GA)-PEG micellar solutions are not toxic to L-929 cells.
 16
 17
 18
 19
 20
 21
 22
 23
 24
 25
 26

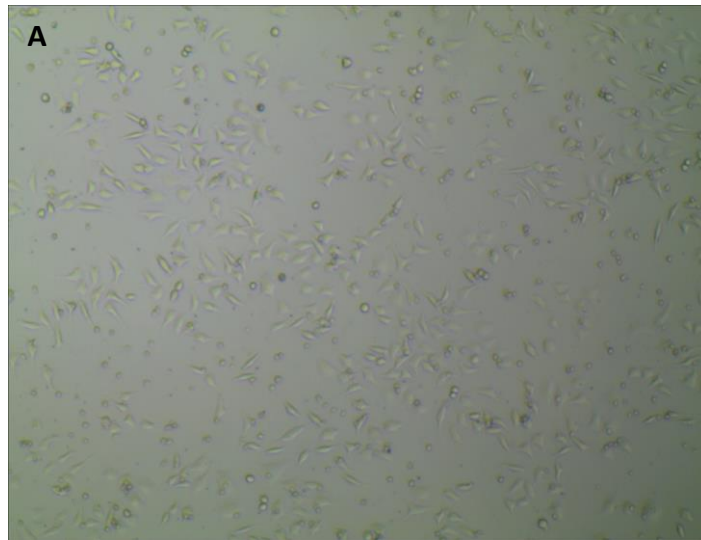


27
 28
 29
 30
 31
 32
 33
 34
 35
 36
 37
 38
 39
 40
 41
 42
 43
 44
 45
 46
 47
 48
 49
 50
 51
 52
 53
 54
 55
 56
 57 Figure 8. Relative activity of L-929 cells after 24 h (A), 48 h (B), and 72 h (C)
 58 culture with micelle solutions at different concentrations as compared to the negative
 59
 60
 61
 62
 63
 64
 65

control. Data are presented as the mean \pm SD (n = 3)

Agar diffusion test

Agar diffusion test is another method of choice used to evaluate the cytotoxicity of biomaterials according to ISO 10993-5⁴⁶. It is based on the fact that neutral red preferentially acts on acid regions of living cells such as proliferating DNA and lysosomes, thus dyeing them red⁴⁷. Therefore, the toxicity of P(CL/GA)-PEG micellar solutions is related to the decolorization caused by undyed damaged cells and cell injury morphological signs⁴⁸. As shown in Fig. 9, L-929 cells were decolorized and shrank in the positive control. In contrast, no decolorization was observed in P(CL/GA)-PEG2K 1 micellar solution and the negative control. Similar findings were observed for other copolymer micellar solutions. These results indicate that P(CL/GA)-PEG micelles present no cytotoxicity.



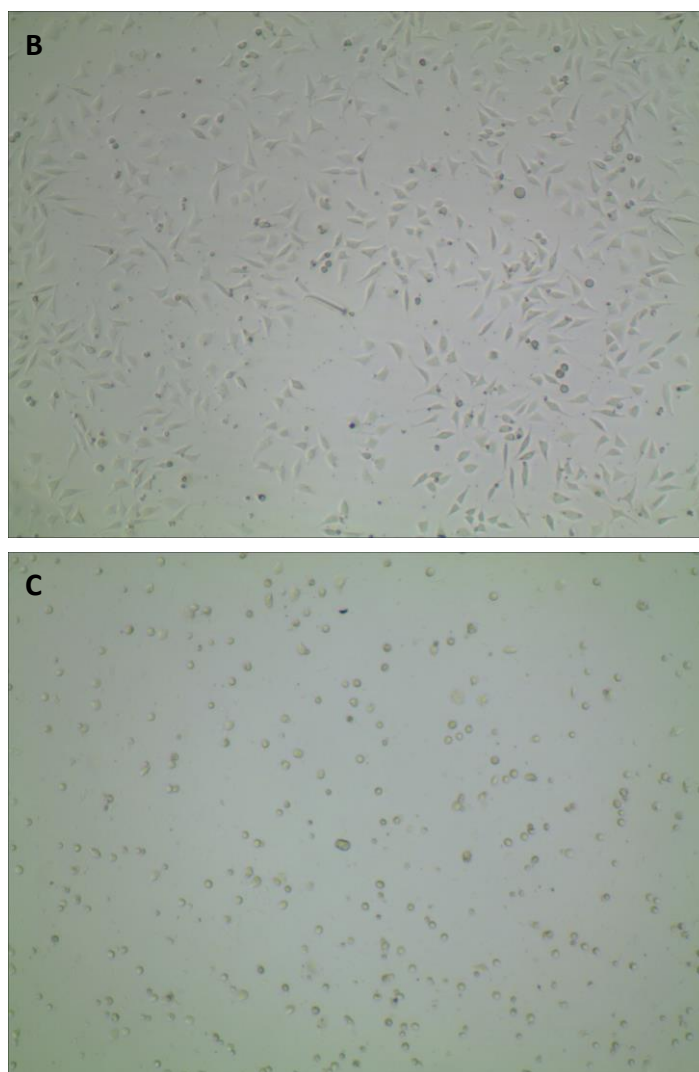


Figure 9. Microscopic images of L-929 cells after 24 h exposure to the negative control (A), P(CL/GA)-PEG2K 1 (B) and positive control (C) in agar diffusion test.

