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Toxicity study of reclaimed water on human embryonic kidney cells

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HIGHLIGHTS

- Twenty-five micropollutants were found at the ng L^{-1} level in water samples.
- Wastewater influent (WTI), wastewater effluent (WTE), and MBR permeate (MBRP) have cyotoxic effects on HEK293 cells.
- WTI and WTE significantly decreased the number of HEK293 cells.
- WTI, WTE, and MBRP induced molecular toxicity through affecting expressions of cell cycle regulatory proteins.
- WTI, WTE, and MBRP induced molecular toxicity through affecting expressions of cell apoptosis-related regulatory proteins.

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ABSTRACT

The importance of evaluating the toxic effects associated with the use of reclaimed water has been increasing. The purpose of this research was to investigate the cytotoxicity and molecular toxicity of reclaimed water on the human embryonic kidney 293 (HEK293) cells. The culture medium was synthesized using the reclaimed water samples. Wastewater treatment plant influent (WTI) and effluent (WTE), containing micropollutants at the nanogram per liter level, decreased cell proliferation (93.4–98.9% and 91.5–96.6% of the control, respectively) and increased cell damage (103.6–117.5% and 100.7–109% of the control, respectively) at all exposure times, except for a decrease in cell damage observed after an 8-h exposure to WTE. Membrane bioreactor permeate (MBRP) increased cell proliferation (102.1–106.7% of the control) and decreased cell damage at 8 and 12 h (92.4 and 98.4% of the control, respectively), but slightly increased cell damage at 24 h and later time points (101.1–104.9% of the control). All three water samples induced cell apoptosis (120.9–123.4% of the control). They also affected the expression of cell-cycle regulatory proteins ($p16^{INK4a}$, $p27^{Kip1}$, cyclin-dependent kinases 2 and 4, cyclin D1, and cyclin E) and apoptosis-related regulatory proteins (*p*-JNK, Bcl-2, caspase-9, and caspase-3). In conclusion, all three water samples showed cytotoxicity and molecular toxicity in the HEK293 cells, and the results of the cell-cycle and apoptosis regulatory protein expression after WTI and WTE treatments were consistent with the results of the cytotoxicity.

1. Introduction

Wastewater reclamation and reuse are broadly considered as the means to overcome worldwide water scarcity (Asano et al., 2007). Potentially, for being used as river recharge water, agriculture water, middle water, and even drinking water, reclaimed water for different uses can be obtained through different treatment processes. However, it still contains many kinds of micropollutants, such as pharmaceuticals, endocrine disruptors (EDCs), and other chemicals (Kay et al., 2017; Padhye et al., 2014; Salgado et al., 2011; Sim et al., 2010; Ternes et al., 2007), owing to the difficulties faced during wastewater treatment (Andresen and Bester, 2006; Kim et al., 2007a; Li et al., 2015; Rodriguez-Mozaz et al., 2015; Wu et al., 2012; Yang et al., 2011). Therefore, an interest in understanding the potential human health effects associated with the

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direct and/or indirect use of reclaimed water containing known and unknown micropollutants has increased rapidly (Chen et al., 2013; Daughton and Ternes, 1999; Gee et al., 2015; Jia et al., 2015; Mehinto et al., 2015; Schwab et al., 2005).

Most risk assessment studies for reclaimed water have focused on the human health effects of a single micropollutant or a mixture of micropollutants, using different experimental models. Föllmann and Wober (2006) studied the cytotoxic, genotoxic, mutagenic, and estrogenic effects of two fire retardants on the V79 hamster fibroblast cells and human endometrial cancer Ishikawa cells. Pomati et al. (2006) investigated the cytotoxic effects of a mixture of 13 micropollutants at their environmental concentrations on the HEK293 cells. For sensitive detection of the cytotoxic and genotoxic effects of anticancer drugs, Novak et al. (2017) used the zebrafish liver (ZFL) cell line. Ren et al. (2008, 2009) developed a micropollutant-sensitive cellular model consisting of the primary cultured rabbit renal proximal tubule cells and studied the expression of cell-cycle regulatory proteins and transporters. They successfully detected subtle effects of micropollutants at their environmental concentrations on cytotoxicity, DNA synthesis, protein expression, and ion uptake. However, these cellular models were developed to investigate the effects of a single or multiple compounds recovered from wastewater or reclaimed water, and not to investigate the toxic effects of the water samples themselves.

