Rosadas, M., Sánchez Espinel, C., Peleteiro, M., Sousa, A., Ribeiro, V., González-Fernández, Á., & Oliveira, A. L. (2026). *Beyond DNA removal: assessing the immunological response to decellularized rabbit dermal matrices*. Abstract from V Jornada IBEROS+, Santiago de Compostela, Spain.

Decellularization aims at producing extracellular matrix (ECM) scaffolds with minimal immunogenicity while preserving their native structure and bioactivity, creating biomaterials with high potential for tissue engineering and regenerative medicine. The efficiency of DNA removal is often used as the primary indicator of successful decellularization, potentially overlooking other factors that may influence host response, for instance endotoxin presence. In this study, decellularized rabbit dermal matrices (dRDMs) obtained through two distinct decellularization protocols – one yielding residual DNA below the proposed safety threshold of 50 ng/mg dry tissue and the other above this threshold – were evaluated for their immunological performance. The sterility and absence of endotoxins were also verified. Complement activation assays using human blood plasma, as well as assays measuring reactive oxygen species (ROS) production, apoptosis induction (by staining with Annexin V/propidium iodide (PI)), activation marker expression, cytokine secretion, and migration, were preformed using human peripheral blood mononuclear cells (PBMCs). Overall, after both decellularization processes matrices did not elicit a pronounced immune activation, with no ROS production or apoptosis detected. PBMC activation remained comparable to negative controls, except for monocytes and B lymphocytes, which showed some signs of activation. Cytokine analysis indicated a mild inflammatory profile while still including detectable anti-inflammatory cytokine IL-10 levels. Interestingly, the matrices with lower DNA content consistently induced higher responses across multiple assays, likely due to detergent residues, structural alterations exposing immunogenic epitopes, or damage-associated molecular patterns (DAMPs) generated during the decellularization process. Leachate testing revealed negligible immunotoxicity. Preliminary migration assays suggested some directed cell migration toward both matrices, particularly the one with lower DNA content. A final comparison includes a commercial decellularized bovine dermal matrix (Matriderm®) as a control for the migration assays. These findings reinforce that DNA quantification alone is insufficient to define the biocompatibility of decellularized matrices and that other process-related factors may play a critical role in modulating immune responses.