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Anti-cancer activity of ZnO chips by sustained zinc ion release

Seong-Hee Moon[a,b,1], Won Jin Choi[c,1], Sik-Won Choi[a], Eun Hye Kim[a], Jiyeon Kim[d], Jeong-O Lee[c,*], Seong Hwan Kim[a,*]

[a] Laboratory of Translational Therapeutics, Korea Research Institute of Chemical Technology, Daejeon 305-343, Republic of Korea
[b] Department of Strategy and Planning, Korea Institute of Science and Technology Information, Seoul 130-741, Republic of Korea
[c] Advanced Materials Division, Korea Research Institute of Chemical Technology, Daejeon 305-343, Republic of Korea
[d] Department of Biomedical Laboratory Science, School of Medicine, Eulji University, Daejeon 301-768, Republic of Korea

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ABSTRACT

We report anti-cancer activity of ZnO thin-film-coated chips by sustained release of zinc ions. ZnO chips were fabricated by precisely tuning ZnO thickness using atomic layer deposition, and their potential to release zinc ions relative to the number of deposition cycles was evaluated. ZnO chips exhibited selective cytotoxicity in human B lymphocyte Raji cells while having no effect on human peripheral blood mononuclear cells. Of importance, the half-maximal inhibitory concentration of the ZnO chip on the viability of Raji cells was 121.5 cycles, which was comparable to 65.7 nM of daunorubicin, an anti-cancer drug for leukemia. Molecular analysis of cells treated with ZnO chips revealed that zinc ions released from the chips increased cellular levels of reactive oxygen species, including hydrogen peroxide, which led to the down-regulation of anti-apoptotic molecules (such as HIF-1α, survivin, cIAP-2, claspin, p-53, and XIAP) and caspase-dependent apoptosis. Because the anti-cancer activity of ZnO chips and the mode of action were comparable to those of daunorubicin, the development and optimization of ZnO chips that gradually release zinc ions might have clinical anti-cancer potential. A further understanding of the biological action of ZnO-related products is crucial for designing safe biomaterials with applications in disease treatment.

1. Introduction

Multifunctional zinc oxide (ZnO) has been used in various forms such as nanoparticles (NPs) or nanorods for biomedical applications including biosensing, imaging, drug delivery, and clinical implants [23,31,44,46]. Since several studies have reported the toxicity of ZnO materials, their potential to induce cell death has been explored in cancer biology. The anti-cancer activity of ZnO nanomaterials has been described, and additive or synergistic effects of ZnO NPs with anti-cancer compounds (or drugs) on the induction of apoptosis in cancer cells also have been reported [10,43].

The effects of zinc ions might explain ZnO-induced cytotoxic and apoptotic activity. Zinc ions released from ZnO materials induce oxidative stress-mediated cell death [3,6,30,40], and the strong correlation between ZnO NP-induced cytotoxicity and free zinc ion concentration also suggests a requirement for ZnO dissolution for effective cytotoxicity [36]. Consistently, extracts exhibit more cytotoxicity in suspended cells than do nanostructured ZnO chip coatings *per se* [30]. These findings motivated us to fabricate a ZnO chip that gradually releases zinc ions and to evaluate its anti-cancer activity. The cytotoxicity of the ZnO chip was compared to that of daunorubicin (an anti-cancer drug used to treat leukemia) in human B lymphocyte Raji cells. After using inductively coupled plasma (ICP) atomic emission spectroscopy (AES) to analyze the zinc ion concentration released from the ZnO chip, we evaluated the cytotoxicity of zinc ions released from the chip in Raji cells and human peripheral blood mononuclear cells (PBMCs). In addition, using a cellular peroxidase activity assay, antibody array, and Western blot analysis, we investigated the mechanism underlying the anti-cancer activity of zinc ions.

2. Materials and methods

2.1. Fabrication of the ZnO chip

ZnO chips were fabricated by coating ZnO onto circular glass slides that fit in a 6-well culture plate using an atomic layer deposition (ALD) method. ALD is a thin film-coating method based on the sequential use of a gas-phase chemical reaction. A single cycle

* Corresponding authors.
E-mail addresses: jolee@krict.re.kr (J.-O. Lee), hwan@krict.re.kr (S.H. Kim).
[1] These authors contributed equally to this study.

