Beyond DNA Removal: Assessing the Immunological Response to Decellularized Rabbit Dermal Matrices

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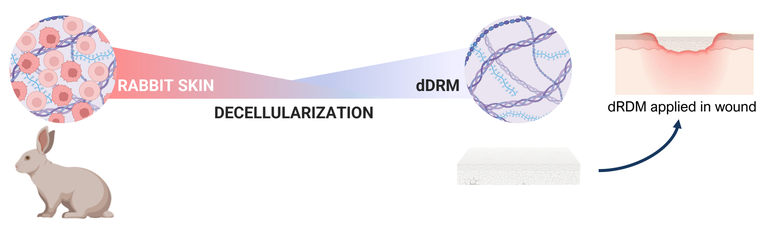
INTRODUCTION

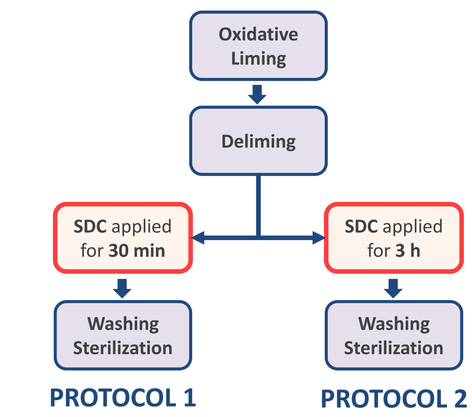
Decellularized matrices have attracted considerable attention in tissue engineering and regenerative medicine due to their ability to preserve the biochemical composition and microarchitecture of native tissues [1]. Although DNA removal is commonly used as the main indicator of decellularization efficiency, other factors—such as endotoxin contamination, damage-associated molecular patterns, and residual reagents—can significantly influence the host immune response [2,3].

In this study, decellularized rabbit dermal matrices (dRDM) produced using two different protocols—resulting in DNA levels below or above the proposed safety threshold of 50 ng/mg dry tissue—were evaluated for their immunological performance.



MATERIALS AND METHODS

DECELLULARIZATION PROTOCOL



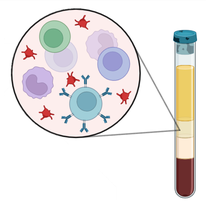
For all the assays were used 8mm punched samples.

ENDOTOXIN QUANTIFICATION

≈ 100mg of sample were incubated in 40mL of endotoxin free water at 37°C for 24 hours. Endotoxin concentration of the leachable was quantified using LAL Assay.

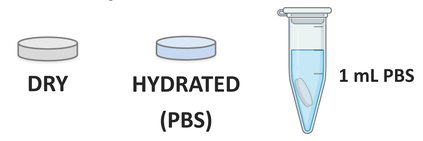
STERILITY VERIFICATION

✓ Turbidity test
✓ Agar LB incubation test



Peripherical blood mononuclear cells (PBMCs) were isolated from fresh blood samples from healthy donors before each assay through Ficoll density gradient centrifugation.

Samples of dRDM of both protocols were analyzed in 3 forms:



ROS PRODUCTION INDUCTION

4H INCUBATION
- dRDM samples
- Positive control: PMA
- Negative control: just cells

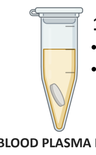
PBMCs → H_2DCFDA (DCFH-DA) ROS assay → Flow cytometry

ANNEXIN V ASSAY

OVERNIGHT INCUBATION
- dRDM samples
- Positive control: PMA, Camptothecin
- Negative control: just cells

PBMCs → Annexin V Assay → Flow cytometry

COMPLEMENT ACTIVATION



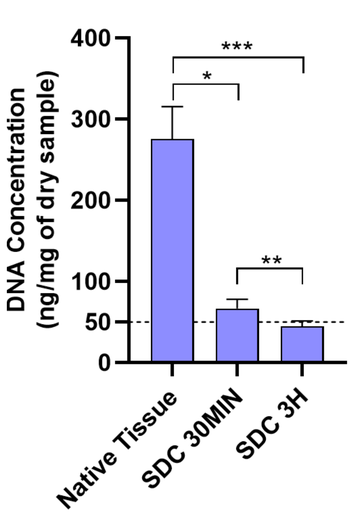
1H INCUBATION
- dRDM samples
- Positive control: Zymosan A
- Negative control: just plasma

BLOOD PLASMA POOL → **Western Blot for C3, C3a and C3b identification**

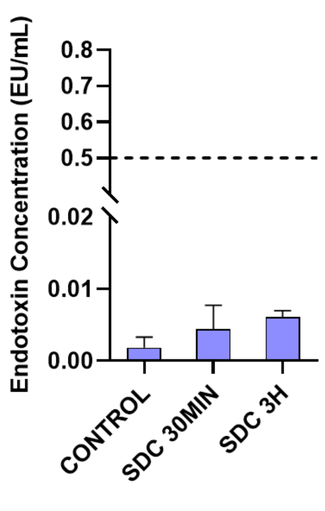
Significant activation was considered when the band had ≥2 times the % relative intensity o the negative control.

RESULTS AND DISCUSSION

DNA Quantification



Endotoxin Quantification (EU/mL)



dRDMs were successfully obtained using both protocols, which significantly reduced DNA content. The SDC 3H protocol met the recommended threshold (<50 ng DNA/mg dry tissue), while the SDC 30 min protocol also achieved low residual DNA levels (xxxx). Endotoxin levels in both dRDMs were below the FDA limit (0.5 EU/mL) for this type of medical device.

