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Modeling of cancer metastasis and drug resistance *via* biomimetic nano-cilia and microfluidics

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ABSTRACT

Three-dimensional (3D) tissue culture platforms that are capable of mimicking *in vivo* microenvironments to replicate physiological conditions are vital tools in a wide range of cellular and clinical studies. Here, learning from the nature of cilia in lungs – clearing mucus and pathogens from the airway – we develop a 3D culture approach *via* flexible and kinetic copolymer-based chains (nano-cilia) for diminishing cell-to-substrate adhesion. Multicellular spheroids or colonies were tested for 3–7 days in a microenvironment consisting of generated cells with properties of putative cancer stem cells (CSCs). The dynamic and reversible regulation of epithelial–mesenchymal transition (EMT) was examined in spheroids passaged and cultured in copolymer-coated dishes. The expression of CSC markers, including CD44, CD133, and ABCG2, and hypoxia signature, HIF-1α, was significantly upregulated compared to that without the nano-cilia. In addition, these spheroids exhibited chemotherapeutic resistance *in vitro* and acquired enhanced metastatic propensity, as verified from microfluidic chemotaxis assay designed to replicate *in vivo*-like metastasis. The biomimetic nano-cilia approach and microfluidic device may offer new opportunities to establish a rapid and cost-effective platform for the study of anti-cancer therapeutics and CSCs.

1. Introduction

3D tissue cultures have emerged as invaluable cancer models for a wide range of clinical issues that exhibit microenvironmental heterogeneity as well as for tumors *in vivo* [1,2]. Compared with traditional two-dimensional (2D) cell cultures, cells cultured in a 3D manner differ considerably in cellular morphology, mass transport properties, and complex cell–matrix and cell–cell interactions [3,4]. Moreover, the malignant phenotypes of cancer cells in 2D dramatically diminish and the effects of chemotherapeutic drugs or selective inhibitors employed on cell–cell communication, EMT and CSCs are also reduced, whereas the behavior of cells cultured in 3D will respond more closely to *in vivo* conditions [5–9]. Therefore, 3D culture platforms have served as versatile tools to explore fundamental cell biology, tissue engineering, and drug development, thereby increasingly becoming a potential pre-clinical model between traditional cell cultures and *in vivo* experiments – allowing a reduction in whole-animal testing, thereby saving cost [1,10–12].

Various techniques of 3D culture platforms have been established, including the use of plastic plates coated with organic (such as agarose [13] and collagen [14]) or inorganic (such as poly-HEMA [15] and hydrogel [16]) matrices, active control of cell suspension by physical forces [17,18], and layer-by-layer assemblies [19]. These platforms provide a suitable environment for 3D cultures, however, challenges still remain. For example, the matrix coating might interfere with the microscopic imaging and fluorometric assays [20], thus questioning the feasibility of integration with other

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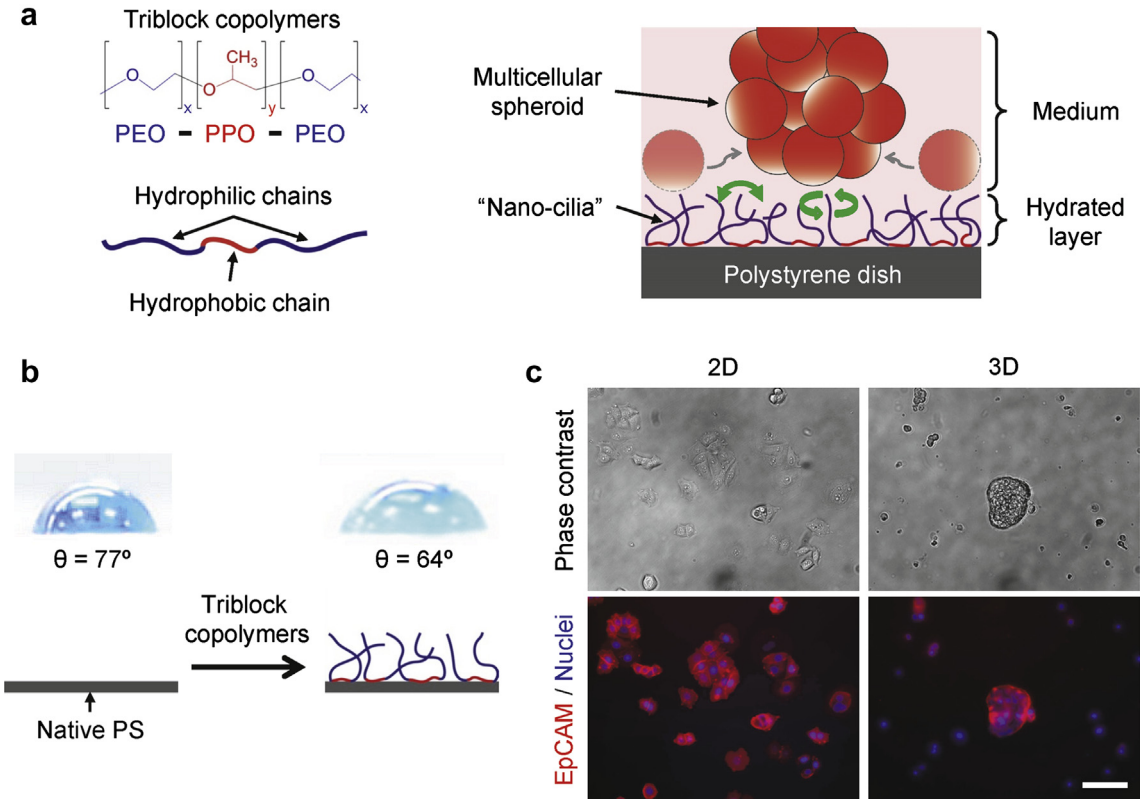

