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Development of a new tri-block copolymer with a functional end and its feasibility for treatment of metastatic breast cancer

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ABSTRACT

We have developed nanomedicine vehicle based on a biocompatible tri-block copolymer, poly(ethylene glycol)-*block*-poly(lactic acid)-*block*-poly(ethylene glycol) (PEG-PLA-PEG) by simple approach without toxic linker to escalate therapeutic efficacy of anticancer agent by enhanced targeting to metastasized breast cancers. The synthesized ABA type copolymer had a low polydispersity index and formed small, highly stable spherical micelles. Furthermore, a functional group at the end site of the copolymer can be decorated with imaging agents and targeting moieties. The doxorubicin loaded micelles (DLM) showed higher drug-loading capacity, faster drug release, and better cell toxicity compared to those using di-block copolymers. DLM efficiently delivered to the metastatic breast cancers in brain and bone and suppressed growing of metastasis. In demonstration of treating metastasized animal model, we present a tri-block copolymer as a potential nanomedicine vehicle to efficiently deliver anticancer drug and to effectively treat metastatic breast cancer.

1. Introduction

Cancer research in the past several decades have made rapid progress to provide better understanding cancer biology, greater reversal of cancer, and further contributed to prolong survival of cancer patients. However, we still encounter several challenges in developing anticancer drugs including the relevant cancer animal model reflecting specific tumor type, the novel anticancer drug carriers with better delivering efficiency to the target lesion, and the real time *in vivo* monitoring to evaluate therapeutic effects [1–3]. Breast cancer is the most common type of malignant neoplasm in women worldwide and metastasis accounts for the majority of deaths [4,5]. Breast cancer preferentially metastasizes to bone and brain, where it is associated with reduced quality of life from pain and progressive neurologic impairments [6,7]. The novel systemic therapy to control multisystem metastasis is necessary.

Currently, nanomedicines using biocompatible polymer-based carriers are promising for the delivery of hydrophobic anticancer agents with high targeting capability [2,8–10]. Self-assembling into micelles having a hydrophobic core and a hydrophilic corona with high-solubilizing capacity and biophysicochemical stability is an exceptional characteristic of amphiphilic block copolymers for formulation availability and clinical efficacy of drug vehicles [11–14]. The various arrangements of segmented blocks consisting of hydrophobic block (A-block) and hydrophilic block (B-block) can provide physicochemical modification of carriers, resulting in control of biodegradability, change of micelle sizes, increased loading capacity, and controlled drug release. Therefore, copolymers with various structures as AB di-block, ABA, BA-linker-AB, ABC tri-block, multi-block, and star/grafted copolymers were developed to innovate a favourable drug delivery systems with favourable properties such as biodegradability, nanosizes, increased loading capacity, and controlled drug release [15–18]. For example, tri-block copolymers with BA-linker-AB arrangement were initially developed for thermosensitive polymers instead of non-degradable Pluronic® by Kim's group [19]. The tri-block copolymers can be utilized for versatile applications such as emulsifiers for microemulsion, high stable carriers due to two arm PEG, thermo-sensitive drug delivery systems, and biological modifying agents like Pluronic® [19–22]. However, these BA-linker-AB copolymers could not provide the

