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In-Vitro Human Cell Experimental Models in Heavy Metal Research

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Abstract: The study of heavy metal toxicity within the field of toxicology presents challenges due to the significant detrimental impact that even tiny quantities of these metals may have on living organisms. Hence, researchers need to choose an appropriate experimental model, that is safe, meets the needs of the experiment and most closely reflects the real toxicity effects on living things. This review focusses on the utilisation of *in-vitro* experimental models involving human cells to investigate the toxic effects of heavy metals. Specifically, it examines the cellular mechanisms, responses, oxidative stress, and genotoxicities associated with heavy metal exposure. The aforementioned discoveries not only contribute to the understanding of the adverse health effects associated with excessive heavy metal exposure but also hold significant promise for developing targeted and efficient strategies to safeguard human and environmental health.

Key words: Human cell models, heavy metal, heavy metal research.

Introduction

Heavy metals are common environmental pollutants that come from both natural and human-made sources and can be found spread widely in the environment. In recent years, there has been an increase in ecological and global public health concerns about these metals causing environmental contamination. In addition, human exposure has increased because of the wide usage of heavy metals in numerous industrial, agricultural, residential, and technical applications (Bradl, 2002). Their toxic effects affect a spectrum of organ systems and cellular processes, ranging from neurotoxicity to genotoxicity. In order to gain a comprehensive understanding of the complexities associated with heavy metal-induced toxicity and to develop effective preventive and therapeutic strategies, researchers use *in-vitro* experimental models to isolate and analyse specific cellular responses to heavy metal exposure.

Within the field of heavy metal research, the use of *in-vitro* experimental models has been highly important in understanding the complicated nature of heavy metal toxicity. These models serve as a crucial link between the controlled laboratory setting and the actual effects of heavy metal exposure on human health. *In-vitro* human cell experimental models involve the culture and eventual manipulation of human-derived cells or tissues in laboratory settings, providing a suitable controlled

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and ethical platform for investigating these mechanisms and effects of heavy metal toxicity and the potential therapies against them.

This review offers a concise analysis of the efficacy and benefits of different *in-vitro* experimental models in the field of heavy metal research. It presents a comprehensive summary of presently employed for *in-vitro* cytotoxicity studies and delves into broader applications for these methods, which have the potential to revolutionise heavy metal exposure risk assessment and treatment strategies.

Experimental Models

Before conducting a research project, it is very important to determine the experimental model that is appropriate to the study to be conducted. The main purpose of employing an experimental model is to acquire knowledge about the underlying principles that regulate the behaviour of intricate and typically inaccessible complex cellular systems. By breaking down these complex systems into simpler, easier-to-handle parts, researchers can find different factors, change certain situations, and closely look at the outcomes of their scientific studies. The utilisation of this regulated methodology enables a methodical examination of the issue, verification of relevant hypotheses, and the compilation of experimental data, all of which collectively enhances comprehension of the biological system being studied. Experimental models are utilised in the field of biology and clinical research to examine processes believed to be implicated in human diseases, as well as to assess and then choose potential drug candidates that can impact these processes. This is done with the aim of progression their advancement into clinical trials (Fereira et al., 2005). Experimental models can be classified into four distinct categories, namely *in-vivo*, *ex-vivo*, *in-vitro* and *in-silico* studies. Each of these categories has a unique and significant function in scientific research. These terms refer to the different methods used in biological and medical study, including looking at living things in their natural state (*in-vivo* and *ex-vivo*), the analysis of isolated cells and tissues (*in-vitro*), and the utilisation of computing models (*in-silico*) (Gan et al., 2022). Different approaches possess particular and distinct strengths and limits, thereby prompting researchers to select the best suitable methodology per their research objectives and the characteristics of the phenomenon under investigation. A description of each of these models is summarised in Table 1.

In-vitro Experimental Model

For the human cell experimental model, the most common model is the *in-vitro* model which will be discussed in great detail below. The term "*in-vitro*" originates from a Latin phrase and corresponds to "in the glass". It is used in the context of scientific research to refer to experiments or studies conducted outside of a living organism, typically in a controlled laboratory setting (Vinken, 2020). Cell culture refers to the methods employed for culturing human, animal, or insect living cells within a controlled artificial setting to encourage their growth, such as using a petri dish or culture flask (Verma et al., 2020). The cells utilised for these cultures are commonly obtained from either multicellular eukaryotes, pre-existing cell lines, or established cell strains (Segeritz & Vallier, 2017; Verma et al., 2020). This represents the core characteristics of an *in-vitro* experimental model. Experimental models conducted *in-vitro* commonly utilise distinct cell lines that are derived from either human or non-human sources, encompassing both normal and pathological tissue types which are identified and propagated for experimental use.

