Ultrasonics Sonochemistry 28 (2016) 1–6

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

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

Short Communication

Sonocatalytic injury of cancer cells attached on the surface of a nickel–titanium dioxide alloy plate

Kazuaki Ninomiya [a], Hirotaka Maruyama [b], Chiaki Ogino [c], Kenji Takahashi [b,*], Nobuaki Shimizu [d,*]

[a] Institute for Frontier Science Initiative, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan
[b] Faculty of Natural System, Institute of Science and Engineering, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan
[c] Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe 657-8501, Japan
[d] Institute of Nature and Environmental Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan

ARTICLE INFO

Article history:
Received 30 April 2015
Received in revised form 18 June 2015
Accepted 18 June 2015
Available online 19 June 2015

Keywords:
Ultrasound
Titanium dioxide
Sonocatalytic reaction
Nickel–titanium alloy plate
Cancer cells

ABSTRACT

The present study demonstrates ultrasound-induced cell injury using a nickel–titanium dioxide (Ni–TiO_2) alloy plate as a sonocatalyst and a cell culture surface. Ultrasound irradiation of cell-free Ni–TiO_2 alloy plates with 1 MHz ultrasound at 0.5 W/cm^2 for 30 s led to an increased generation of hydroxyl (OH) radicals compared to nickel–titanium (Ni–Ti) control alloy plates with and without ultrasound irradiation. When human breast cancer cells (MCF-7 cells) cultured on the Ni–TiO_2 alloy plates were irradiated with 1 MHz ultrasound at 0.5 W/cm^2 for 30 s and then incubated for 48 h, cell density on the alloy plate was reduced to approximately 50% of the controls on the Ni–Ti alloy plates with and without ultrasound irradiation. These results indicate the injury of MCF-7 cells following sonocatalytic OH radical generation by Ni–TiO_2. Further experiments demonstrated cell shrinkage and chromatin condensation after ultrasound irradiation of MCF-7 cells attached on the Ni–TiO_2 alloy plates, indicating induction of apoptosis.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Stents are small, tubular, and self-expandable mesh devices that have been long used to relieve stenosis caused by luminal obstructions because of malignancies in the esophagus, intestines, and bile duct [1,2] and by thrombi in blood vessels. Stents are generally made from a flexible mesh material, such as stainless steel or nitinol (alloy of nickel and titanium, and are called as bare-metal stents. After stent insertion, in-stent restenosis (reoccurrence of stenosis) is a common adverse event that caused by the proliferation of cancer cells. To ameliorate in-stent restenosis, drug-eluting stents have recently been introduced, comprising bare-metal stents coated with a matrix containing antiproliferative drugs that prevent cancer cell growth. However, drug release is exhausted within several months, and the antiproliferative effects are lost [2].

Ultrasound has been commonly used for medical diagnosis because of its ability to penetrate tissues with low energy attenuation. Ultrasound also has therapeutic uses, typically in cancer therapy. These include hyperthermic cancer therapy based on the thermal effects of high-intensity focused ultrasound [3] and sonodynamic cancer therapy based on the sonocatalytic effects of a combination of low-intensity ultrasound and a sonosensitizer which involves preferential uptake and/or accumulation in tumor tissues and subsequent activation by ultrasound irradiation [4]. Umemura et al. [5] suggested the sonodynamic therapy for cancer cells using hematoporphyrin, which was formally used as a photosensitizer for photodynamic therapy [6]. However, the clinical application of photodynamic therapy is limited to cancers in surface regions because of the inability of photo energy to penetrate deep tissues.

Titanium dioxide (TiO_2) is a photocatalyst that generates reactive oxygen species (ROS) under ultraviolet irradiation [7,8]. The ensuing ROS have been exploited to kill cancer cells [8,9], degrade harmful chemicals, and inactivate microorganisms. However, PDT remains limited to the near-surface applications of TiO_2 photocatalysts.