Fig. 1. 3D spheroid culture with triblock copolymer (nano-cilia)-based locomotion. (a) Illustrations show the configuration of the triblock copolymers (nano-cilia) system utilized for 3D multicellular spheroid cultures, in which *x* and *y* indicate the monomer units. Through the hydrophobic–hydrophobic interaction, the hydrophobic PPO chains will bind to the PS surface and beat the hydrophilic PEO chains freely in medium, thereby diminishing the cell-to-substrate adhesion and directing cell–cell interactions to organize a cellular spheroid. (b) DI water drop with blue dye on PS surface before and after treatment with copolymers (1% Pluronic F108). A contact angle change of 13° was observed. (c) Morphology of MCF7 cells cultured in conventional 2D monolayers and 3D spheroids, as shown by phase contrast (top panel) and immunofluorescence (bottom panel; cell adhesion molecule stained with EpCAM, nuclear with Hoechst) images. Scale bar, 100 μm.

technologies, such as micro- and nano-fluidics. In addition, complexity arising from the use of external systems is less attractive than more simplistic approaches. Other challenges include the use of organic matrices or engineered nanoparticles for 3D cultures, which might have certain effects on the cells, both biochemically and physiologically.

Cancer metastasis and drug resistance are important malignant phenotypes of tumors *in vivo* that cause roughly 90% mortality in human-associated cancers [21]. During cancer invasion and metastasis, EMT plays a crucial role and purportedly generates cells with properties of CSCs that reveal self-renewal, tumorigenesis, and drug resistance in malignant tumors [22]. Recent studies have demonstrated that EMT and putative stemness may be induced in cellular spheroids cultured in serum-free medium supplemented with adequate mitogens – such as the basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) – using low-attachment culture dishes [5,23], but this approach might be costly and ineffective. In addition, the population of CSCs can be enriched *via* the selective cell-surface markers, such as $CD44^{+}/CD24^{-/low}$ and CD133 [24,25]. Nevertheless, other studies present that non-stem-like cancer cells can be triggered forcedly or can be stochastically reversed to progenitor/stem-like cancer cells [26,27], suggesting that there is a dynamic regulation between stem and non-stem conditions and further indicating that existing methods based on CSC markers may be unreliable. Consequently, new methods need to be developed, particularly for the study of tumor microenvironment and heterogeneity.

Our recent work has demonstrated that triblock copolymers can direct human ovarian epithelial cancer cell reprogramming and EMT independent of soluble factors [28]. The copolymer mainly contains two separate and hydrophilic PEO chains (approximately 45 nm in length) that kinetically swing themselves freely in solutions and sterically repulse proteins, indicating that this is a biomimetic version resembling the way in which cilia function on lung mucosal epithelia and clear mucus/pathogens out of the airways. Therefore, triblock copolymers may be called biomimetic "nano-cilia" or "molecule cilia" [29]. However, the time and cost by using this method might not be economic. Here, to address the drawbacks and limitations mentioned above, we present a cost-effective and purpose-tailored 3D spheroid culture platform to identify dynamic EMT/MET process and further effectively enrich stem-cell-like/drug resistance cells from a pool of cancer cells. Furthermore, we determine the phenotypic characterization altered by the hypoxia microenvironment in nano-cilia and perform the primary evaluation of feasibility for the study of tumor cell biology.

2. Materials and methods

2.1. Cell lines

Human ovarian cancer cell line SKOV3 (HTB-77, ATCC) was maintained in Dulbecco's modified Eagle medium (DMEM, 31600-034, GIBCO, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (FBS, SV30014, Hyclone, South Logan, UT, USA), 1% penicillin/streptomycin (P/S, 15140, GIBCO), and 1.5 g/L D-(+)-glucose (G5400-250G, SIGMA, St. Louis, MO, USA). Human breast cancer cell line MCF7 (HTB-22, ATCC) was maintained in DMEM/F12 (12400-024, GIBCO), supplemented with 10% FBS and 1% P/S. Panc 02.03B human pancreatic cancer cells (provided from