Biomaterials 35 (2014) 278–286

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

A pH and thermosensitive choline phosphate-based delivery platform targeted to the acidic tumor microenvironment

Xifei Yu [a, b], Xiaoqiang Yang [a, b], Sonja Horte [a, b], Jayachandran N. Kizhakkedathu [a, b, c], Donald E. Brooks [a, b, c, *]

[a] Centre for Blood Research, 2350 Health Sciences Mall, University of British Columbia, Vancouver V6T 1Z3, Canada
[b] Department of Pathology and Laboratory Medicine, UBC, Canada
[c] Department of Chemistry, UBC, Canada

ARTICLE INFO

Article history:
Received 16 July 2013
Accepted 16 September 2013
Available online 7 October 2013

Keywords:
Choline phosphate
Cell adhesion
Tumor
Drug delivery
Thermal and pH sensitive

ABSTRACT

Solid tumors generally exhibit an acidic microenvironment which has been recognized as a potential route to distinguishing tumor from normal tissue for purposes of drug delivery or imaging. To this end we describe a pH and temperature sensitive polymeric adhesive that can be derivatized to carry drugs or other agents and can be tuned synthetically to bind to tumor cells at pH 6.8 but not at pH 7.4 at 37 °C. The adhesive is based on the universal reaction between membrane phosphatidyl choline (PC) molecules and polymers derivatized with multiple copies of the inverse motif, choline phosphate (CP). The polymer family we use is a linear copolymer of a CP terminated tetraethoxymethacrylate and dimethylaminoethyl (DMAE) methacrylate, the latter providing pH sensitivity. The copolymer exhibits a lower critical solution temperature (LCST) just below 37 °C when the DMAE is uncharged at pH 7.4 but the LCST does not occur when the group is charged at pH 6.8 due to the ionization hydrophilicity. At 37 °C the polymer binds strongly to mammalian cells at pH 6.8 but does not bind at pH 7.4, potentially targeting tumor cells existing in an acidic microenvironment. We show the binding is strong, reversible if the pH is raised and is followed rapidly by cellular uptake of the fluorescently labeled material. Drug delivery utilizing this dually responsive family of polymers should provide a basis for targeting tumor cells with minimal side reactions against untransformed counterparts.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

The rapid growth characteristic of solid tumors relies on enhanced metabolic rates that generate acidic products in the microenvironment around individual tumor cells although the internal pH in these cells remains in the normal, roughly neutral range [1]. This general observation of an acidic external pH (pHe) has been observed and verified in a wide variety of *in vitro*, *in vivo* and clinical settings by a number of electrophysiological and spectroscopic techniques [2–5]. In working towards an enhanced therapeutic window for cancer treatment, the low pHe has been recognized as a potential feature that can be utilized in targeting tumor cells that does not require specific recognition of a particular molecule or pathway [6–10]. This is a very active field of research and a number of approaches for treatment are being examined [6–24].

Ideally one would like a distinct marker for each target tumor cell and be able to direct drug delivery or imaging species to each with a particular recognition system. Folate receptors can play that role in some classes of tumors and therapy directed to this feature has received much attention [11–14]. However, while they are widely distributed, a significant to majority fraction of many tumors does not show overexpression of this receptor [11]. An estimate of the typical number of receptors per cell, assuming the reported value of ~20 pM/mg membrane protein [15], is less than 10^4 copies per cell, which may limit the association reactions utilized. The other very general approach to targeting has been to take advantage of the leaky circulation that develops in solid tumors allowing preferential passage of nanoparticulate systems into the tissue, a process known as the enhanced permeability and retention (EPR) [16,17] effect. This allows a variety of polymer–drug conjugates, polymer–protein conjugates, polymeric micelles, liposomes, antibody–drug conjugates, therapeutic antibodies, and nanoscale devices to be exposed to the tumor environment [6–24]. In many cases these delivery systems carry a pH sensitive element that releases drug at low pH [6–15,18–24]. Concentrations of these

* Corresponding author. Centre for Blood Research, 2350 Health Sciences Mall, University of British Columbia, Vancouver V6T 1Z3, Canada. Tel.: +1 604 822 7081; fax: +1 604 822 7742.
E-mail address: don.brooks@ubc.ca (D.E. Brooks).

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.09.052

carriers are low however due to dilution in the bloodstream and critical micelle concentration limitations for micellar systems. As there is generally no mechanism to enhance local concentrations near the tumor cells the delivered elements can be slow (days) to take effect [24].