CONCLUSIONS

In this work, P(CL/GA)-PEG diblock copolymers with various compositions were obtained by ring-opening polymerization of ϵ -caprolactone (CL) and glycolide (GA) using mPEG2000 or mPEG5000 as initiator and $\text{Sn}(\text{Oct})_2$ as catalyst. The effect of copolymer composition on the micellar size, critical micelle concentration, drug loading and drug release properties was elucidated. All the micelles are spherical in shape, and the micelle size is larger for copolymers with longer PEG blocks. In contrast, the CMC of copolymers increases with decreasing the overall hydrophobic block length. The drug loading is closely related to the length of PCL blocks and

1 copolymer composition. Higher drug loading is obtained for micelles with longer PCL
2 blocks. Drug release is mainly dependent on copolymer degradation. Micelles
3 prepared from mPEG2000 initiated copolymers exhibit faster release than those of
4 mPEG5000 initiated copolymer micelles, and higher GA content leads to faster
5 paclitaxel release. Moreover, drug release rate is enhanced in the presence of lipase
6 from *Pseudomonas* sp., thus indicating that drug release is favored by copolymer
7 degradation.
8

9
10 On the other hand, the hemocompatibility and cytocompatibility of micelles were
11 evaluated. Low hemolytic ratio, low effect on the dynamic clotting time and plasma
12 recalcification time indicate good hemocompatibility of the various micellar solutions.
13 MTT and agar diffusion tests suggest that all micellar solutions present very low
14 cytotoxicity on cell adhesion and proliferation. Therefore, these bioresorbable
15 P(CL/GA)-PEG micelles, with excellent stability, high drug loading content,
16 prolonged drug release and good biocompatibility could be promising for applications
17 as drug carrier.
18
19
20
21
22
23
24
25
26
27
28
29
30

31 **ACKNOWLEDGEMENTS**

32
33 This work was supported by National Natural Science Foundation of China (No.
34 81570287), Science and Technology Development Plan of Shandong Province (No.
35 2015GGX102017 and 2018GGX102016), Achievement Transformation Plan of
36 Qingdao (No. 16-6-2-17-nsh and 16-6-2-9-nsh).
37
38
39
40
41
42
43
44

45 **REFERENCES**

- 46 1. Kim, S.C.; Kim, D.W.; Shim, Y.H.; Bang, J.S.; Oh, H.S.; Wan, K.S.; Seo, M.H.
47 *J. Controlled Release.* **2001**, *72*, 191.
48
- 49 2. Singla, A.K.; Garg, A.; Aggarwal, D. *Int J Pharm.* **2002**, *235*, 179.
50
- 51 3. Yang, L.; Wu, X.H. *Pharm Res.* **2009**, *26*, 2332.
52
- 53 4. Xie, J.; Wang, C.H. *Pharm Res.* **2005**, *22*, 2079.
54
- 55 5. Reddy, L.H.; Bazile, D. *Adv Drug Deliv Rev.* **2014**, *71*, 34.
56
57
58
59
60
61
62
63
64
65

- 1 6. Sparreboom, A.; Van, T.O.; Nooijen, W.J.; Beijnen, J.H.; *Anti-cancer drugs*. **1996**,
- 2 7, 78.
- 3
- 4 7. Chiellini, E.; Solaro, R.; *Advanced Materials*. **1996**, 8, 305.
- 5
- 6 8. Wang, S.; Chen, H.; Cai, Q.; Bei, J. *Polym Adv Technol*. **2001**, 12, 253.
- 7
- 8 9. Li, S.; Garreau, H.; Pauvert, B.; Mcgrath, J.; Toniolo, A.; Vert, M.
- 9 *Biomacromolecules*. **2002**, 3, 525.
- 10
- 11 10. Allen, C.; Han, J.; Yu, Y. *J Controlled Release*. **2000**, 63, 275.
- 12
- 13 11. Soppimath, K.S.; Aminabhavi, T.M.; Kulkarni, A.R.; Rudzinski, W.E. *J*
- 14 *Controlled Release*. **2001**, 70, 1.
- 15
- 16 12. Bougard, F.; Giacomelli, C.; Mespouille, L.; Borsali, R.; Dubois, P.; Lazzoroni,
- 17 *R. Langmuir*. **2008**, 24, 8272.
- 18
- 19 13. Liu, T.J.; Liu, S.; Hu, X.L.; Li, X. *Chem Res Chinese Universities*. **2011**, 27, 628.
- 20
- 21 14. Moghimi, S.M.; Hunter, A.C.; Murray, J.C. *Pharmacol Rev*. **2001**, 53, 283.
- 22
- 23 15. Geng, Y.; Discher, D.E. *Polymer*. **2006**, 47, 2519.
- 24
- 25 16. Robert, P.; Mauduit, J.; Frank, R.M.; Vert, M. *Pharma Sci*. **1993**, 3, 197.
- 26
- 27 17. Williams, D.F. *Biomaterials*. **2008**, 29, 2941.
- 28
- 29 18. Sun, X.; Liu, X.; Li, C.; Wang, Y.; Liu, L.; Su, F. *Journal of Applied Polymer*
- 30 *Science*. **2018**, 135, 45732.
- 31
- 32 19. Gong, C.; Shi, S.P.; Kan, B.; Gou, M.; Wang, X.; Li, X. *Int J Pharm*. **2009**, 365,
- 33 89.
- 34
- 35 20. Rajagopal, K.; Mahmud, A.; Christian, D.A.; Pajerowski, J.D.; Brown, A.E.;
- 36 Loverde, S.M. *Macromolecules*. **2011**, 43, 9736.
- 37
- 38 21. Dominguez, A.; Fernandez, A.; Gonzalez, N.; Iglesias, E.; Montenegro, L. *J Chem*
- 39 *Educ*. **1997**, 74, 1227.
- 40
- 41 22. Cai, S.; Vijayan, K.D.; Lima, E.; Discher, D. *Pharm Res*. **2007**, 24, 2099.
- 42
- 43 23. Biological evaluation of medical devices Part 4: Selection of tests for interactions
- 44 with blood [S]. ISO 10993-4:2009.
- 45
- 46 24. Standard Practice for Assessment of Hemolytic Properties of Materials [S].
- 47 ASTM F756-2000.
- 48
- 49
- 50
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60
- 61
- 62
- 63
- 64
- 65

