## Spatio-Temporal Characterization of Gene Expression Changes in Porcine Wound Healing

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The purpose of this study was to investigate the spatiotemporal characteristics of wound healing using high-throughput gene expression analysis.

The investigation was conducted using 6 pigs, each with 12 wounds. The wound edge and center were sampled from days 0, 1, 7, 9, 11, 13, 15, 16, till wound closure day 21, totaling 150 samples. Tissue was processed for RNAseq by the high-throughput and precise next-generation sequencing (NGS) techniques with the illumina NovaSeq platforms.



Our primary aim was to identify genes that dynamically change their expression during healing.

We hypothesized that synchronous expression of a cluster of genes may indicate a shared regulatory mechanism, such as expression by the same cell type or response to the same stimulus in different cells. We searched for clusters of genes with strong correlations in their expression patterns. The identified clusters were validated using the Gene Ontology (GO) resource. Only clusters with statistically significant p-values and clearly defined sets of GO terms were included in the analysis. To better understand the dynamics of each gene cluster, we plot one gene from each cluster, showing all replicates.

findings suggest a more probable association with how samples are collected.



13 gene clusters were identified: 3 clusters with maximum on days 1-4 correspond to immune response, 3 clusters with minimum gene expression on day 1 and consistently increasing after that are related to extracellular matrix development, 2 clusters are related to epithelization. As expected, "epithelization" clusters at the wound edge consistently maintain high expression levels, while in the wound center, expression diminishes on day 1 post-injury, returning to baseline by the end of the healing. Epithelial genes appear at wound center only during the later stages of healing correlating with histological re-epithelialization.

3 gene clusters exhibit a notable correlation between genes inside each cluster and possess specific GO annotations yet display non-smooth dynamics. The first of these clusters is labeled by GO as "Hair", "Nuscei" and "Lipid".



Our study highlights the dynamic spatiotemporal changes in immune response, extracellular matrix formation, and epithelialization during acute wound healing, while also emphasizing the challenges posed by wound tissue heterogeneity. Understanding these dynamics and addressing sampling challenges are essential for gaining accurate insights into wound healing mechanisms and developing improved therapeutic approaches. The data obtained in this study can serve as a valuable reference for future investigations in wound transcriptomics.