In spite of the above progress there is still an urgent need for approaches that have reduced toxicity to healthy tissue while killing tumor cells. Most of the work in this area that uses an acidic pH as the target to identify tumor cells does not distinguish between pHe and the internal acidic pH within lysosomes encountered when and if the delivery system is taken up by tumor cells. The present work describes an approach to the delivery problem by focusing on *adhesion* of the polymeric delivery system to the plasma membrane of cells that exist in a reduced pH microenvironment which is followed by uptake of the polymer into the cellular cytoplasm. In particular we illustrate a system that adheres strongly to cells at physiological temperature and pH 6.8, (which seems to represent the acidic milieu surrounding many types of cancer cells in solid tumors [1,6–24]), but does not adhere to cells at the same temperature and pH 7.4, conditions reflecting normal tissue. This adhesion increases the surface concentration of the platform remarkably so a very high local concentration of the agent is available at the cell surface to deliver a drug or label.

In our case adhesion is provided by a linear copolymer with the capacity to bind to any mammalian cell through the association of multiple phosphatidyl choline (PC) lipids in the cell membrane with multivalent choline phosphate (CP) groups carried by the copolymer. While an individual CP–PC interaction is too weak to form a stable bond in water, multivalent CP–PC interactions have been shown recently to provide a strong bond whose strength increases with the number of CP groups available per polymer molecule [25,26]. Such multivalent associations are essentially irreversible on the relevant time scales (hours to days), even in the presence of competitive inhibitors, However, we have developed a method to reverse or inhibit such binding by using linear polymeric carriers that exhibit a lower critical solution temperature (LCST) [27]. Below the LCST such polymers are in an extended hydrated H-bonded configuration that allows multiple CP groups access and attachment to PC-containing cell membranes. Above the LCST the polymers collapse hydrophobically to a configuration that inhibits CP binding and the macromolecule either does not bind or, if bound below the LCST, is released from the cell.

With the low pHe characteristic in mind we have designed into the copolymer a pH sensitivity that essentially eliminates binding at pH 7.4 above the LCST of 36.5 °C but allows strong CP-based binding at 37 °C if the pH is ~6.8. The LCST is provided by the use of a dedrivatized tetraethyleneoxide methacrylate as the major comonomer [27–30]. The pH sensitivity is provided by the inclusion of one or two 2-(dimethylamino)ethyl methacrylate (DMAEMA) monomers in the copolymer that are ionized by protonation at pH 6.8 but are largely uncharged at pH 7.4. Addition of the positive charge changes the H-bonding and solubility of the copolymer in such a way that the LCST no longer is observed up to 100 °C so the polymer remains in an extended configuration and readily bonds to PC rich membranes. Such binding moreover results in rapid uptake of the polymer into nucleated cells. The evidence behind these statements is provided below and in the Supporting information.

2. Materials and methods

2.1. Synthesis of the smart biomembrane adhesive

2.1.1. Materials [25–27]

All chemicals were purchased from Sigma–Aldrich and used without further purification unless otherwise mentioned. Tetraethylene glycol (99%) and pentaethylene glycol (98%) were purchased from Alfa Aesar and used without further purification. Fresh whole blood was drawn into EDTA from consenting volunteers under a protocol approved by the UBC Clinical Research Ethics Board.

2.2. Synthesis of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanol, AEO_4

In a dried 1 L one-necked flask, equipped with a dropping funnel containing triethylamine (25.3 g, 0.25 mol) and a drying tube, was placed tetraethylene glycol (194.2 g, 1.0 mol), tosyl chloride (38.1 g, 0.2 mol) and 500 mL anhydrous THF. The triethylamine was added dropwise over 4 h to the mixture at 4 °C and was stirred at room temperature (RT) overnight. The reaction mixture was filtered to remove the solid and extracted with methylene chloride (DCM). A reaction mixture was collected after the rotary evaporation of DCM. The mixture, sodium azide (16.3 g, 0.25 mol) and DMF (150 mL) were transferred to a one-necked flask equipped with a water cooled condenser. The reaction mixture was stirred for 18 h at 80 °C and was cooled to RT. The reaction mixture was filtered to remove the solid and extracted with DCM. The extract was dried over $MgSO_4$ and the crude product was collected after the rotary evaporation of methylene chloride. The final product of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanol, AEO_4, was purified by flash chromatography and ethyl acetate and hexane (1:1, $R_f = 0.2$) to yield a clear oil, yield was 23.8 g (54%).

^{1}H NMR (δ: ppm, 400 MHz, $CDCl_3$): AEO_4, 3.72–3.61 ($-CH_2CH_2O-$, m, 14H), 3.42–3.39 (N_3CH_2-, t, 2H), see Fig. S1.