In-vitro models are extensively utilised across a multitude of scientific disciplines, including but not limited to biology, pharmacology, toxicology, and medical research. Under specific conditions, cells do have the capacity to proliferate outside their original organ or tissue. This is achieved by providing them with a medium that contains nutrients and growth supplementations, as well as mimicking their *in-vivo* parameters in terms of temperature, pH, CO_2, O_2, osmolality, and nutrition (Arango et al., 2000; Verma et al., 2020). Furthermore, these cultured cells necessitate the presence of anaseptic setting together with the continued provision of nutrients for their proliferation and the maintenance of optimal incubation conditions. Laboratories offer a regulated and isolated setting in which to investigate biological processes, cellular reactions, and the impacts of different chemicals or interventions on cells and tissues. Cell culture models are a distinct subgroup of *in-vitro* models that concentrate on the cultivation and manipulation of cells inside a controlled setting to examine cellular behaviour, functions, and reactions (Yao & Asayama, 2017). The diverse advantages of these cell culture systems are as follows:

(a) They are developed for use in a controlled testing environment and are less effective when exposed to systemic influences from an open environment.

Table 1: Types of experimental models

Model	*Phrase meaning*	*Experimental details*	*Examples*
In-vivo	Within the living	• Tests are carried out inside a living body, such as a human subject, an animal subject, or a plant subject. • Academic researchers The biological process was examined within the framework of the complete organism, enabling the exploration of interactions and responses that take place inside a living system • Normally require ethical consideration	• Clinical trials • Animal testing • Observances of flora in their native habitat.
Ex-vivo	Out of the living	• The practise of conducting experiments or measurements on tissues, cells or organs within an artificial environment, while minimising any alterations to the natural conditions of the organism. • Tissue/cells/organ were placed in controlled sterile condition up to 24 hours	• Organ transplantation • Tissue biopsies
In-vitro	In glass	• Pertains to scientific investigations that are carried out only within a controlled laboratory setting, typically involving the use of isolated cells, tissues, or biological substances. • *In-vitro* investigations are frequently employed to scrutinise distinct cellular or molecular mechanisms within a regulated environment. • The conducted experiments do not encompass the utilisation of a living organism.	• Cell culture experiment • Enzyme assays • Molecular analysis including polymerase chain reaction and genome sequencing
In-silico	Derived from the term silicon	• With respect to computer-based programs, simulations and modelling to forecast or replicate biological occurrences. • This methodology offers notable benefits for investigating complex systems while minimising practical and ethical concerns.	• Bioinformatics • Simulated testing of new pharmaceuticals • Computational biology

(b) Their cellular standardisation has the potential to reduce variability in different experimental settings.
(c) The dose range being studied can be easily assessed using a variety of test methods that could include different cellular and tissue models at the same time.
(d) Time-dependent studies can be carried out, enabling prompt and regulated investigation of samples.
(e) Cell culture testing procedures are characterised by their efficiency and affordability, with the usage of a minimal quantity of test material and the waste being generated.
(f) *In-vitro* techniques have the capacity to be conducted utilising human cells and tissues including transgenic cells, resulting in minimal use of laboratory animals for experimental studies (Takhar & Mahant, 2011)

Cell Cultures in Heavy Metal Research

The application of the cell culture technique has played a crucial role in studying the fundamental mechanisms and effects of heavy metal toxicity in various types of cells. The field of cell culture research has surpassed conventional experimental models, enabling more ethical investigation of heavy metal toxicity with minimal harm being inflicted upon living beings. This approach has resulted in crucial insights into the mechanisms of heavy metal toxicity, the regulation of cellular responses and homeostasis, induction of oxidative stress, as well as genotoxic responses and regulation both prompt and delayed that are being observed and measured. The information obtained from cell culture studies not only enhances our comprehension of heavy metal toxicity at the cellular and molecular levels but also influences public health understanding and interventions designed to protect human and environmental health from the harmful consequences of environmental heavy metal contamination.

In-vitro Mechanisms of Heavy Metal Toxicity

The utilisation of cell culture research has played a pivotal role in elucidating the fundamental pathways associated with heavy metal toxicity. Choi et al. (2018)

conducted a study to examine the cumulative harmful impact of airborne particulate matter which contains heavy metals, specifically lead, arsenic, and nickel, on the A549 human lung cell line. According to the analysis of this experiment, the metal with the most toxicity, shown by its low IC_{50} value, representing acute lethal concentration, was nickel. In a previous investigation conducted by Rossi et al. (1996) the Human Intestinal Cell Line CaCo-2 (CaCo-2), which originates from a human colon carcinoma specimen, was employed to assess the potential toxicity of cadmium, zinc and copper in human cellular tissue.

