Local Fracture Conditions And Circulating Inflammatory Markers Correlate With Host Cell Attachment To Implants

Sarah M. Romereim[1], Aatif Jabbar[1], Matthew Smykowski[1], Hailey Bennett[2], Xue Ma[2], Nicholas Bernthal[3], Joseh Wenke[4], EMIT* Group, Rachel Seymour[1,2], Joseph R. Hsu[1,2], Bailey V. Fearing[1,2]
*EMIT: Christine Churchill[1], Catherine Young[1], Madhav Karunakar[1], Kevin Phelps[1], Suman Medda[1], Sarah Pierrie[1], Laurence Kempton[1]

[1]Atrium Health Musculoskeletal Institute, Charlotte, NC; [2]Wake Forest University School of Medicine, Winston-Salem, NC; [3]Orthopaedic Surgery, University of California Los Angeles School of Medicine, Los Angeles, CA; [4]University of Texas Medical Branch, Galveston, TX

sarah.romereim@advocatehealth.org

Disclosures: Nicholas Bernthal (3B-Daiichi Sankyo, Zimmer Biomet; 6-Zimmer Biomet), Joseph Hsu (2-Stryker, Smith & Nephew; 3B-Stryker, Smith & Nephew)

INTRODUCTION: Orthopaedic implant-related infections in open fractures are highly prevalent (~20%) and difficult to treat. A robust host cell response and endogenous cell attachment to an implant is critical for protection from bacterial adhesion and biofilm formation. Here, we utilize external fixator (ex-fix) pins as a model of human host cell attachment to implants to investigate the effect of local fracture conditions and systemic circulatory factors on cell phenotype and inflammatory response to orthopedic implants.

METHODS: This study was approved by the Atrium Health Institutional Review Board (IRB00091189). Patients receiving external fixation for fractures or for joint stabilization following soft tissue injury were consented for collection of ex-fix pins and blood samples. The cohort (n=41) included 28 male and 13 female patients. Medical data (demographics, injury characteristics, treatment information, and documentation of complications including infection) were entered/stored in REDCap. Upon removal, ex-fix pins were immediately treated with an enzymatic solution to isolate the adherent cells followed by Ficoll Paque Plus density gradient separation of red blood cells from mononuclear cells. The resulting implant-adherent cell population was stained for flow cytometric analysis. Four main cell populations were identified as fibroblasts ($CD45^-/CD90^+$), fibrocytes ($CD45^+/CD90^+$), innate lymphoid ($CD45^{int}/CD90^{bright}$), and leukocytes ($CD45^+/CD90^-$). Within the leukocytes, three subgroups were identified as monocytes ($CD11b^{bright}/CD68^-$), macrophages ($CD11b^{int}/CD68^+$), and other leukocytes. Serum was isolated from each blood sample collected prior to ex-fix pin removal, and cytokine concentrations were subsequently quantified with a 40-target human inflammation antibody array. Statistical analysis including 2-way ANOVA with Šidák multiple comparisons, unpaired T-tests, linear regression, and Pearson's correlation was performed in GraphPad Prism version 10.6.0.

RESULTS: In this on-going study, the ex-fix pins collected included pins placed in a fractured bone as well as pins placed in adjacent, uninjured bones as part of injury stabilization. A total of 55 samples were collected, including 28 pin sets from fractured bones and 27 pin sets from uninjured bones. Overall, the predominant cell type present was leukocytes (mean 85.9% ±19.5% std dev) followed by fibroblasts (9.8% ±17.6%) with a small contribution by fibrocytes (1.5% ±2.2%) and innate lymphoid cells (2.8% ±3.1%). The leukocyte subtypes were 55.6% ±27. 2% monocytes, 19.9% ±16.6% macrophages, and 24.5% ±18.5% other leukocytes. There was substantial sample-to-sample variation, and we sought to determine which demographic or injury characteristics might contribute to this variation. There were no significant differences in cell type prevalence based on patient age/sex or based on whether the implant location of the ex-fix pin was a fractured bone or an uninjured bone. Within the pins that were implanted in a fractured bone, however, there were significant differences in the cell types based on whether the fracture was open or closed (Fig. 1, n = 28, $p<0.0001$) but no differences in the leukocyte subtypes ($p=0.1121$). In addition to the local fracture conditions, the systemic inflammatory status of the patient was investigated via circulating cytokine concentrations. There were 18 circulating cytokines that were positively correlated with macrophage adhesion to implants (e.g. several interleukins, IFNg, G-CSF, MCSF; Pearson's $r>0.3$, $p<0.05$). Elevated circulating MCP-1/CCL2 was also positively correlated with fibroblast adhesion to ex-fix pins and negatively associated with leukocyte adhesion.

DISCUSSION: The present study observed differential cell type adhesion to orthopedic implants based on both local fracture conditions (open vs. closed) and circulating inflammatory markers. Our data suggests that ex-fix pins implanted in bones with open fractures have a lower percentage of adherent leukocytes but no difference in leukocyte subtypes. Circulating MCP-1/CCL2 concentration was correlated with lower leukocyte adhesion, and 18 other circulating inflammatory markers were correlated with a higher percentage of macrophages within the leukocyte population. This suggests there is a distinct difference in the way host cells respond to implants in the context of open trauma irrespective of systemic inflammation, and that the adherent leukocyte subtypes are affected by the systemic inflammatory status. Further patient accrual and planned in vitro and animal model studies will provide mechanistic insight into the potential difference in cell type recruitment, adhesion, or proliferation under different injury conditions, and this may provide discrete treatment targets for improved host cell integration with implants via implant surface coatings or modifications.

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides a greater understanding of how injury and treatment characteristics in orthopedic implant surgeries impact endogenous immune cell response and attachment to implants. Toward the goal of preventing orthopaedic implant-associated infections, these data provide the cellular context present for any implant coatings/applications which could impact their efficacy.

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IMAGES AND TABLES:

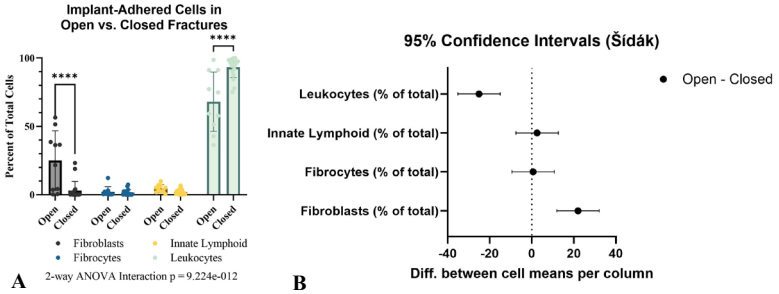

Figure 1. Significant differences in the implant-adhered cell types identified via flow cytometry were identified in open vs. closed fractures, n=28 (A), and the 95% confidence intervals are also shown (B).