Is Decellularized Rabbit Dermis a Viable Option for Skin Wound Healing and Regeneration?

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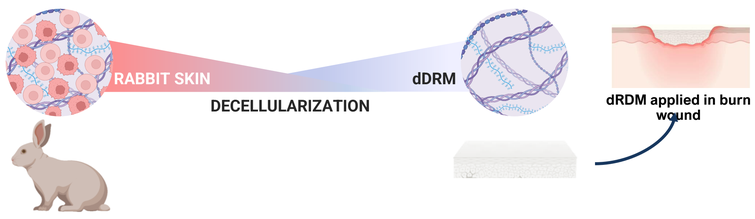
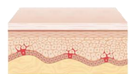
INTRODUCTION

Burn wounds remain a major clinical challenge, requiring effective coverage to restore the skin barrier and support healing. Autologous grafts are the standard treatment but are limited in deep or extensive burns [1]. Decellularized allografts and xenografts offer alternatives by removing immunogenic material while preserving ECM components [2]. 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 that exceeds 5000 skins/day at the world-leading company Cortadoria Nacional de Pêlo, and studies its potential for skin regeneration.



MATERIALS AND METHODS

COMPANY'S PROTOCOL ANALYSIS



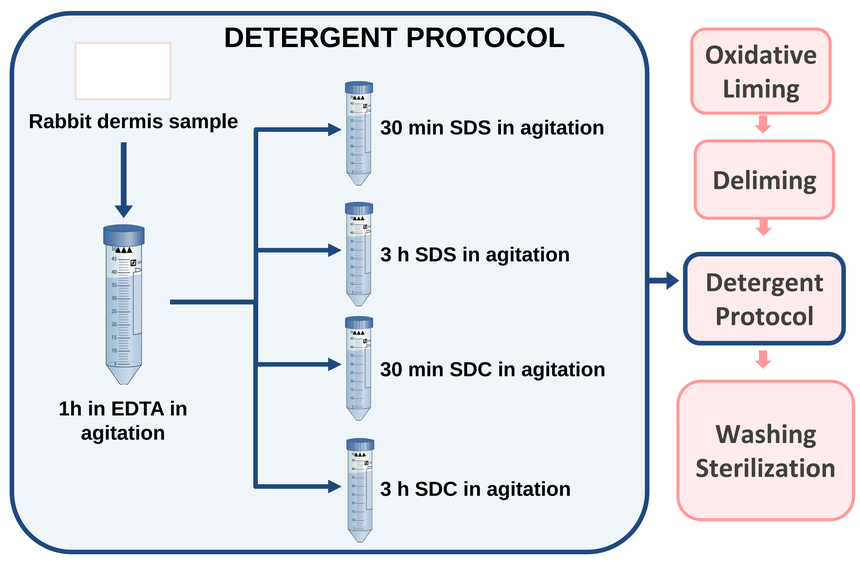
The protocol for skin preparation for tannery included treatments also used for decellularization.

- **Skin Selection** — Skin selection according to the company's specifications.
- **Oxidative Liming** — Hair and epidermis removal. Calcium hydroxide, hydrogen peroxide, sodium hydroxide, and specific enzymes. (pH = 12)
- **Mechanical Processing** — Mechanical removal of the subcutaneous fat tissue.
- **Deliming** — Removal of oxidative liming reagents, specially calcium hydroxide. pH decreases until around 7.
- **Bating** — Enzyme application.
- **Pickling** — Application of formic acid (pH = 3,5).

Which treatments were worth including in the final decellularization protocol?

DECELLULARIZATION PROTOCOL

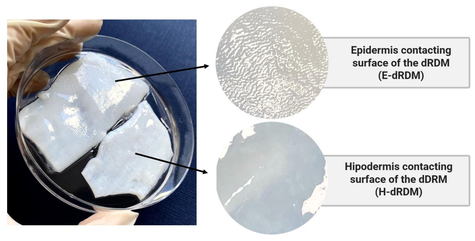
To complement the treatments outlined in the company's protocol, a rapid detergent-based protocol was developed. Were studied two detergents, SDS and SDC, for two application timings, 30 minutes and 3 hours.