In a study conducted by Al-Ghafari et al. (2019), the authors investigated the immediate impact of lead and cadmium on osteoblasts in human bone, which is recognised as a significant location for heavy metal accumulation that can result in eventual potential harm. The results of this investigation revealed that osteoblasts exhibited considerable cytotoxicity when exposed to minute concentrations of 0.1 μM of lead and cadmium. Furthermore, it was observed that cadmium demonstrated a higher level of cytotoxicity compared to lead, as seen by its lower IC_{50} values at all examined time points in this study.

Cellular Responses of Heavy Metal Toxicity

Different cell types are known to respond differently to heavy metal exposure. Thus cell culture experiments can allow researchers to study specific cellular responses in isolation. In a study conducted by Rossi et al. (1996), it was demonstrated that metals such as cadmium, copper, and zinc exhibited greater toxicity when administered to the basolateral side of the cells compared to the apical side. This finding implies a potentially hazardous effect on intestinal cells from the systemic circulation because of this spatial arrangement of cells in human tissue. Various types of poisoning may be relevant in situations where intoxication occurs by inhalation. Previously, this research group demonstrated that treating $CaCo_2$ cells with 400 μM of zinc sulphate and copper sulphate resulted in an enhanced accumulation of mRNA for metallothionein. Metallothionein is a protein that plays a crucial role in maintaining heavy metal homeostasis and facilitating cell differentiation. Research conducted by Scarino et al. (1992) has shown that heavy metals can inhibit protein synthesis in $CaCo_2$ cells, with a more significant impact being seen in undifferentiated cells as opposed to differentiated cells.

The cytotoxicity of lead and cadmium towards human osteoblast cells is accompanied by a significant decline in mitochondrial membrane potential, as well as reductions in the activities of mitochondrial complex I and mitochondrial complex III. Furthermore, this particular exposure resulted in a decrease in the rate of oxygen intake by osteoblasts, thereby inhibiting their aerobic metabolism. Additionally, it led to an increase in the formation of lactate in the cells (Al-Ghafari et al., 2019).

Cellular Oxidative Stress of Heavy Metal Toxicity

Choi et al. (2018) in their study not only analysed cell death caused by exposure to airborne particulate matter but also showed that Glutathione (GSH), was a more sensitive biological endpoint, with a similar trend toward mortality, and indicated that nickel was the most harmful metal in their experimental setting. In a study conducted by Pan et al. (2023), the researchers exposed human normal embryonic kidney cells (HEK293) and human hepatocellular carcinoma cells (HepG2) to particulate matter with dimensions smaller than 2.5μm, commonly referred to as $PM_{2.5}$. The results of this study indicate that the presence of hexavalent chromium (Cr(VI)) in $PM_{2.5}$ has a significant harmful effect on HepG2 and HEK293 cells. The toxicity is mostly caused by triggering cytotoxicity via the oxidative stress pathway. Additionally, Cr(VI) inhibits cell proliferation by inducing cell cycle arrest specifically in the S-phase.

The depletion of cellular antioxidants and the enhancement of cellular oxidants following heavy metal exposure in osteoblast cells were demonstrated by Al-Ghafari et al. (2019). They also demonstrated that lead and cadmium significantly reduced the enzymatic antioxidant superoxide dismutase (SOD) and catalase (CAT); as well as the level of glutathione in the osteoblast cell culture. Moreover, this metal exposure also increased the level of the lipid peroxidation marker, thiobarbituric acid reactive substances (TBARS).

Yavuz and Doganlar (2019) conducted a study to assess the effects of a mixture of metals (copper, zinc, lead, and iron) present in permissible amounts in drinking water, on a human aortic vascular smooth muscle cell (HAVSMC) line. The findings of this investigation revealed conflicting effects regarding enzymatic antioxidants. It indicated a notable rise in the expression of antioxidant enzymes, implying that the mixture of metals that were exposed to the HAVSMC cell line induced the production of reactive oxygen species (ROS) and triggered the activation of the antioxidant system to scavenge these ROS toxicants.

Researchers investigating non-enzymatic antioxidant effects on cell lines exposed to heavy metals in addition to enzymatic antioxidants. Egiebor et al. (2013)

studied the harmful effects of four heavy metals (Hg, Cd, As, Pb, and Mix) on MCF-7 breast cancer cells, analysing their kinetic signature in the presence and absence of glutathione, a renowned non-enzymatic antioxidant. The study revealed that MCF-7 cells treated with the glutathione-scavenging compound L-buthionine sulfoximine (LBSO) before chemical exposure experienced increased cell death in a shorter timeframe compared to cells exposed to just these metals. The increased occurrence of cell death in the presence of LBSO is likely because LBSO removes cellular glutathione and enhances the impact of harmful substances towards the cell line.