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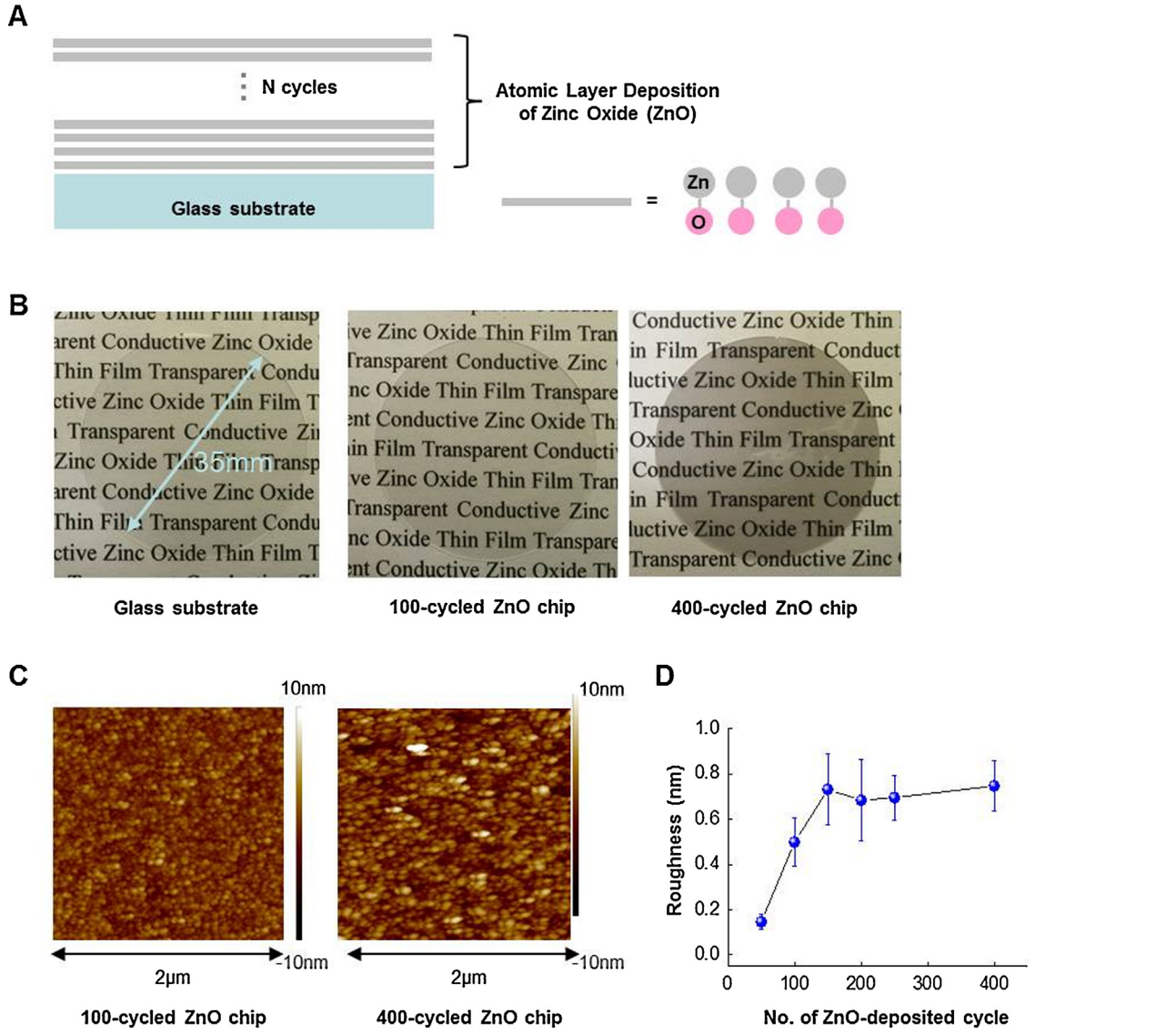

Fig. 1. Growth of ZnO thin films and its characteristics. (A) A schematic depiction of ALD-processed ZnO thin film with different thickness. (B) A 6-well plate-sized glass substrate was used to prepare chips deposited by ZnO thin films. The transparent 100-cycle and 400-cycle ZnO chips are shown. Also shown are topological images (C) and a plot of surface roughness (D), analyzed by AFM, *versus* the number of ALD cycles, respectively.

of ALD consists of a pulse of diethyl zinc (DEZ) followed by a purge process. This step is then followed by a subsequent pulse of water (oxidant) to form a layer of ZnO. The ALD method enables atomic-scale control of the thickness of ZnO thin films, with a chip undergoing 200 cycles of this process characterized as a 200-cycle ZnO chip. ALD was performed in a Lucida D-100 chamber using DEZ (Sigma-Aldrich, MO, USA) with water as the reactant and oxidant, respectively. ALD was carried out under full saturation conditions, with DEZ–purge–water–purge cycles controlled at 0.5 s–10 s–0.1 s–30 s. The deposition temperature was fixed at 150 °C.

2.2. ICP-AES analysis

After the ZnO chip was incubated with cell culture media, the concentration of zinc ions in the media was analyzed using an iCAP 6500 (Thermo Scientific, MA, USA).

2.3. Cell culture

A549 cells and Raji cells were purchased from ATCC (VA, USA) and cultured with DMEM and RPMI-1640 containing 10% heat-inactivated fetal bovine serum and 1% antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) in a humidified atmosphere of 5% CO_2 at 37 °C, respectively. All cell culture materials were purchased from Hyclone (UT, USA). Human PBMCs and culture medium were purchased from Zen-Bio Inc. (NC, USA).

2.4. Apoptosis analysis

Cells (2×10^5 cells/ml) were incubated in the culture plate with the ZnO chip or with daunorubicin (Selleckchem, TX, USA) or the extracts containing zinc ions, for the indicated time. Then, using Muse™ Annexin V and the Dead Cell Assay kit (Millipore, MA, USA), live and apoptotic populations were measured in a Muse™ Cell Analyzer (Millipore).