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"Calcium Sulfate Bone Void Fillers: Are There Unintended Immune Consequences?"

Introduction: Wounds resulting from open fractures carry a risk of infection, leading orthopaedic surgeons to use wound treatment methods, like calcium sulfate beads mixed with antibiotics, which both fill bone voids and deliver local antibiotics to prevent infection. However, studies have shown that high calcium environments stimulate inflammatory differentiation in immune cells, potentially resulting in soft tissue damage. This study assesses whether calcium sulfate beads cultured with undifferentiated macrophages induce in vitro pro-inflammatory macrophage polarization. Additionally, this study will evaluate the macrophage phenotype in vivo, in the context of a murine freeze burn injury model, with or without calcium sulfate beads.

Methods: Murine RAW 264.7 macrophages were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin at 37°C with 5% CO_2 and exposed to sterile calcium sulfate beads (Biocomposites®) for 48 hours. Beads were secured with 3D-printed polylactic acid inserts in 35mm diameter tissue culture plates, and polylactic acid inserts without beads served as control. Inserts were cleaned and UV-sterilized before use. After 48hrs, supernatant was collected, and cells were fixed in zinc buffered formalin and stained for phalloidin/DAPI to quantify cell number/morphology after image acquisition from confocal microscopy. Supernatant was stored for cytokine analysis using the LEGENDplex™ Mouse Macrophage Panel (13-plex) to quantify pro-inflammatory (CXCL1, IL-18, IL-23, IL-12p70, IL-6, TNF-α, IL-12p40, IL-1 β) and anti-inflammatory (TGF-β1, CCL22, IL-10, G-CSF, CCL17) cytokines. For in vivo work, 32 female CD-1 mice were randomly assigned to control or bead-implant groups, receiving freeze burn injury to the vastus lateralis followed by calcium sulfate bead placement (experimental) or no bead (control). Mice were euthanized at 3 or 14 days, and tissues (muscle, bone, bone marrow) were collected for RNA isolation and histology.

Results: The in vitro results suggest calcium sulfate did not induce polarization, with most analytes remaining below the limit of detection. However, TNF-α and IL-10 levels were significantly elevated following calcium sulfate bead exposure compared to controls. CCL22 was detectable, but its levels did not differ significantly between calcium sulfate-treated and control cells.

Conclusion: The lack of detectable M1 and M2 cytokines suggests that calcium sulfate did not provide a strong stimulus for polarization toward either a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype in RAW 264.7 macrophages. Evaluation of in vivo outcomes is ongoing.