2.3. Synthesis of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl methacrylate, AEO_4MA

In a dried 500 mL one-necked flask, equipped with a dropping funnel containing methacryloyl chloride (12.5 g, 0.12 mol) and a drying tube, was placed triethylamine (30.4 g, 0.3 mol), 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanol (21.9 g, 0.1 mol) and 200 mL anhydrous THF. The methacryloyl chloride was added dropwise over 4 h to the mixture at 4 °C and was stirred at RT overnight. The reaction mixture was filtered to remove the solid and extracted with DCM. A reaction mixture was collected after the rotary evaporation of DCM. The extract was dried over $MgSO_4$ and the crude product was collected after the rotary evaporation of methylene chloride. The final product of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl methacrylate, AEO_4MA, was purified by flash chromatography and ethyl acetate and hexane (1:4, $R_f = 0.2$) to yield a clear oil, yield was 19.7 g (68%).

^{1}H NMR (δ: ppm, 400 MHz, $CDCl_3$): AEO_4MA, 6.14, 5.58 ($-CH_2{=}C-$, 2s, 2H), 4.33–4.30 ($-CH_2OOC-$, t, J = 1.60 Hz, 2H), 3.78–3.74 ($-OCH_2CH_2OOC-$, t, J = 1.64 Hz, 2H), 3.70–3.66 ($-OCH_2CH_2-$, m, 10H), 3.41–3.38 ($-CH_2N_3$, t, J = 1.68 Hz, 2H), 1.96 ($-CH_3$, s, 3H), is shown in Fig. S2.

ESI-TOF-MS (Positive), m/z: 310.14 (Mw + 1Na), see Fig. S3.

2.4. Reversible addition–fragmentation chain transfer (RAFT) [31] copolymerization of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl methacrylate and 2-(dimethylamino) ethyl methacrylate, $p(AEO_4MA_x\text{-}co\text{-}DMAEMA_y)$

To a flame-dried 25 mL Schlenk flask equipped with a magnetic stir bar and under argon atmosphere, at RT, were added AEO_4MA (2.9 g, 10.1 mmol), 2-(dimethylamino)ethyl methacrylate (DMAEMA, 0.1 g, 0.64 mmol), 2-cyano-2-propyl dodecyl trithiocarbonate (34.5 mg, 0.1 mmol), AIBN (0.4 mg, 0.0024 mmol), p-toluenesulfonic acid (22.8 mg, 0.12 mmol) and ethanol (3 mL) into the flask. The reaction flask was sealed by a rubber septum and the reaction mixture was degassed through three cycles of freeze–pump–thaw. After the last cycle, the reaction mixture was recovered back to RT and stirred for 10 min before being immersed into a pre-heated oil bath at 65 °C. After 48 h, the polymerization was quenched by quick immersion of the reaction flask into liquid nitrogen. The reaction mixture was precipitated into ethyl ether three times to afford $p(AEO_4MA_{40}\text{-}co\text{-}DMAEMA_{1.25})$ as a light yellow waxy solid, yield was 1.3 g (42%). M_n^{NMR} = 11,200 g/mol, M_n^{GPC} = 18,600 g/mol, M_w/M_n = 1.10. ^{1}H NMR (δ: ppm, 400 MHz, $CDCl_3$): $p(AEO_4MA_{40}\text{-}co\text{-}DMAEMA_{1.25})$, 4.08–3.95 ($-CH_2OOC$), 3.75–3.55 ($-OCH_2CH_2-$), 3.38–3.30 ($-CH_2N_3$), 3.35–3.25 ($-CH_2SSC-$), 2.48, 2.36 ($(CH_3)_2N-$, 2s), 2.17 ($(CN)C(CH_3)_2$, S), 1.96–1.5 ($-CH_2-$), 1.5–1.15 ($-C_{10}H_{20}-CH_3$), 1.15–0.78 ($-CH_3$), see Fig. S4.

2.5. "Click" reaction between $p(AEO_4MA_{40}\text{-}co\text{-}DMAEMA_{1.25})$ and prop-2-ynyle choline phosphate (p-CP) [25]

Prop-2-ynyle choline phosphate (200 mg), $p(AEO_4MA_{40}\text{-}co\text{-}DMAEMA_{1.25})$ (200 mg), copper sulfate pentahydrate (1.25 mg), sodium ascorbate (2.5 mg) and methanol (5 mL) were transferred to a 20 mL one-necked flask. The reaction mixture was stirred for 24 h at RT. Finally, dialysis was used to remove the excessive prop-2-ynyle choline phosphate and the copper catalyst with MWCO 1000 membrane. After freeze-drying, $p(CPEO_4MA_{40}\text{-}co\text{-}DMAEMA_{1.25})$, DR(20.8K)-$CP_{40}$, was collected, yield was 272 mg, LCST(DR(20.8K)-CP_{40}) = 36.5 °C. M_n^{NMR} = 20,800 g/mol, M_n^{GPC} = 24,500, M_w/M_n = 1.12. The proton and phosphorous NMR spectra are shown in Fig. S5.

Download English Version:

https://daneshyari.com/en/article/6217

Download Persian Version:

https://daneshyari.com/article/6217

Daneshyari.com