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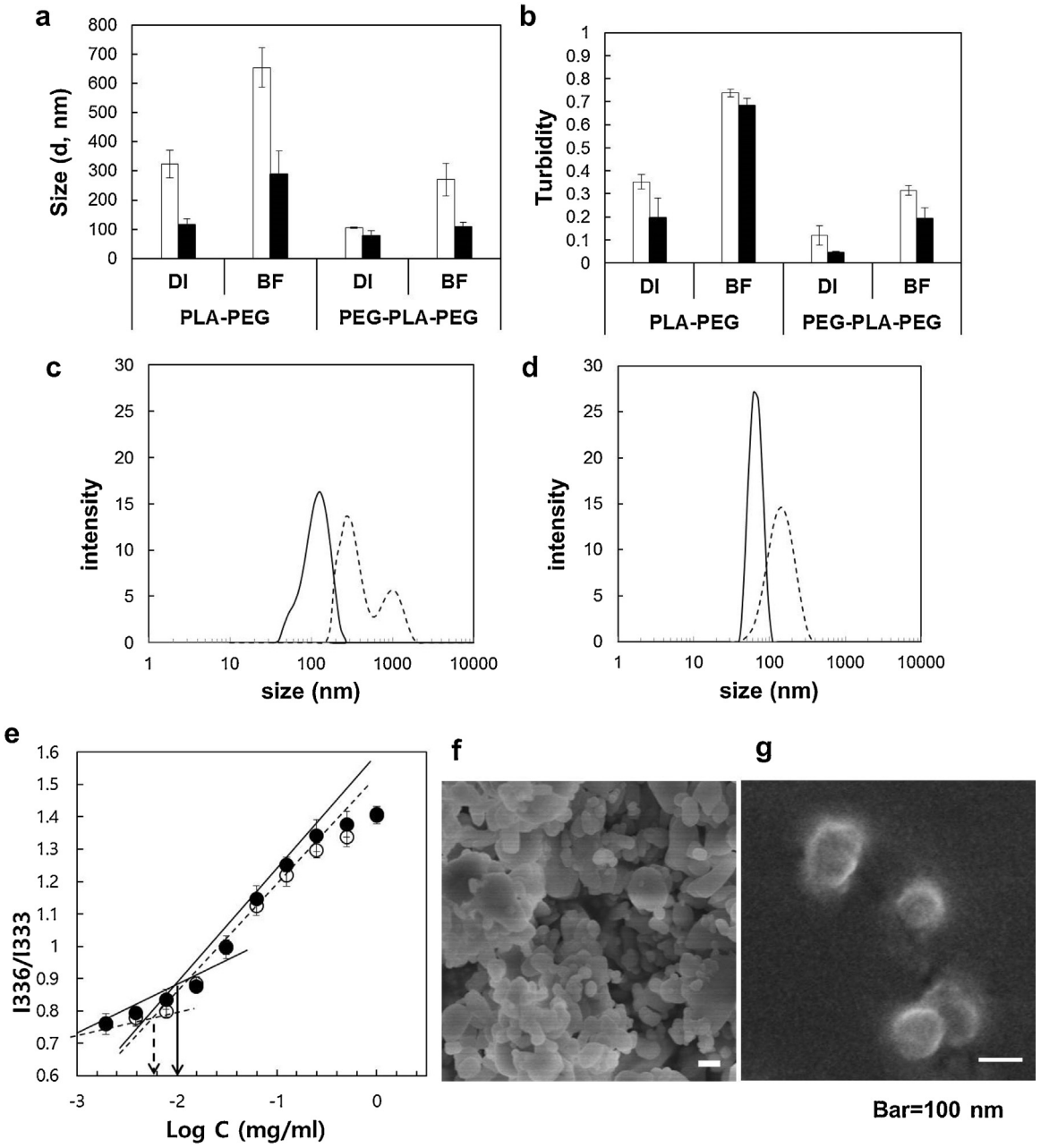

Fig. 1. Physicochemical characterizations of micelles from di- and tri-block copolymers depended on preparation.
(a) Sizes and (b) turbidity of micelles prepared by diafiltration (DI) and bottom-flask (BF) methods without sonication (white bar) and with sonication (black bar) (n = 3, mean ± SD). Micelles prepared from PEG-PLA-PEG with sonication had smaller size and low turbidity. The smallest particle size (77 ± 18.9 nm) and lowest turbidity (0.118 transmittance) was for PEG-PLA-PEG by DI with sonication. Particle size distribution of micelles in PLA-PEG (—) and PEG-PLA-PEG (—) solutions prepared by DI (c) without sonication and (d) with sonication for 1 min. Micelles of di-block copolymers and tri-block copolymers made by DI showed bimodal and unimodal size distribution, respectively. After additional sonication, size distributions of both micelle types changed to unimodal distribution and particle size distribution of tri-block copolymer micelles was sharper and narrower. (e) Plot of intensity ratios I_{336}/I_{333} from excitation spectra as a function of PLA-PEG (n = 3, mean ± SD). Arrows, CMC of di-block 10 μg/ml (open dot) and tri-block 13 μg/ml (closed dot) copolymers. (f–g) FE-SEM of DOX-loaded micelles prepared from (f) PLA-PEG and (g) PEG-PLA-PEG copolymer after sonication. Bar, 100 nm.

functional groups at the end of polymer, resulting in limited applications in the theragnosis and active targeting due to absence of points to modify.

In this study, we developed a tri-block copolymer of PEG-PLA-PEG with a functional group at the end of polymer for nanosized hydrophobic drug carriers efficiently targeted to metastatic tumors in the brain and the bone. Treatment efficacy was monitored in animal model of metastasized triple negative human breast cancer by appending an imaging probe, an improvement over other tri-block copolymers with toxic linkers [19,23,24].

2. Materials and methods

2.1. Materials

PEG (MW 2000), methoxy-PEG (mPEG, MW 2000), L-lactide (3,6-dimethyl-1,4-dioxite-2,5-dione), *N*,*N*-dicyclohexylcarbodiimide (DCC), stannous octoate (Tin(II)2-ethylhexanoate), 4-dimethylaminopyridine (DMAP), dimethylsulfoxide (DMSO), and triethylamine (TEA) were from Sigma-Aldrich (St. Louis, MO, USA). Tetrahydrofuran (THF), acetonitrile (ACN),and dichloromethane (DCM) were purchased from