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Comparison of the toxicity of sintered and unsintered indium-tin oxide particles in murine macrophage and epidermal cells

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ABSTRACT

Indium-tin oxide (ITO) is used to produce flat panel displays and several other technology products. Composed of 90% indium oxide (In_2O_3) and 10% tin oxide (SnO_2) by weight, ITO is synthesized under conditions of high heat via a process known as sintering. Indium lung disease, a recently recognized occupational illness, is characterized by pulmonary alveolar proteinosis, fibrosis, and emphysema. Murine macrophage (RAW 264.7) and epidermal (JB6) cells stably transfected with AP-1 to study tumor promoting potential, were used to differentiate between the toxicological profiles of sintered ITO (SITO) and unsintered mixture (UITO). We hypothesized that sintering would play a key role in free radical generation and cytotoxicity. Exposure of cells to both UITO and SITO caused a time and dose dependent decrease of the viability of cells. Intracellular ROS generation was inversely related to the dose of both UITO and SITO, a direct reflection of the decreased number of viable RAW 264.7 and JB6/AP-1 cells observed at higher concentrations. Electron spin resonance showed significantly increased hydroxyl radical (•OH) generation in cells exposed to UITO compared to SITO. This is different from LDH release, which showed that SITO caused significantly increased damage to the cell membrane compared to UITO. Lastly, the JB6/AP-1 cell line did not show activation of the AP-1 pathway. Our results highlight both the differences in the mechanisms of cytotoxicity and the consistent adverse effects associated with UITO and SITO exposure.

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1. Introduction

Indium-tin oxide (ITO) thin films are widely used in the electronics industry for the production of transparent electrodes for liquid crystal display screens, along with flat panel displays, alkaline batteries, and various other technological applications. With unique properties such as high electrical conductivity and transparency, ITO is in high demand. Composed of 90% indium oxide (In_2O_3) and 10% tin oxide (SnO_2) by weight, ITO is synthesized under conditions of high heat via a process known as sintering. During this process, loose metal powders are bonded together first through local bonding between adjacent particles, followed by a later stage of pore-rounding and shrinking, ultimately transforming into solids at temperatures below their melting point (Hoganas, 2013; Lison et al., 2009).

With the increase in ITO demand and production in recent years, indium lung disease has been recognized amongst exposed workers (Nakano et al., 2016; Nakano et al., 2015). In 2003, the first case of indium-associated pulmonary toxicity was reported in Japan, one of the world's largest manufacturers of ITO. The individual was diagnosed with interstitial pneumonia, where histopathological analysis of the lung biopsy revealed alveolar spaces filled with red blood cells, fibrin, cholesterol clefts, alveolar macrophages, and numerous fine particles, consistent with ITO particle inhalation (Homma et al., 2003). Amongst workers with indium lung disease, other reported clinical features include cough, fibrosis, abnormal pulmonary function tests, pulmonary alveolar proteinosis (PAP), and emphysematous changes (Nakano et al., 2016; Cummings et al., 2012).

In cultured murine macrophage (RAW 264.7) and human bronchial epithelial cells (BEAS-2B), indium compounds have been shown to induce a pro-inflammatory response via activation of the nucleotide oligomerization domain-like receptor protein 3 inflammasome (NLRP3), which has been implicated in the development of pulmonary fibrosis (Badding et al., 2015; Biswas et al., 2011). In addition, rats exposed to SITO have exhibited pulmonary alveolar proteinosis with the presence indium in the blood plasma used as a marker of exposure (Badding et al., 2016). Various studies have also suggested that the observed toxicities associated with indium compounds, can in part be attributed to their ability to generate reactive oxygen species (ROS). Indium is capable of interacting with hydrogen peroxide (H_2O_2) via Fenton-like reactions to produce •OH, with the downstream effects being nucleic acid

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damage, lipid peroxidation and activation of programmed cell death pathways (Lison et al., 2009; Leonard et al., 2004; Jomova and Valko, 2011; Badding et al., 2014).

Previous work done by Leonard et al. has utilized ITO which had been in contact with metalworking fluid (MWF). It is not unusual for MWF, often used to reduce heat and friction and to remove metal particles in machining and grinding operations, to be contaminated with gram negative bacteria (Badding et al., 2016, 2014). Work-related asthma, hypersensitivity pneumonitis, chronic bronchitis and impaired lung function have all been associated with occupational exposure to MWF (Gilbert et al., 2010).

The ITO collected and used in the current work has never been in contact with MWF. Though the main goal of this study was to ascertain the differences between UITO and SITO in cells, we were also interested in whether or not contact with MWF played a role in the toxicities associated with ITO previously observed in murine macrophage RAW 264.7 cells, in which the ITO was baked to de-activate the endotoxin (Badding et al., 2016). The murine epidermal JB6 cell line, stably transfected with the AP-1 luciferase plasmid, was used to study the tumor promoting potential of ITO, since only limited evidence exists about its carcinogenic properties (Nagano et al., 2011).