Some studies have investigated the potential effects of wastewater and reclaimed water on human cells. Žegura et al. (2009) investigated the cytotoxic and genotoxic effects of wastewater, surface water, and drinking water samples using the human hepatoma cells (HepG2). Ragazzo et al. (2017) and Shi et al. (2009) also studied the toxic effects of wastewater and disinfected water using the HepG2 cells. Friha et al. (2015) made progress in the cytotoxic and stress response disruption study of textile wastewater influent and MBRP using the human Caco-2 cells. Etteieb et al. (2015) and Leush et al. (2014a) assessed cytotoxicity and estrogenicity of treated wastewater on the Caco-2 and MCF-7 cells. Leush et al. (2014b), in a separate study, performed 13 in vitro assays involving reclaimed water, drinking water, and rainwater. In a study by Escher et al. (2013), 20 laboratories applied 103 unique cell-based bioassays to reclaimed water, stormwater, surface water, and drinking water samples. Leusch and Snyder (2015) summarized the bioanalytical tools for assessing the recycled water quality. Although these methods are useful for detecting the toxic effects of water samples, they still have some limitations because the toxicity of diluted water samples may be different from that of the original undiluted samples.

In this study, we developed a new method to detect the cytotoxic effects of wastewater and reclaimed water without diluting the water samples. In this method, the cell culture medium was prepared by dissolving the nutrient medium (powdered form) in the water samples to be tested. We also assessed the molecular toxicity of wastewater and reclaimed water using two typical indicators of cell function: cell-cycle and apoptosis regulatory protein expression (Nurse, 1994; Nuñez et al., 1998). HEK293 cells, a representative human embryonic cell line, were selected for toxicological testing (Yu et al., 2001; Pomati et al., 2006; Zhang et al., 2015). Thus, the purpose of this study was to investigate the toxic effects of undiluted samples of WTI, WTE, and MBRP on human cells that were cultured on media directly prepared from the water samples to be tested.

2. Materials and methods

2.1. Water samples

Four water samples, namely WTI, WTE, MBRP, and reverse osmosis (RO) permeate (control), were collected from different water sources. The raw domestic wastewater and secondary effluent were obtained from a wastewater treatment plant (A2/O process). MBRP was collected from a lab-scale MFMBR system (module type, flat), where the feed wastewater was the same as the influent of the wastewater treatment plant. The RO permeate was used as the control water sample, and obtained from a lab-scale reverse osmosis system (module type, spiral wound), where the influent of RO permeate system was tap water.

2.2. Analysis of water quality

Solid chemical oxygen demand (SCOD) was quantified according to the standard methods (APHA, 1998), and turbidity was measured using a turbidimeter (Thermo Fisher Scientific, USA). The ammonium (NH_4^+), phosphate (PO_4^{3-}), fluoride (F^-), chloride (Cl^-), sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}) ions were analyzed using Dionex LC 20 chromatography (Dionex Corporation, USA).

Thirty-one typical micropollutants were monitored in the autoclaved wastewater and reclaimed water samples, and their concentrations have been shown in Table S1. The concentrations of the 31 micropollutants from the non-autoclaved wastewater and reclaimed water samples have been shown in Table S2. Analytes were extracted using the hydrophilic balance glass cartridges from Waters Corporation (Milford, MA, USA). All compounds were extracted by solid-phase extraction and analyzed using either liquid or gas chromatography coupled with tandem mass spectrometry, according to the previously published methods (Trenholm et al., 2006; Vanderford et al., 2003; Vanderford and Snyder, 2006).

2.3. Cell culture

The HEK293 cells were purchased from the American Type Culture Collection (ATCC CRL-1573). For routine culturing and subculturing of these cells, a standard culture medium, namely Dulbecco's modified Eagle's medium (DMEM; Invitrogen, USA), supplemented with 10% fetal bovine serum (FBS; Invitrogen, USA) was used. The cells were maintained in a CO_2 incubator at 37 °C with 5% CO_2. Next, they were washed with Dulbecco's phosphate-buffered saline (PBS; Invitrogen, USA) solution and harvested using trypsin (Invitrogen, USA).

For evaluating the toxicity of water samples, four culture media were prepared using WTI, WTE, MBRP, and the control water samples, under the same conditions. DMEM powder (Invitrogen, USA) and sodium bicarbonate (Sigma–Aldrich, USA) were dissolved in each water sample and the pH was adjusted to 7.4 using HCl or NaOH. Next, the media were autoclaved at 121 °C and 0.1 MPa for 15 min. Finally, 5 mL of horse serum (Invitrogen, USA) was added to 500 mL of each autoclaved medium.

2.4. Antibodies

The primary antibodies for cyclin-dependent kinase 4 (CDK4), cyclin-dependent kinase 2 (CDK2), cyclin D1, cyclin E, $p16^{INK4a}$, $p27^{Kip1}$, Bcl-2, caspase-3, caspase-9, and β-actin were procured from Santa Cruz Biotechnology (USA). The primary antibodies for total-JNK and phospho-JNK were purchased from Cell Signaling Technology (USA). Goat anti-mouse IgG, used as the secondary antibody, was procured from Santa Cruz Biotechnology (USA).

2.5. Sulforhodamine B (SRB) assay

Cell proliferation was assessed using the SRB assay. The