Previously, we showed that TiO_2 can act as a sonocatalyst that produces hydroxyl (OH) radicals under conditions of ultrasound irradiation without ultraviolet irradiation [10]. The sonocatalytic effects of TiO_2 particles have been applied to the degradation of specific types of chemicals [11], and to the inactivation of microorganisms [12–16]. To apply the sonocatalytic effects of TiO_2 nanoparticles to cancer cells, we recently demonstrated the sonocatalytic cancer cell injury using surface-modified TiO_2 nanoparticles which specifically binds to cancer cells. Subsequent sonocatalytic activation generated OH radicals and caused cell death [17–20].

* Corresponding authors.
E-mail addresses: ktkenji@staff.kanazawa-u.ac.jp (K. Takahashi), nshimizu@staff.kanazawa-u.ac.jp (N. Shimizu).

http://dx.doi.org/10.1016/j.ultsonch.2015.06.015
1350-4177/© 2015 Elsevier B.V. All rights reserved.

However, to the best of our knowledge, no previous studies have assessed sonocatalytic injury of cancer cells attached on the surface of a TiO_2 plate.

Therefore, the present study demonstrates the sonocatalytic cancer cell injury using nickel–titanium dioxide (Ni–TiO_2) alloy plates as both sonocatalyst and cell-culture surface. This is the first report to demonstrate a sonocatalytic injury of cancer cell cultured on Ni–TiO_2 plate. The present data contribute to the development of non-invasive bedside treatments for in-stent restenosis using combinations of low-intensity ultrasound and Ni–TiO_2 stents.

2. Material and methods

2.1. Ni–TiO_2 alloy plates

Experiments were performed using Ni–TiO_2 and nickel–titanium (Ni–Ti) alloy plates of 10 mm × 8 mm (ACTMENT Co., Ltd, Kasukabe, Japan) as catalysts and controls, respectively. Ni–TiO_2 alloy plates were prepared by direct oxidation of Ni–Ti alloy plates and were immersed in an aqueous solution containing 10% (w/w) hydrogen fluoride. Subsequently, acid-cleaned substrates were washed out using sufficient amounts of water and were then immersed in a solution of hydrogen peroxide (10%) for two days. The Ni–Ti alloy plates were then subjected to direct anodic oxidation in electrolyte and atmospheric oxygen twice, as described previously [21]. The specimens were cleaned ultrasonically and sterilized using a steam autoclave at 121 °C for 15 min prior to use.

Terephthalic acid (TA) and 2-hydroxyterephtalic acid (2HTA) were purchased from Nakalai Tesque (Kyoto, Japan). All other chemicals were of reagent grade.

2.2. Cell cultures on Ni–TiO_2 alloy plate

Human MCF-7 breast cancer cells, purchased from the RIKEN Cell Bank (Tsukuba, Japan), were used as model cancer cells which show adhesive property to Ni–Ti and Ni–TiO_2 plates. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Nakalai Tesque, Kyoto, Japan) supplemented with 10% (v/v) fetal bovine serum (FBS; Life Technologies Corporation, Carlsbad, CA, USA). The culture media were supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B (Nakalai Tesque), and the cells were maintained at 37 °C in a 5% CO_2 atmosphere.

The cells were harvested using a solution containing 2.5 g/l trypsin and 1 mM EDTA (Nakalai Tesque), and viable cell numbers were determined by manual counting using a hemocytometer and trypan blue exclusion. Cell culture on the Ni–TiO_2 and Ni–Ti alloy plates was performed after suspension of 8×10^4 cells in 1 ml of culture medium and inoculation onto sterilized Ni–TiO_2 or Ni–Ti alloy plates in 24-well microtiter plates (well area, 180 mm^2). After incubation for 24 h, the attached cells were washed twice with Dulbecco's phosphate-buffered saline without calcium and magnesium (D-PBS(−)) and were then used in experiments.

