Systemic Toxic Reactions due to CoCrMo Wear Particles on the Liver cells using a Dynamic Microfluidic Bioreactor

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INTRODUCTION: The prevalence of total hip replacements (THR) has shown a steady upward trend in recent years, with an estimated yearly incidence of over 450,000 cases in the United States[1]. It primarily involves the use of metal components, namely Cobalt Chromium-Molybdenum (CoCrMo) and Titanium-based alloys. However, it is important to note that significant clinical evidence has brought attention to cases of early THR failures and the possible negative impacts on patients (locally and systemically) resulting from the release of metal particles and ions. The investigation of the adverse effects of released byproducts from hip implants on different types of cells has predominantly been conducted using static cell cultures by researchers[2,3]. However, this technology is limited in its ability to accurately recreate the dynamic nature of the *in-vivo* joint environment. Therefore, there is a need to carry out further extensive research on the adverse effects caused by these byproducts using a microfluidic system that accurately replicates the cell-particle interactions with increasing dosimetry of the released products. In our previous ORS abstract[4], we reported the possible risk of toxicity on liver cells under static conditions, with short time exposed to higher concentration. In this study, we hypothesis that under dynamic condition, CoCrMo wear particle may cause liver toxicity even in low concentration with longer period of exposure. Hence, the objective of the study is to investigate and compare the toxicity effect on the liver cells at 10 ppm of CoCrMo particles with 3 days of exposure.

METHODS: 1) Particle characterization: The CoCrMo particles were obtained from Bioengineering Solutions, Chicago and were prepared using sterile deionized water. All additional medium and staining reagents were obtained from Thermo Fisher. 2) Static cultures: In this study, HepG2 -human hepatocellular carcinoma (ATCC) were cultured and maintained using Dulbecco's Modified Eagle Medium (DMEM) at a temperature of 37°C with a 5% CO_2 incubator. The cells were then treated with a concentration of 10 ppm of CoCrMo for a period of 3 days. The assessment of cell viability was conducted through alamarBlue and live/dead staining techniques, whilst the evaluation of nuclear integrity was performed by DAPI staining. 3) Microfluidic Channel Preparation: The slides were prepared for the microfluidic system using a series of procedures. In brief, immersing the slides in nitric acid, deionized water, and 70% ethanol for a duration of 1 hour at each stage, aiming to achieve thorough sterilization. Prior to autoclaving, the other components of the microfluidic system were subjected to a cleansing process including soap, deionized water, and a 70% ethanol solution to remove any traces of previous usage. 4) Dynamic cultures: a) HepG2 (liver) cells were cultured using DMEM media at 37° C with 5 % CO_2 and were treated with 10 ppm CoCrMo for three days under constant media flow rate of 200 uL/min[5] in a microfluidic system **(Fig. 1a).** Cell-particle interaction were continuously monitored with attached miniatured version of live cell analysis through CytoSMART Lux2 camera and placed all the testing system in a well-controlled incubator. Cell viability was assessed using alamarBlue and live/dead staining while nuclear integrity was assessed with DAPI staining. Statistical analysis was performed on the alamarBlue data using GraphPad Prism software.

RESULTS: (a) Cell Viability: The cell viability data revealed a significant ($p<0.05$) decrease between controls and the treated static culture after 3 days. However, there was a significant increase in cell viability between the control and treated in dynamic cultures after 3 days **(Fig. 1b)**. Live/dead staining showed an increase in dead cells (red) compared to live cell (green) in the treated images for both the dynamic and static cultures **(Fig. 1c)**. Additionally, when comparing the static and dynamic cultures, there was decreased cell viability in the static cultures as seen by the greater number of red cells in the image **(Fig. 1c)**, thus validating the hypothesis that CoCrMo particles could be a potential source of systemic toxicity to hepatocytes.(b) Cell Morphology: Both the control images for the dynamic (ciii) and static (ci) cultures show a larger amount of nuclear staining and typical growth pattern expected of HepG2 cells **(Fig. 1c)**. Treatment images for dynamic (civ) and static (cii) both show less nuclear staining, along with a more atypical, spaced pattern of growth as opposed to the controls. (c) Cell-particle interaction: Real-time imaging and video from the Lux2 camera were recorded and presented in **Fig. 1e**. This assist in the visualization of the cell-particles interactions with the HepG2 cells in the dynamic system, as a function of exposed time. The control images show the absence of particles and minimal change in the morphology of the cells between the 0th hour (ei), and day 3 (72nd hour) (eii), respectively. However, for the treated cells, in the 0th hour (eiii), the presence of particles are evident as black dots in the image. However, at the day 3, there is only a minimum amount of particles as can be seen in the **Fig. 1e** (iv); interestingly, significant changes in the cell morphology are portrayed in the images.

