

Unlocking the potential of decellularized rabbit dermal matrices for advancing skin regeneration

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Purpose

Burn wounds represent a significant challenge in medical care, particularly due to the complexity of dermal reconstruction. The use of autologous grafts as substitutes is the standard option, however, it may not be suitable to deep and extensive burns (1). An alternative approach involves employing artificial collagen-based dermal substitutes, which do not meet the full requirements of the dermis extracellular matrix (ECM) in terms of biochemical composition and architectural features (2). Thus, skin xenografts have emerged as a suitable option involving the need for decellularization to remove the immunogenic material preserved ECM for skin regeneration (3). Focused on these valences, this study describes for the first time a refining protocol for decellularizing rabbit dermis, a valuable agro-food by-product which exceeds 5000 skins/day. The presented approach allowed to obtain highly preserved decellularized dermal matrices with microarchitecture and biochemical properties similar to that of human dermis.

Methods

Rabbit skins by-product were processed at Cortadoria Nacional de Pêlo S.A., following a set of pioneer methodologies involving chemical, enzymatic and mechanical processing. The obtained purified rabbit dermis was further processed through selected chemical decellularization agents (SDS and SDC) with varying exposure periods, to achieve a fast and complete decellularization process with a minimum impact to dermal matrices' microarchitecture, mechanical properties and biochemical composition. The impact of the processing methods and decellularization agents on matrix preservation was examined by morphological analysis (SEM), swelling properties and tensile mechanical behavior, compared to that of human skin. The cellular content and decellularization effectiveness were confirmed by histological analysis and DNA quantification. Human dermal fibroblast (hDFs) were used for testing the in vitro cytocompatibility of the preserved decellularized rabbit dermal matrices (dRDMs). Further characterizations, including GAGs and collagen quantification are ongoing to confirm the dRDM preservation and a newly-formed ECM the seeded cells.

Results

The obtained results indicate that the applied methods and reagents at different pHs influence collagen matrix conformation, affecting the swelling capability and fluid interaction. Morphological analysis revealed different surface properties at the rabbit dermis, showing a flat epidermal-contacting surface with pores resulting from fur removal and another fibrous hypodermis-contacting surface, as well as different topographical effects depending on the decellularization agents and exposure time. Mechanical properties showed different impacts of the decellularization agents on collagen content of the dRDMs but with a good overall integrity throughout rabbit dermis processing. DNA quantification confirmed different

decellularization efficiencies (<50 ng/mg dry weight) depending on the processing stage and decellularization agents. From in vitro characterization it was observed that the obtained dRDMs supported hDFs adhesion and proliferation up to 7 days of culture.

Conclusions

This study marks the first demonstration of successfully clean chemical methods for rabbit dermis processing and decellularization, preserving ECM components and yielding high-quality matrices with superior biological, structural and biomechanical properties to cover large areas of the human body while promoting skin regeneration.

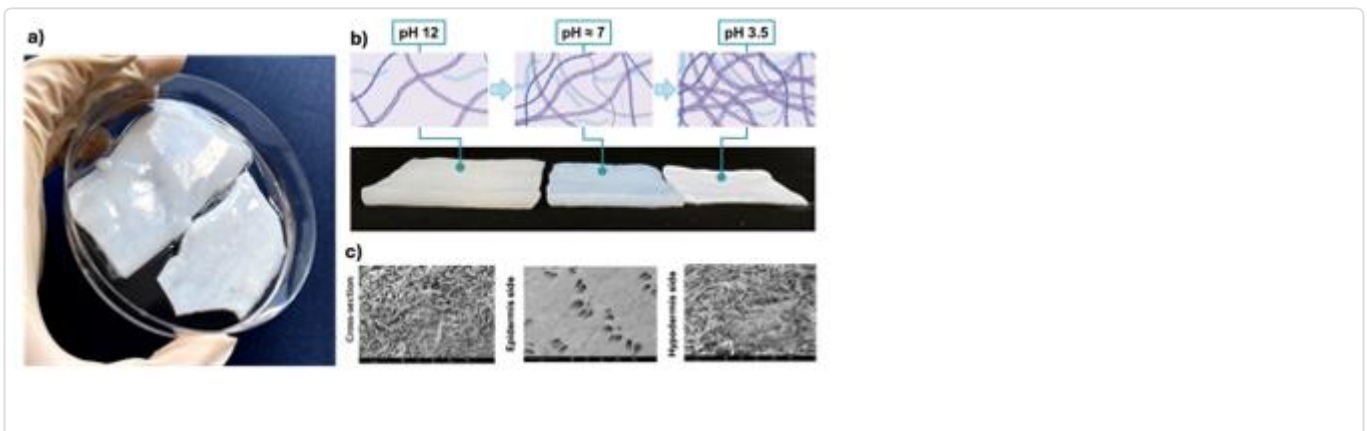
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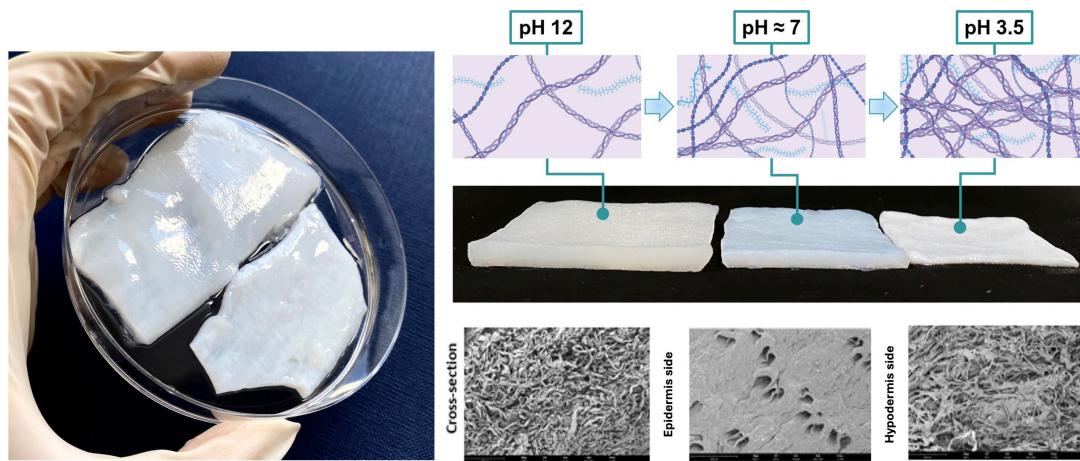
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**Schematic of the rabbit dermis processing**

a) Example of a dRDMs; b) illustration of the influence of pH on collagen conformation and tumescence of the dermis; c) Scanning electron microscopy (SEM) images of the cross-section, epidermal-contacting surface and hypodermiscontacting surface of dRDMs.