Cell Accelerated Corrosion in Hip Taper Junction: A 3D Bioprinting Approach to Study the Cell Migration

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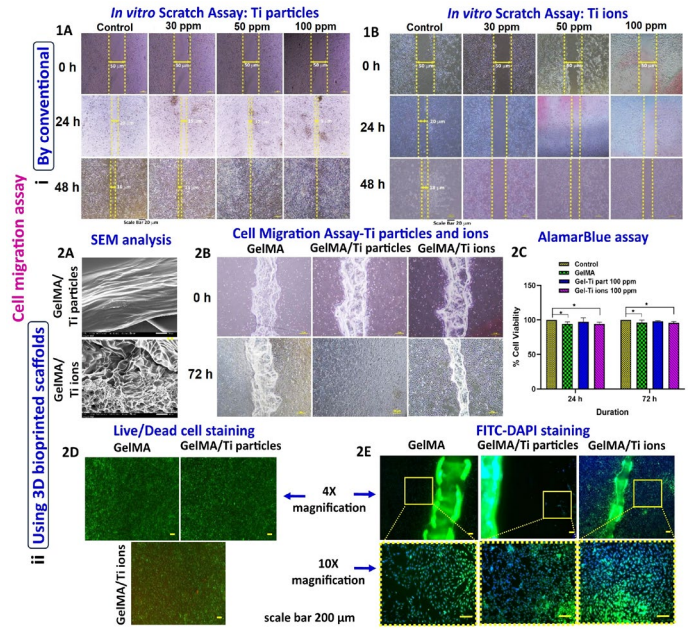
INTRODUCTION: Total hip replacement (THR) is a common procedure for those with severe osteonecrosis and arthritis, with around 330,000 procedures performed each year in the US alone[1]. Substitutes like these often have titanium and cobalt-chrome-molybdenum (CoCrMo) alloys in their design[2]. However, tribocorrosion (wear+corrosion) can cause degradation at the interfaces and pose a possible risk for early failures and released degradation products (metal particles and ions) may lead to side effects such as inflammatory reactions and psedotumor[3]. Other classical corrosion types, Recently, cell accelerated corrosion (CAC) was reported at hip taper junction[3]. Further, the column-like damage in the cell migrated pathways was also observed in the retrieved implant tapered surfaces of the neck and head due to the CAC. However, it is unclear how the cells migrate to the metal interface and the underlying mechanisms associated with CAC. Therefore, we adopted two approaches to investigate cell migration. The first approach involved growing a monolayer culture of MG63 cells and creating a scratch, and cells are treated with Ti particles and Ti ions. The second approach involved the use of 3D bioprinted scaffolds loaded with Ti particles and ions and cultured with MG63 cells. We hypothesize that 3D bioprinted scaffolds will mimic the *in vivo* implant environment and controlled release of the loaded Ti particles and ions and how influence the cell-particle/ion interaction. This will assist us in understanding the pathways of cell migration in the presence of metal particles and/or ions than the conventional migration assay method.

METHODS: (i) *In vitro* scratch assay: MG63 cells were cultured till monolayer formation, created a scratch, and treated with different concentrations of Ti particles and ions (30, 50, and 100 ppm) respectively, and incubated. The optical microscope images were taken at various intervals (0, 24, and 48 h) to observe the migration of the cells in the scratch region. (ii) Fabrication of Ti particles and ions loaded 3D bioprinted Scaffolds: The calculated amount of Gelatin methacrylate (GelMA) 10 % wt/vol polymer was homogenized with 100 ppm Ti particles and ions. The 3D bioprinted hydrogel scaffolds were fabricated using Regen HU 3D bioprinter. The fabricated scaffolds were denoted as GelMA, GelMA/Ti particles, and GelMA/Ti ions. (iii) Characterization of the scaffolds: The surface morphology of the scaffolds was analyzed by SEM-EDS. (iv) *In vitro* cell culture assays with MG63 cells: Cell migration assay with 3D bioprinted scaffolds: This study was performed by culturing the MG63 cells and seeded directly with 3D bioprinted scaffolds GelMA, GelMA Ti particles 100 ppm, and GelMA Ti ions 100 ppm and images were taken at the duration of 0, 24, 48, and 72 h to evaluate, how the cell migration behavior in the presence of Ti particles and Ti ions, compared to GelMA (control). MG63 osteoblast-like cells were cultured using MEM with FBS (10 %) and antibiotic-antimycotic (1%) solution. The cells were cultured directly in a petri dish containing 3D bioprinted scaffolds for 24 and 72 h. Subsequently, the cells were added with AlamarBlue reagent and incubated for 4 h. The optical density (OD) was measured at 570 and 600 nm and calculated the % cell viability. Live/Dead cell staining: This study was performed for the duration of 24 and 72 h with 3D printed scaffolds and Live/Dead cell staining added. Similarly, Cell proliferation and nuclear integrity imaging: The scaffolds with MG63 cells and incubated for 72 h and stained using FITC-DAPI and subsequently imaged using a confocal laser scanning microscope (CLSM). Triplicate of the samples (N=3) used in this study. GraphPad Prism (8.3.0) Software was used for calculating the significance by TWO-way ANOVA (*$p < 0.05$).