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
25. Shen, X.; Su, F.; Dong, J.; Fan, Z.; Duan, Y.; Li, S. *J Biomater Sci Polym Ed.* **2015**,
26, 497.
26. Liu, J.X.; Yang, D.Z.; Shi, F.; Ca, Y.J. *Thin Solid Films.* **2003**, 429, 225.
27. He, Z.; Schulz, A.; Wan, X.; Seitz, J.; Bludau, H.; Alakhova, D.Y. *J Control
Release.* **2015**, 208, 67.
28. Hu, Y.; Darcos, V.; Monge, S. *J Mater Chem B.* **2014**, 2, 2738.
29. Liu, X.; Shen, X.; Sun, X.; Peng, Y.; Li, R. *Polymers for Advanced Technologies.*
2018, 5.
30. Mutlu, H.; Lutz, J.F. *Angew Chem Int Ed Engl.* **2014**, 53, 13010.
31. Jiang, Z.; You, Y.; Deng, X. *Polymer.* **2007**, 48, 4786.
32. Yang, L.; Qi, X.; Liu, P.; El, G.A.; Li, S. *Int J Pharm.* **2010**, 394, 43.
33. Yang, L.; Zhao, Z.; Wei, J.; El, G.A.; Li, S. *Journal of colloid and interface
science.* **2007**, 314, 470.
34. Jelonek, K.; Li, S.; Wu, X.; Kasperczyk, J.; Marcinkowski, A. *Int J Pharm.* **2015**,
485, 357.
35. Yokoyama, M. *Journal of Experimental & Clinical Medicine.* **2011**, 3, 151.
36. Zhu, J.; Hayward, R.C. *Journal of the American Chemical Society.* **2008**, 130,
7496.
37. Forrest, M.L.; Won, C.Y.; Malick, A.W.; Kwon, G.S. *Pharmazie Die.* **2008**, 63,
235.
38. Jelonek, K.; Li, S.; Kasperczyk, J.; Wu, X.; Orchel, A. *Materials Science and
Engineering: C.* **2017**, 75, 918.
39. Ramchandani, M.; Robinson, D. *Journal of Controlled Release: Official Journal
of the Controlled Release Society.* **1998**, 54, 167.
40. Shih, M.F.; Shau, M.D.; Chang, M.Y.; Chioua, S.K.; Chang, J.K.; Cherng, J.Y. *Int
J Pharm.* **2006**, 327, 117.
41. Lee, P.J.; Ho, C.C.; Hwang, C.S.; Ding, S.J. *Surf Coat Technol.* **2014**, 258, 374.
42. Kenausis, G.L.; Vörös, J.; Elbert, D.L. *J Phys Chem B.* **2000**, 104, 3298.
43. Gorbet, M.B.; Sefton, M.V. *Biomaterials.* **2004**, 25, 5681.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

44. Dash, B.C.; Réthoré, G.; Monaghan, M.; Fitzgerald, K.; Gallagher, W. *Biomaterials*. **2010**, 31, 8188.

45. Luo, Y.; Zhang, C.; Xu, F.; Chen, Y. *Polymers for Advanced Technologies*. **2012**, 23, 551.

46. Schmalz, G. *International Endodontic Journal*. **1988**, 21, 59.

47. Wang, Q.; Liu, P.; Liu, P.; Gong, T.; Li, S. *Biomed Mater*. **2014**, 9, 015007.

48. Tan, L.; Liu, Y.; Wei, H.; Ding, L.S.; Peng, S.L. *Soft Matter*. **2012**, 8, 5746.