2.3. Ultrasound irradiation of Ni–TiO_2 alloy plates

Fig. 1 shows the experimental setup for the assay of sonocatalytic OH radical generation and injury of cancer cells on Ni–TiO_2 and Ni–Ti alloy plates. Briefly, Ni–TiO_2 or Ni–Ti alloy plates were set inside 35 mm culture dishes on wires that maintained the space between the alloy plate and the bottom of the culture dish. Assays of OH radical generation were performed by setting cell-free Ni–TiO_2 or Ni–Ti alloy plates in 35 mm culture dishes containing 2 mL of 3 mM TA solution in aqueous NaOH (7.5 mM). While, assays of cancer cell injury were performed using

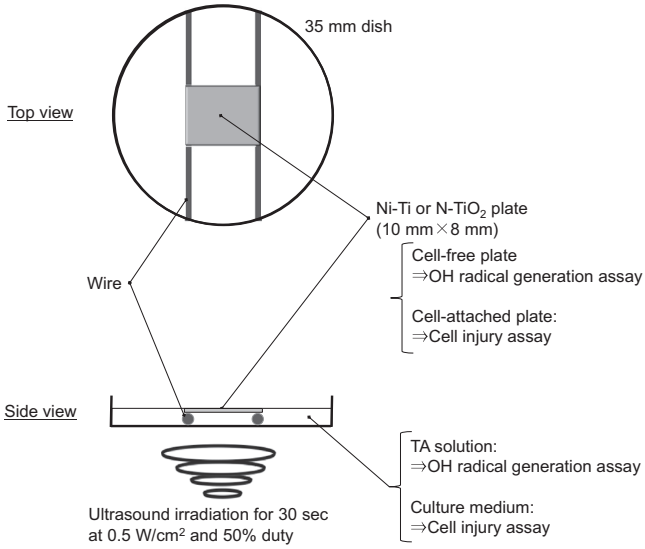

Fig. 1. Experimental setup for sonocatalytic generation of OH radicals and injury of cancer cells on Ni–TiO_2 alloy plates.

cell-attached Ni–TiO_2 or Ni–Ti alloy plates that were set in 35 mm culture dishes containing 2 mL of culture medium with the cells facing down. After covering the surface of the transducer with 3 ml of water, culture dishes were directly placed on the transducer component of the ultrasonic apparatus (Sonic Master ES-2, OG Giken Co. Ltd, Okayama, Japan) in the dark. Ultrasound irradiation was applied from the bottoms of the dishes using the following conditions: frequency, 1 MHz; duty ratio, 50%; output power: 0.5 W/cm^2; and irradiation time, 30 s, gaseous condition: ambient air condition. This ultrasonic irradiation condition was set considering our previous study of sonocatalytic cancer cell injury using TiO_2 particle [18,20]. During ultrasonic irradiation, temperature increase of liquid was 3 °C (from 23 °C to 26 °C), and no standing wave formation. After ultrasound irradiation, OH radical generation in TA solutions and injury of cancer cells on the alloy plates were analyzed.

2.4. Analysis of OH radical generation

OH radical generation was evaluated using TA, which reacts with OH radicals to generate 2HTA with stable fluorescence [22,23]. Fluorescence signals of 2HTA following the reaction of TA with OH radicals were measured using a microplate spectrofluorometer (Gemini XPS, Molecular Devices, Sunnyvale, CA, USA) at excitation and emission wavelengths of 315 nm and 425 nm, respectively. Fluorescent signals were converted to concentrations using an authentic 2HTA external standard and were calibrated using a series of 2HTA dilutions (0.1–100 μM) in NaOH solution.

2.5. Analysis of cell attachment on Ni–TiO_2 alloy plates

The numbers of viable cancer cells on Ni–TiO_2 and Ni–Ti alloy plates were determined using scanning electron microscopy (SEM) images or ATP assays. Subsequently, cell density was determined as the number of viable cells per unit area of the alloy plate.

For the SEM-based measurement, the number of cells on the alloy plate was directly determined by manually counting on the acquired image (Fig. S1 is a sample of low magnification SEM image used for cell counting). Specifically, cell attached Ni–TiO_2 and Ni–Ti alloy plates were then washed twice with D-PBS(−), and fixed in 2.5% glutaraldehyde in D-PBS(−) for 15 min at room temperature (RT). Samples were then washed three times using D-PBS(−), and dehydrated in ethanol solutions of increasing

Download English Version:

https://daneshyari.com/en/article/1266581

Download Persian Version:

https://daneshyari.com/article/1266581

Daneshyari.com