RESULTS: The scratch assay was performed on MG63 cells monolayer in 6 well plates and treated with Ti particles and ions with varying concentrations such as 30, 50, and 100 ppm respectively. The migration of the cells in the scratch region was photographed for the duration of 0, 24, and 48 h, respectively (**Figure 1 A, B**). The 3D bioprinted scaffolds loaded with Ti particles and ions were successfully fabricated using the 3D bioprinter. The SEM-EDS analysis of the Ti particles and ions loaded scaffolds were shown in **Figure 2A** with smooth surface and porous characteristics with GelMA/Ti particles and GelMA/Ti ions respectively. The cell migration behavior was observed over a period of 72 h. GelMA and Ti particles migrated better than the Ti ions group (**Figure 2B**). The AlamarBlue assay was used to evaluate the cell viability of the GelMA, Ti particles, and Ti ions loaded scaffolds for the duration of 72 h, which showed good cell viability for the duration of 24 and 72 h (**Figure 2C**). Likewise, Live/Dead staining showed good viability with the scaffolds for the duration of 72 h (**Figure 2D**). The MG63 cells' migration characteristics of the scaffolds showed good migration with Ti particles and ions for the duration of 72 h. However, the Ti particles group showed cell migration from the periphery of the cell culture plate and migrated toward the scaffolds. Whereas Ti ions showed migration from all around the region of the cell environment. In addition to that cell proliferation and nuclear integrity of the scaffolds with MG63 cells stained with FITC and DAPI showed good cell proliferation and nuclear integrity (**Figure 2E**).



DISCUSSION: Our findings showed that the addition of titanium particles in the scratch region showed higher migration than Ti ions. Additionally, the cell migration towards 3D bioprinted scaffolds is due to Ti particles and ions in a biomimetic way. In accordance with this, the existence of Ti particles may encourage cell migration, which may have an impact on the effectiveness and durability of hip implants. When exposed to titanium particles, we saw a substantial increase in cell migration. Although the underlying mechanism is unclear, the biochemical changes mediated by cell migration at the crevices of the taper junction could be a driving factor for the cell movement at the taper junction. Further, the increased cell migration may be due to the activation of stimuli and growth factors that activate signaling pathways which are attributed to more cell migration[4]. The limitations of this study include a shorter time, carried out with only MG63 cells and Ti-products. To completely comprehend how these migratory patterns are triggered and how they affect the functionality and longevity of hip implants, further study is required.

SIGNIFICANCE: The study demonstrated a novel method for cell migration using 3D bioprinted scaffolds in the presence of Ti particles and ions, to indicate the underlying mechanism in cell accelerated corrosion in Hip Taper Junctions.

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The major findings of the study include: **Figure 1**: **A, B** - *In vitro* scratch assay showed higher MG63 cell migration with Ti particles than ions. **Figure 2A** - SEM analysis showed a smooth and porous nature. **2B** - 3D bioprinted scaffolds MG63 cell migration in the presence of Ti particles and ions. **2C** - AlamarBlue, **2D** - Live/Dead, and **2E** - FITC-DAPI staining showed improved cell viability, adhesion, and proliferation with nuclear integrity respectively. This supports the 3D bioprinted scaffolds are nontoxic and supports the migration of the cells towards Ti particles and ions in a controlled manner.