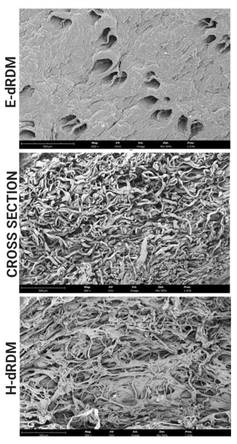
RESULTS AND DISCUSSION

COMPANY'S PROTOCOL ANALYSIS

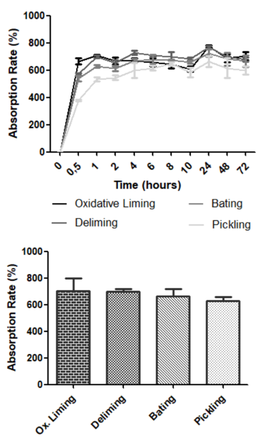
MORPHOLOGICAL ANALYSIS



SEM IMAGES

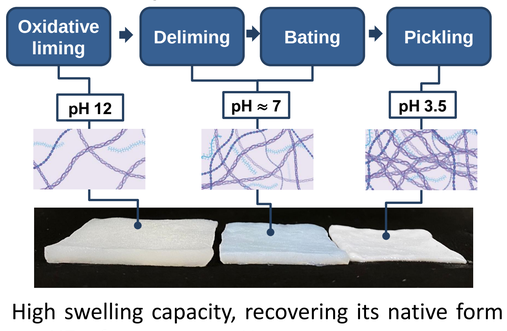


SWELLING PROPERTIES



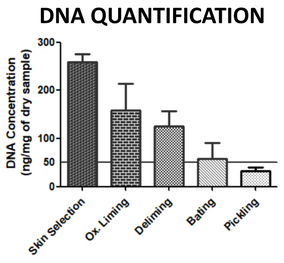
The rabbit dermal matrix closely resembles human dermis, with a porous top surface (E-dRDM) and a fibrous cross-section and bottom (H-dRDM).

pH RESPONSIVENESS

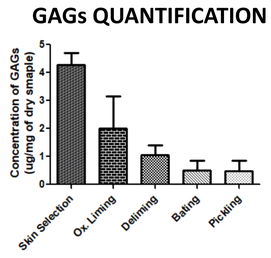


High swelling capacity, recovering its native form at pH7, after extreme pH exposure

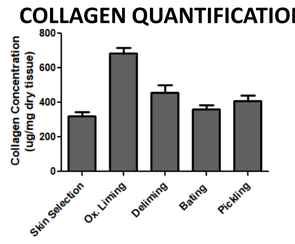
DNA QUANTIFICATION



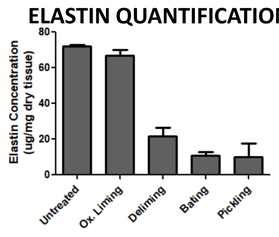
GAGs QUANTIFICATION



COLLAGEN QUANTIFICATION

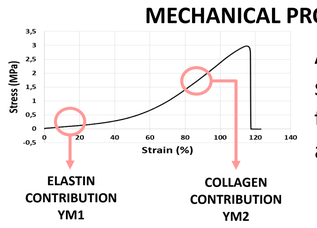


ELASTIN QUANTIFICATION

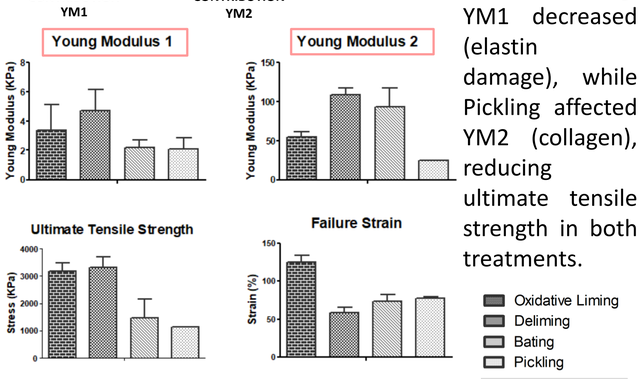


dsDNA quantification revealed a progressive reduction in DNA content after each treatment, with final values reaching the 50 ng dsDNA/mg dry tissue threshold for effective decellularization [3]. Collagen concentration increased during Oxidative Liming, while elastin and GAGs were markedly reduced.

MECHANICAL PROPERTIES ANALYSIS

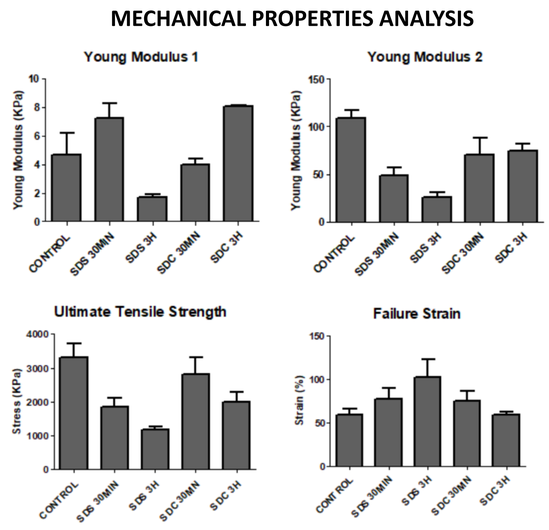


All samples showed similar stress–strain behavior with two zones linked to elastin and collagen contributions



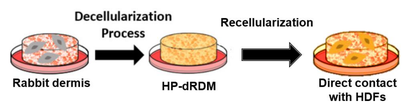
After Bating, YM1 decreased (elastin damage), while Pickling affected YM2 (collagen), reducing ultimate tensile strength in both treatments.