DISCUSSION: These *in vitro* conditions are used to simulate more realistic *in vivo* conditions as compared to static cultures by mimicking the blood flow that hepatocytes receive. Based upon the findings of the alamarBlue assay, this can be seen with the viability of the static treated cultures being significantly reduced, whereas the viability in the treated dynamic cultures is significantly increased. A potential explanation for this finding is that the particle concentration seeded into both static and dynamic cultures was 10 ppm. However, the dynamic setup had a constant flow of media which might have caused the particle to be pushed into the media reserve, thus diluting the actual particle concentration to closer to 0.1 ppm. As blood flow remains constant *in vivo*, it is not unreasonable to assume that a similar phenomenon could happen in patients who have received THRs. Additionally, the findings of altered cell morphology and lower viability in both the dynamic and static treatment cultures compared to control cultures suggest that CoCrMo particles do have some degree of toxic effect to hepatocytes. However, it is again noteworthy that this toxicity appears to be to a greater extent in static cultures as the lack of fresh media flow potentially allows for particles to interact with the surface of the hepatocytes for longer periods. These findings suggests that dynamic cultures might offer a better solution to estimating the effects of systemic toxicity of hip implants than static cultures can allow for. This study has several limitations; further studies will be continued by considering different dosimetry of CoCrMo particles with other biological assays. Further, the microfluidic bioreactor could be interfaced with hip simulators to investigate the chronic conditions in the patients[6]. In summary, our hypothesis aims to drive the field of research toward the development of safer hip implant designs.

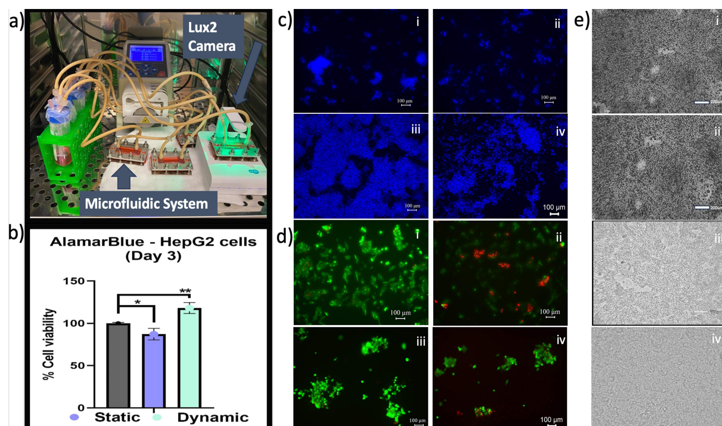

Figure 1 (a) Microfluidic system setup and Lux2 camera setup; **(b)** AlamarBlue assay data; **c(i-iv)** DAPI stain after 3 days of dynamic control (iii), dynamic treatment (iv), static control (i), and static treatment (ii) cultures; and **d(i-iv)** live/dead imaging after 3 days of dynamic control (iii), dynamic treatment (iv), static control (i), and static treatment (ii) cultures **e(i-iv)** Real-time microscopy of the microfluidic system with control at 0-hour (i), control at 3 days (ii), treatment at 0-hour (iii), and treatment at 3 days (iv) is shown.

SIGNIFICANCE: The results herein highlight a concern for potential systemic toxicity to hepatocytes in patients who have received THRs based on findings from static and dynamic cultures. Furthermore, dynamic cultures might more closely mimic toxicology research from implant derived CoCrMo particles to hepatocytes as it has the ability to stimulate the constant blood flow the liver receives *in vivo.* However, further testing and validation is required with varying concentrations of particles to fully understand cell-particle interactions and the role the particles in systemic cell toxicity.

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