Cellular Genotoxic Effect of Heavy Metal Toxicity

Cadmium, arsenic, and nickel are examples of heavy metals that have been classified as carcinogenic substances. The precise mechanism by which carcinogenesis occurs is still unknown; however, it has been observed that heavy metal exposure may potentially cause genetic damage by inhibiting the essential proteins involved in DNA repair processes and also inducing double strand breaks (DSBs) in the DNA (Morales et al., 2016). The assessment of genotoxicity has been significantly aided by cell culture research, which has examined mutation rates, DNA repair mechanisms, and chromosomal aberrations in cultured cell lines exposed to heavy metals.

In previous studies conducted by Morales et al. (2015, 2016), a cohort of researchers successfully generated and maintained viable cell line using HEK293 cells, wherein an Alu-Alu recombinant-Puro (AARP) construct was integrated at the FRT site. The cell line that was created was subjected to exposure with cadmium chloride, nickel chloride, and arsenic trioxide. After a duration of 48 hours of exposure to a concentration of 1.0μM $CdCl_2$, 100μM $NiCl_2$ and 1.0μMAs O_3, it was shown to be adequate in inducing mutagenicity and facilitating the deletion of genomic sequences, hence enabling the expression of the puromycin resistance gene. In addition, they also demonstrated that the repair outcomes of double-stranded DNA breaks vary depending on specific heavy metal exposure, resulting in the accumulation of a diverse set of mutagenic alterations in the genome.

Exposure to lead (Pb) or cadmium (Cd) is known to cause cellular redox stress, leading to the activation of nuclear transcription factor Nrf2, which plays a role in regulating redox balance. This activation was seen to be 2.32-fold and 2.98-fold higher in response to exposure to hazardous amounts of lead or cadmium, respectively (Al-Ghafari et al., 2019). Cells may resist the damaging effects of xenobiotic and redox stressors by activating the transcription factor known as nuclear factor E2-related factor 2 (Nrf2). When exposed to certain cellular stimuli like redox stress, Nrf2 moves from the cytoplasm to the nucleus. This translocation helps in the transcription of several cytoprotective genes, such as those involved in antioxidant and detoxification enzymes. (Al-Ghafari et al., 2019; Tonelli et al., 2018).

Furthermore, it has been observed that the presence of heavy metals can lead to modifications in the gene expression of the heat-shock proteins HSP27, HSP60, and HSP70. This suggests that the toxicity of metal mixtures in HAVSMC can result in protein misfolding (Yavuz & Doganlar, 2019). The aforementioned study also indicated a positive correlation between the upregulation of the EX01 gene, which functions as a DNA repair gene, associated with both the duration and concentration of exposure. This correlation suggests the presence of DNA damage and subsequent repair processes after exposure to heavy metals.

Meanwhile, Miller et al. (2004) aimed to evaluate the capacity of two heavy metals of military significance, namely depleted uranium (DU) and a reconstituted combination of tungsten, nickel, and cobalt (rWNiCo), to activate stress genes in human liver cancer cells (HepG2). The researchers showed a complicated profile with a considerable dose-dependent increase with DU and rWNiCo, leading to genomic instability and DNA strand breakage. These mostly happened through the activation of gene promoters such as hMTIIA FOS, p53RE, Gadd153, Gadd45, NFκBRE, CRE, HSP70, RARE, and GRP78. These promoters are linked to the synthesis of metallothionein and DNA damage. This work demonstrated that DU and rWNiCo may trigger gene expression via many different signal transduction pathways, potentially contributing to the toxicity and tumorigenicity of both DU and rWNiCo.

Conclusion

Cell culture research plays a vital role in unraveling the intricate cellular dynamics of heavy metal toxicity. Scientists employ controlled lab settings to simulate and investigate the impacts of heavy metal exposure on different cell types, leading to significant insights into the complex mechanisms underlying their harmful effects at both cellular and molecular levels. The capacity to examine distinct cellular reactions, such as oxidative stress, genotoxicity, and modified gene expression, has paved the way for a more comprehensive

understanding of heavy metal toxicity. These insights improve our knowledge of the health risks associated with high levels of heavy metal exposure and provide great promise for developing targeted and effective methods to protect human and environmental well-being. In essence, the ongoing investigation into the toxicity of heavy metals using cell culture research highlights the crucial significance of scientific inquiry in protecting human health and the environment against the widespread hazards presented by heavy metals.

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Author contribution

NSNY penned the first draft of the text, and all of the writers provided feedback on the earlier versions of the article. NNSMS was the principal investigator for the research grant that supported this study. MNFN contributed to designing the outlines of the literature study. All authors read and approved the final manuscript. VFK and WMZWY supervised the work involving the research activity planning and execution.

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Availability of data and materials

The authors certify that the data and material supporting the findings of this study are available within the article.

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