A novel ECM Platform for Skin Regeneration: Balancing Structural Integrity with Low Immunogenicity

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Rabbit skin is an abundant agri-food by-product which can be up-cycled for generating xenogeneic extracellular matrix (ECM) scaffolds for skin tissue engineering, regeneration and modelling. When decellularized, rabbit dermis can preserve native collagen–elastin architecture and bioactive cues that support cell behaviour [1]. However, decellularization parameters strongly influence ECM preservation, and trigger residual immunogenic components such as damage-associated molecular patterns (DAMPs) [2].

Decellularized rabbit dermal matrices (dRDMs) were produced using optimized chemical decellularization protocols and evaluated for decellularization efficiency, physicochemical properties and biochemical composition, using SEM, tensile testing, FTIR, proteomics, DNA, GAGs, collagen, and elastin quantification. Immunogenicity of dRDMs was assessed by endotoxin evaluation and using human peripheral blood mononuclear cells (PBMCs) to measure complement activation, ROS, apoptosis, activation markers and cytokines. dRDMs capacity to support human dermal fibroblasts (hDF) and keratinocytes (HaCaTs) adhesion and proliferation was assessed for 14 days, via Alamar Blue, BrdU, SEM, and DAPI/phalloidin staining.

The dRDMs preserved native collagen and elastin networks. GAGs content was comparable to human dermis. DNA remained <50 ng/mg dry tissue, indicating efficient decellularization. No significant immune activation occurred: ROS and apoptosis were absent. PBMC activation matched controls with mild monocyte/B-cell responses. Cytokine profiles revealed modest inflammatory signaling with IL-10 production. Notably, lower DNA matrices induced stronger immune response, suggesting that biocompatibility depends on processing factors like detergent residues or DAMPs rather than DNA alone. *In vitro*, hDF and HaCaT viability and proliferation were maintained for 14 days on dRDM, supporting its potential for dermo-epidermal reconstruction in advanced co-culture models. Overall, dRDMs exhibited structural, biochemical, and

immunological features supporting their potential as a robust dermal substrate for skin tissue engineering.

References

[1]Rosadas et al., *Front. Biomaterials*, 2024.
[2]Kasravi et al., *Biomaterials Research*, 2023.

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