In addition, though inhalation exposure to ITO is the primary cause for concern in relation to the development of pulmonary alveolar proteinosis, the potential toxicities associated with dermal exposure still need to be investigated. The Centers for Disease Control estimates that >13 million workers in the United States are occupationally exposed to >82,000 chemicals that can be absorbed through the skin (U.S.G.A.O, 2005). Studies done in mice have shown that topical exposure to UITO does in fact cause immune stimulation and has the potential to penetrate both intact and breached skin (Brock et al., 2014). With occupational exposure to indium on the rise (Tolcin, 2016; Hines et al., 2013), a better understanding ITO toxicity is needed to allow for improved worker health and safety.

2. Materials and methods

2.1. Indium collection

The indium used in this study was collected from a United States ITO production facility from production processes (NIOSH, 2012). In contrast to both SITO and UITO particles used in previous studies in our lab, ITO particles in this study were never exposed to MWF.

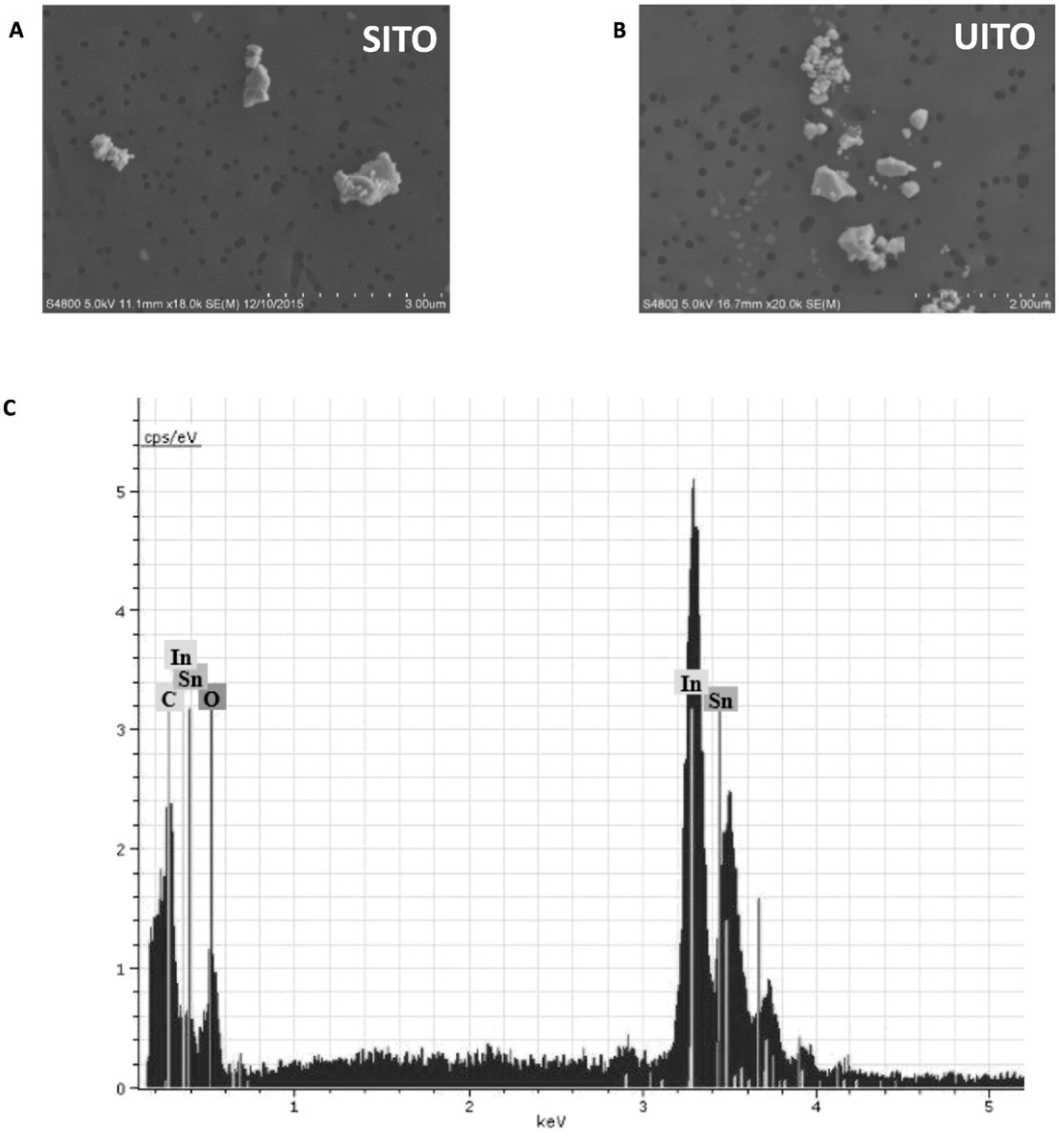

Fig. 1. Electron Micrsoscopy and Elemental Analysis of SITO and UITO. Images obtained from field emission scanning electron microscopy (FE-SEM) confirm that both SITO (A) and UITO (B) particles were <5 μm in diameter. Images were aquired using 20,000× magnification using a 5.0 kV accelerating voltage. (C) Representative elemental analysis for both SITO (pictured) and UITO particles confirm the presence of indium, tin, carbon and oxygen.