MECHANICAL PROPERTIES ANALYSIS

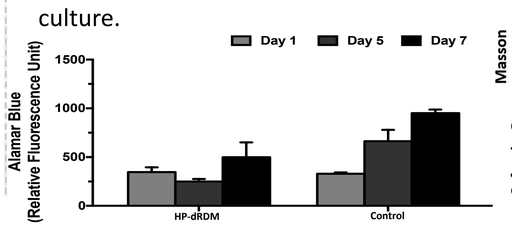


SDS treatment applied for 3H had a negative effect on YM1 and YM2, resulting in a weaker ultimate tensile strength. On the other hand, SDC treatments were able to maintain properties with no differences from the control. The SDC 3H treatment showed better overall results.

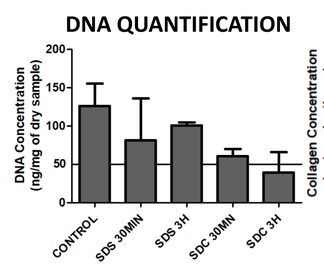
IN VITRO ASSAYS



Preliminary direct contact assays showed that human dermal fibroblasts (hDFs) were able to adhere and proliferate in the matrix up to 7 days of culture.



DNA QUANTIFICATION



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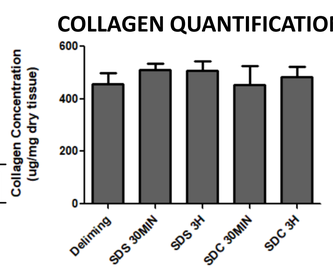

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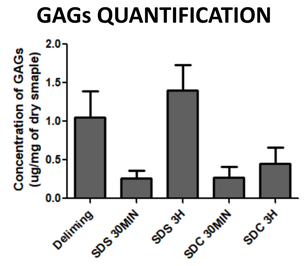
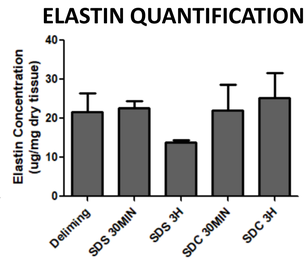


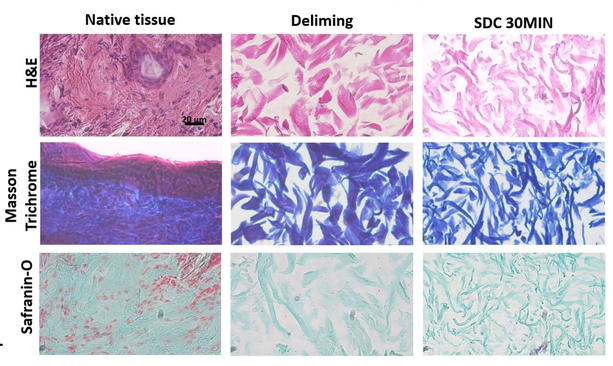
ELASTIN QUANTIFICATION



SDC treatments proved more effective in DNA removal. Collagen content was preserved across all protocols, although mechanical analysis suggested that SDS may damage collagen fibers. Elastin quantification further indicated that SDS not only induced structural damage but also reduced elastin concentration within the matrix.

GAG content decreased to 0.5 µg/mg, a level still sufficient to support cellular responses. However, SDS-treated samples showed inconsistent GAG values, likely due to interference from residual SDS.

HISTOLOGY



Histology analysis revealed ECM preservation, with collagen fibers shown by Masson's Trichrome and GAGs by Safranin-O staining

CONCLUSIONS

A rabbit skin decellularization protocol was successfully optimized to produce a preserved decellularized rabbit dermal matrix (HP-dRDMs) by combining industrial-scale methods with a fast SDC process. DNA content was reduced below 50 ng dsDNA/mg, meeting decellularization standards, while ECM components—including collagen, elastin, and GAGs—were well preserved, as confirmed by mechanical properties and histological analysis. Furthermore, *in vitro* studies confirmed the cytocompatibility of HP-dRDMs, highlighting their potential for biomedical applications.

REFERENCES

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2. Rosadas, M., et al., (2024). Frontiers in Materials, 12(10), 1285948.
3. Crapo, P. M. (2011) https://doi.org/10.1016/j.biomaterials.2011.01.057

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