Proximity and Cell Density Effects on the Killing Ability of Mg and MgTi Microparticles In-Vitro

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Statement of Purpose:

Magnesium-titanium (MgTi) bimetallic microparticles have been shown to kill cells more effectively at much lower particle concentration than Mg alone.[1] It has also been found that pH plays an important role in killing cells in vitro.[1] However, there may be other mechanisms, such as the production of radical oxygen species (ROS), during particle corrosion that may be contributing to cell death. The mechanisms of cell death due to particle corrosion must be studied in order to determine if the particles will be effective in treating bacterial infections and cancer in vivo. This study explores the effects of cell density and proximity on cell viability in the presence of particles.

Methods:

Mg/MgTi Cytotoxicity at Different Cell Densities

MC3T3 cells (ATCC #: CLR-2593) were placed in 6-well plates (A=9.6 cm^2) with a cell seeding density of 5,000 cells/cm^2, 10,000 cells/cm^2, 20,000 cells/cm^2, or 30,000 cells/cm^2. Different concentrations of Mg and MgTi particles were made in the range from 25 to 3000 µg/mL. MgTi particles were synthesized by sputtering Mg particles (Goodfellow Mg006021) for 5 minutes (1.2 kV at 50 mA, 100-200 mTorr). For each cell density, the concentrations of Mg and MgTi particles measured above were randomly distributed in the wells (n =3). At 24 hours, live/dead assay (Invitrogen #L3224) was performed and fluorescent imaging was used to measure cell viability. Ten fluorescent images per well were taken at random places in the wells and from these images, an average of % cell viability was determined by counting the green (live) and red (dead) cells. A t-test was done at each concentration to see if there was any significant different between Mg and MgTi particles, $p<0.05$.

Proximity Effect of Mg/MgTi Particles

20% pluronic solution was made by dissolving 2.0 g of pluronic F-127 (Sigma-Aldrich P2443) in 10 mL of the complete media at 4 Celsius. After cells (10,000 cells/cm^2) were seeded in 70 mm petri dish, 8 mL of 20% pluronic solution was poured into the dish. The pluronic served to confine the particles in the center while also allowing for diffusion of aqueous species and preservation of cell viability. After the solution gelled, 0.005 g of Mg or MgTi particles was added at the center of the dish. At 24 hours, the particles and pluronic were dissolved in PBS and washed away and live/dead assay was performed. Images were taken at the particle site, the borderline, 1 cm, 2 cm, and 3 cm away from the particle center.

Results:

Mg/MgTi Cytotoxicity at Different Cell Densities

Previous study showed that MgTi particles kill cells much more effectively than Mg alone, where MgTi killed cells completely at 650 µg/mL when cell density was 5,000 cells/cm^2. However, with all conditions remaining the same and only the cell density doubled, cells died completely only after 1250 µg/mL for MgTi (Figure 1). This result implies that fewer cells are killed when more of them are present.

When cell density is 30,000 cells/cm^2, the cells re-organize themselves around the Mg particles, where some even attach to the particle surface, as if the cells are engulfing the particles together (Figure 2). Most of the cells that are close to or attached to the particles are very viable and this phenomenon is only observed when cell density is high.

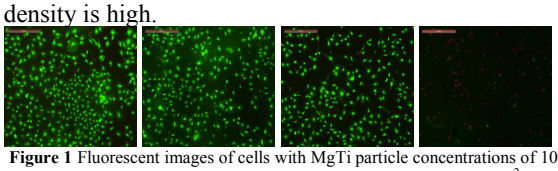

Figure 1 Fluorescent images of cells with MgTi particle concentrations of 100, 500, 750, 1250 µ/mL (Left to Right) for cell density of 10,000 cells/cm^2.

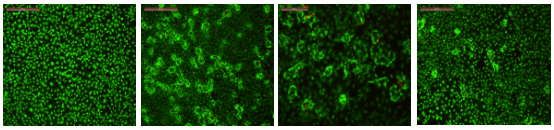

Figure 2 Fluorescent images of cells of control, Mg 100 µg/mL, Mg 100 µg/mL close-up, and MgTi 100 µg/mL (Left to Right) for cell density of 30,000 cells/cm^2.

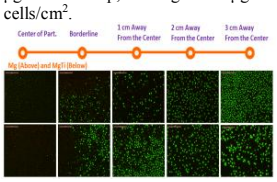

Figure 3 Fluorescent images of cells at various points away from the center of the particles.

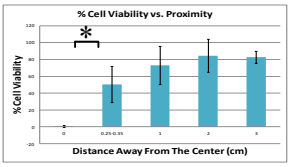

Table 1 % Cell viability versus distance away from the center of the particles, which has an average radius of 0.25 cm, $p<0.05$.

Proximity Effect of Mg/MgTi Particles

The results show that the particles have to be very close to the cells to have a toxic effect. At the boundary where the particles end, an approximately 1 mm thick transition zone is present where the % cell viability increases from 0.64% to 50.4% (Table 1).

Discussion:

These experiments show that the Mg/Ti and Mg particle effects are highly localized to the particulate sites. Also, the killing effect is diminished when the cell density is increased. This may indicate that there are other mechanisms, such as ROS production, that induces cell death where the killing molecule can be depleted or dissipated by large cell densities. Particles have to be actively corroding in the presence of cells for cell death to occur. Based on these findings, further studies will be done to investigate the mechanisms of cell death.

Conclusion:

In conclusion, the cell killing effect of Mg and Mg/Ti particles is a highly localized effect. Also, an increase of cell density increases cell viability for a particular particle concentration.

References:

1. *Kim, J and Gilbert, JL.* WBC, 2012. Vol. 3085.