Cytocompatibility of Hydroxyapatite and Magnesium Oxide Nanoparticles Dispersed in Poly(lactic-co-glycolic acid) (PLGA) Scaffolds for Bone Tissue Engineering

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Statement of Purpose: The objective of this study is to evaluate cytocompatibility of poly(lactic-co-glycolic acid) (PLGA) composites containing either hydroxyapatite (HA) nanoparticles or magnesium oxide (MgO) nanoparticles for potential orthopedic and dental applications. HA nanoparticles (nHA) has been shown to improve human mesenchymal stem cell (MSC) adhesion and osteogenic differentiation[1]. MgO nanoparticles (nMgO) has been shown to enhance HA deposition[4], and reduce bacterial infection[2], providing desirable properties for bone grafting.

Materials and Methods: For this study, PLGA (50:50, MW 100,000 Da; Polyscitech) was first dissolved in chloroform. Nano-HA and nano-MgO powders were then dispersed into the polymer suspension using high-power sonication to give a ceramic/polymer ratio of 3:7. This ratio was chosen for this initial study because it has been shown to give optimal properties for nHA/PLGA composites[1]. Immediately after sonication, the suspension was cast into a Teflon mold, dried in air for 24 hours, and then dried in vacuum for additional 48 hours. The resulting 0.1mm thick composite scaffolds were cut into 10mm by 10mm squares for cell studies. PLGA scaffolds, nMgO, nHA, Ti squares, and glass slide were used as controls and tissue culture polystyrene (TCPS) as the reference. All materials of interest were sterilized before cell culture.

Rat bone marrow derived stromal cells (BMSCs) were used as the model cell to evaluate the cytocompatibility of these nanocomposites of interest. BMSCs were extracted from the femur and tibia of juvenile rats according to the established protocols in our laboratory[3]. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). The media pH was adjusted to 7.4 before their use in cell culture. BMSCs at their second passage were seeded onto the scaffolds and controls at a density of 10,000 cells/cm^2, and incubated for 4, 24, 48, and 72 hours. The media was collected every 24 hours for pH measurements. At the end of each prescribed time point, non-adherent cells were removed by washing with phosphate buffer solution (PBS). Adherent cells were fixed using 4% paraformaldehyde, stained with DAPI to visualize the nuclei of the cells, and counted base on at least 5 fluorescence images for each sample. BMSC adhesion density was calculated as the number of cells per unit area. Experimental samples were run in triplicate.

Results: Cell adhesion improved on the nMgO/PLGA over the nHA/PLGA, the controls, and TCPS reference during the first 24 hours. However, cell density on the nMgO/PLGA scaffold decreased at 48 and 72 hours, while the cell density increased on the Ti, glass, and reference. The nHA/PLGA composite scaffold showed continuous increase in cell density and then stabilized between 48 and 72 hours. The well containing nHA showed improved cell adhesion within the first 4 hours but steadily decreased at subsequent time points. The cell density on the nMgO remained close to zero for all time points. This indicated that free nMgO alone might have a toxic effect on the BMSCs. It is possible that, as the PLGA degraded, the nMgO in the nMgO/PLGA scaffolds released at such a rate that it exceeded the tolerable level for cells. We also observed an increase in pH to 9.18 after the first 4 hours in the wells with nMgO. The difference in pH for the other wells was not statistically significant and remained between 8.1 and 8.4 for all time points. The increase of media pH might be the cause of the observed cell death in the nMgO case, but this does not account for the cell death with nMgO/PLGA at 48 and 72 hours.

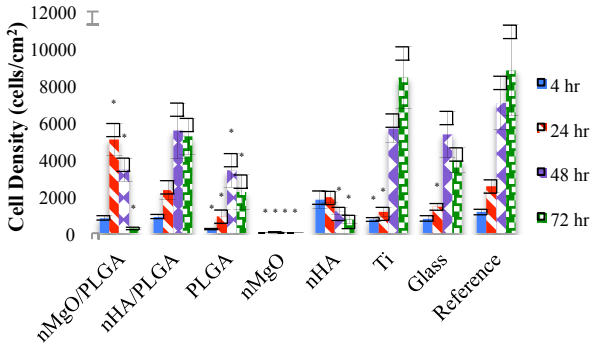

Figure 1: BMSC density on each material at 4, 24, 48, and 72 hours of culture. (mean ± SEM; n=5; *p<0.05 compared to reference)

Conclusion: The nMgO/PLGA composite improved BMSC adhesion density initially but decreased as PLGA degraded. Decreasing the amount of nMgO loading and using a polymer with a slower degradation rate may mediate the toxic effects of MgO while maintaining the adhesion enhancing effects.

References:

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