TITLE

Is Decellularized Rabbit Dermis a Viable Option for Skin wound healing and Regeneration?

Marta Rosadas[1], Teresa Sousa[1], Alda Sousa[2], Viviana P. Ribeiro*[1], Ana L. Oliveira[1]

1 - CBQF-Centro de Biotecnologia e Química Fina–Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal
2 - Cortadoria Nacional de Pêlo, S.A., S. João da Madeira, Portugal.
* vpribeiro@ucp.pt

INTRODUCTION

Burn wounds remain a significant challenge in medical care, requiring effective wound coverage to restore the skin barrier and promote healing or support skin reconstruction. The use of autologous grafts as substitutes is still the standard treatment, however, it is not suitable for deep and extensive burns (1). Decellularized skin allografts and xenografts have emerged as suitable options, using decellularization to remove the immunogenic material present in the tissue while preserving the ECM components and interesting biomolecules (3). Considering that xenografts source availability is significantly higher and free of ethical concerns, this study describes for the first time a protocol for decellularizing rabbit dermis, leveraging a valuable agro-food by-product which exceeds 5000 skins/day at the world-leading company Cortadoria Nacional de Pêlo, and studies its potential for skin regeneration.

MATERIALS AND METHODS

Rabbit skin agro-food by-product was 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 on 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. The cellular content and decellularization effectiveness were confirmed by DNA quantification. Further characterization, include ECM components such as GAGs, elastin, and collagen quantification to confirm the dRDM preservation and a newly formed ECM the seeded cells. Human dermal fibroblasts (hDFs) were used for testing the *in vitro* cytocompatibility of the preserved decellularized rabbit dermal matrices (dRDMs). Further in vitro tests to evaluate the immune and inflammatory response to the dRDMs using human peripheral blood mononuclear cells (PBMCs) are ongoing.

RESULTS AND DISCUSSION

The developed decellularization protocol effectively removed cellular components, achieving DNA concentrations below the 50 ng/mg dry weight threshold while retaining good quantities of GAGs, collagen and elastin essential for wound healing. ECM integrity was preserved, with SEM evidencing a flat epidermal-contacting surface with pores resulting from fur removal and another fibrous hypodermis-contacting surface favorable for cell infiltration and with resemblance with human dermis and thickness in the range

of human skin. The swelling properties demonstrated a pH-responsive behavior, maintaining hydration and flexibility under physiological conditions. Mechanical testing revealed a stress-strain profile similar to human dermis. *In vitro* studies showed no cytotoxic effects induced by the dRDMs (cell viability >70%), and a suitable biocompatibility allowing hDFs adhesion and proliferation over time. The presented approach demonstrated the feasibility of using decellularized rabbit skin as a viable dermal substitute for skin tissue healing and regeneration applications. The incorporation of a sustainable by-product derived biomaterial aligns with circular economy principles, presenting an eco-friendly alternative to conventional dermal substitutes.

CONCLUSION

The highly preserved dRDM obtained presents microarchitecture and biochemical properties similar to that of human dermis, exhibiting promising characteristics, in applications such as wound healing. The incorporation of a sustainable by-product derived biomaterial aligns with circular economy principles, presenting an eco-friendly alternative to conventional dermal substitutes.

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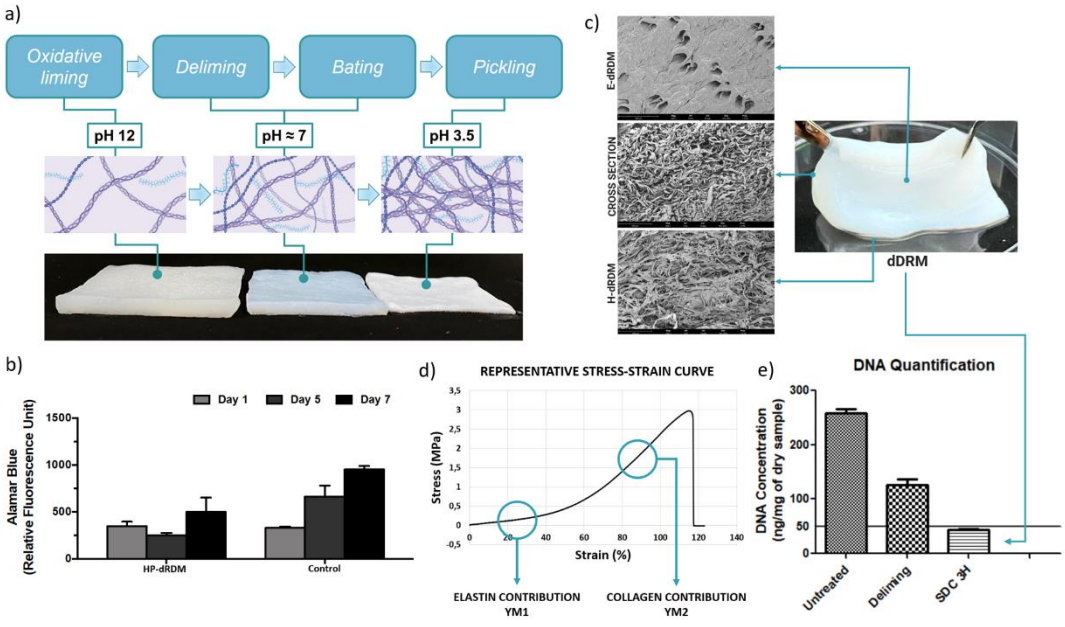

Figure 1- a) pH responsiveness and b) direct contact assay with HDFs c) dRDM SEM images; stress-strain curve from tensile testing; and e) DNA concentration variation.

Keywords: Skin; Wound healing; Biological material; Biocompatibility, leachables, in vitro tests

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