**INTRODUCTION**

Lead (Pb) is regarded as one of the most toxic substances among heavy metals. Pb causes serious illness which resulted in physiological, biochemical, and behavioral dysfunctions in humans, especially in children (Needleman, 2004). Clarkson et al., 1993. It can be exposed to the human body through food intake as well as through many environmental elements such as dust, air, and water. The common route of exposure of Pb is in the blood and eventually it is deposited in the hard tissues such as bone and teeth. The deposition in the latter tissues is permanent (Davies and Campbell, 1992). Several factors are identified which influence the Pb deposition in teeth. These include types of teeth and the presence of caries. Pb is distributed with the highest concentration in the stratum intermedium which is localized in the inner layer of dentin, adjacent to the dental pulp. The mechanism by which Pb is deposited within the matrix of the primary and circumferential dentin is not clear (Larsen et al., 1986). Nevertheless, it has been suggested that the deposition of Pb in teeth is probably due to the similar oxidation number of both Ca and Pb ions. Teeth play an important role in maintaining the homeostasis and function of tissues and play a pivotal role in dealing with invaders such as Pb. These effects have been documented in previous work only through the use of dental cells (Tawadros et al., 2002). Little is known, however, on the behaviour of dental stem cells towards Pb.

**AIM**

To investigate the effects of lead on the proliferation and differentiation of stem cells originating from bone marrow (BM-MSCs), pulp of deciduous (SCDs), permanent (DPSCs) teeth and periodontal ligaments (PDLs).

**METHODOLOGY**

**TEETH EXTRACTION**

BM-MSCs, SCDs, PDLS, and DPSCs were obtained from human donors undergoing endodontic therapy at the Department of Endodontics, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia. BM-MSCs were sourced from BM teeth, SCDs were isolated from mature deciduous teeth, PDLs were isolated from extracted teeth, and DPSCs were cultured from human molars and premolars in accordance with the guidelines of the American Society for Cell Biology (American Society for Cell Biology, 2007) and the guidelines established by the University of Malaya Human Ethics Committee.

**ISOLATION**

BM-MSCs were isolated according to the protocol of Mandard et al. (2005). SCDs were isolated using the method of Prokopi et al. (2014). PDLs were isolated using the method of Kasim et al. (2011). DPSCs were isolated using the method of Abdulkarim et al. (2013).

**PULP PREPARATION**

Five different Pb (Cat No 300800, Sigma-Aldrich) concentrations were used in this study, with 0 (control) and Pb concentrations of 40, 80, 160, and 200 µM. All the cells were transfected to each other at a concentration of 2 x 10^6 viable cells/mL. The cells were incubated for 21 days, at which point the cell viability was determined using the sulforhodamine B (SRB) assay.

**RESULTS**

**PROLIFERATION**

Figure 1: Cytotoxic effect of Pb to BM-MSCs, SCDs, PDLS, and DPSCs (Phase contrast microscope; 10x of magnification) in the presence of various concentrations of Pb (n=3).

- BM-MSCs, SCDs, DPSCs, and PDLs cultures maintained a fibroblastic cell-like morphology in controlled conditions and a small variation was observed in cells exposed to 10 µM of Pb.
- The cell line began to lose their cell culture as the concentration of Pb increased to 160 µM. BM-MSCs shows the worst followed by SCD, DPSCs, and PDLs.

**DIFFERENTIATION**

Figure 3: Differentiation of BM-MSCs, PDLS, and DPSCs at various concentrations of Pb (n=3).

- Osteogenic differentiation (von Kossa staining - 21 days): BM-MSCs showed a weak deposition of calcium in the mineralized matrix starting at a concentration of 40 µM. Whereas, cell lines from dental sources were only affected at 160 µM.
- Chondrogenic differentiation (alcan Blue - 21 days): No differences were noted between the control and treatment groups in all cell lines.
- Adipogenic differentiation (oil Red O - 21 days): BM-MSCs showed less accumulation of lipid vacuoles of cells exposed from 80 up to 160 µM. Whereas for dental derived stem cell, the lipid vacuoles were still obvious even at 160 µM.

**DISCUSSIONS**

- Pb exposure inhibited adhesion with the highest being in BM-MSCs followed by SCDs, PDLS, and BM-MSCs as well as it increased the biological aging of the cells in a dose-dependent manner.
- In this study we identified that the concentration of Pb (40 to 160 µM) suppressed the proliferation of stem cells rather than inducing cell death. One possible explanation is that platelets are present in significant amounts in stem cells or bone marrow mononuclear cells cultures (Assmus et al., 2010; Prokop et al., 2009) and Pb is thought to influence platelets or lysate-based platelets by regulating the levels of growth chemotactic factors (Muller et al., 2011). Thus, we speculate that Pb inhibits growth factors related to the proliferation of stem cells resulting in slow growth of cells.
- All the cell lines from various sources were capable of differentiating into osteoblast, chondrocytes, and adipocytes as observed in the control samples.
- Osteogenic differentiation: BM-MSCs showed reduced differentiation capacity compared to dental derived stem cell. Pb follows the movement of Ca2+ in the body as it utilizes the same ion transporter as calcium, acting like a competitive inhibitor (Alissa et al., 2014). We postulate that the demineralisation process is slow in teeth allowing the cells to dental origin to adapt for more Pb toxicity. The genes such as alkaline phosphatase and osteocalcin has been suppressed by Pb (Zuscik et al., 2007). We speculate suggest that Pb can inhibit osteoblast differentiation due to disturbance of osteogenic pathways.
- Our results showed that when exposed to Pb, chondrogenic differentiation was not observed in all cell lines. This concurred with findings reported by Zuscik et al. (2007) and Cormouc (2009).

**CONCLUSIONS**

- Our study revealed that lead exposure affects the proliferation rate and induces alteration in differentiation of stem cells of dental origin.
- Dental derived stem cells have a potential as screening model for heavy metal since it has demonstrated its ability to withstand the toxicity of Pb better than BM-MSCs.

**REFERENCES**