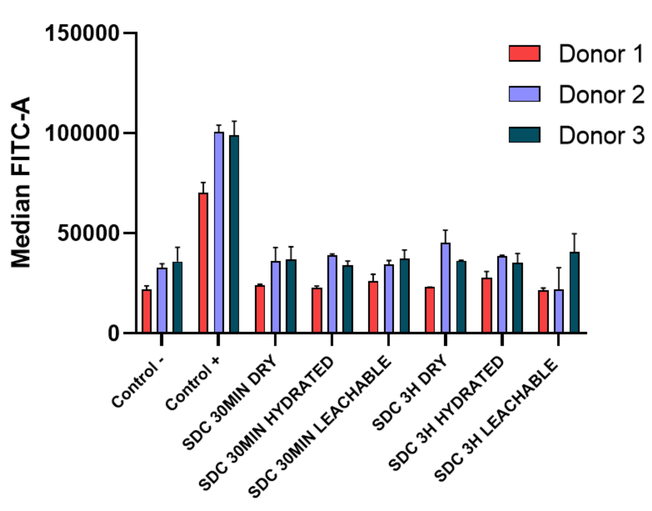
Proteomic analysis

Log10(Abundance)
Max — Min

Protein Name	Gene Symbol	SDC 30MIN	SDC 3H
Collagen type I alpha 1 chain	COL1A1		
Collagen type I alpha 2 chain	COL1A2		
Collagen type III alpha 1 chain	COL3A1		
Collagen type V alpha 2 chain	COL5A2		

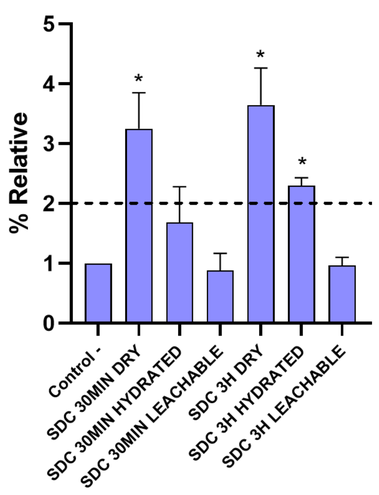
Proteomic analysis revealed that both dRDMs were predominantly collagenous. The SDC 30 min protocol preserved a collagen profile similar to native dermis, while the SDC 3H showed alterations suggestive of collagen degradation, potentially associated with damage-associated molecular patterns (DAMPs) release and compromised tissue integrity.

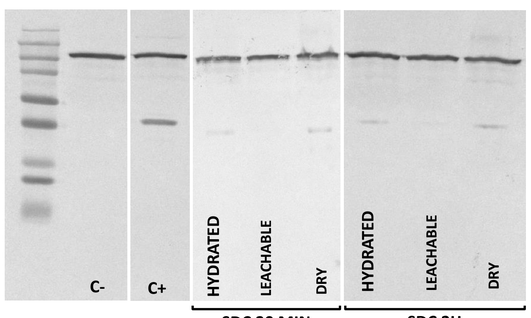
ROS Production



All conditions showed ROS levels comparable to the negative control, while the positive control induced a marked increase in ROS. These results indicate that dRDMs and their released products induce minimal immune activation and oxidative stress.

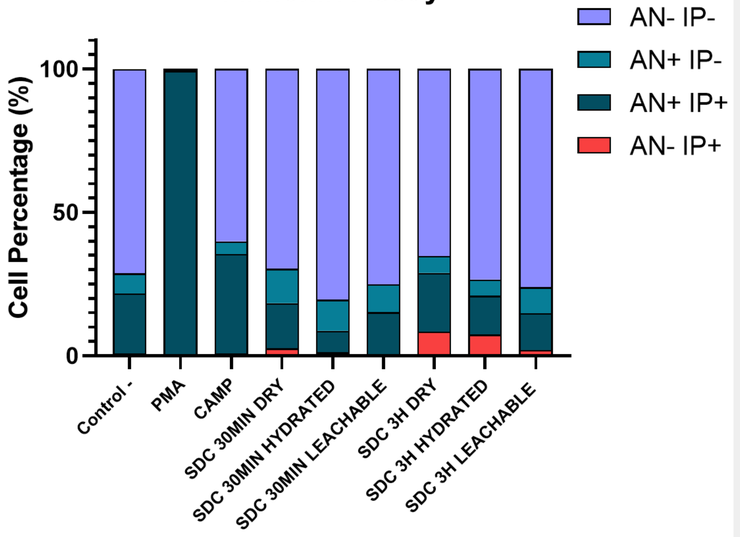
Complement Activation





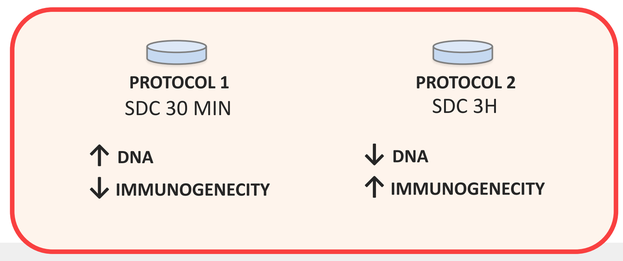
Dry matrices from both protocols induced complement activation, likely due to exposure of collagen-associated triggering moieties while dehydrated. Among hydrated matrices, only the SDC 3H dRDM showed significant activation, although at lower levels than the positive control.

Annexin Assay



Annexin V assays showed comparable levels of healthy cells across all samples and the negative control. Hydrated dRDMs produced with the SDC 30 min protocol showed the highest viability and lowest apoptosis/necrosis, while dry matrices and hydrated SDC 3H dRDMs exhibited increased membrane damage (AN−/IP+). Leachables had only minor effects, indicating minimal detergent contribution.

Overall, the results suggest that cell responses were mainly driven by physical stress at the cell–matrix interface, exacerbated by collagen degradation and matrix dryness.



CONCLUSIONS

Two decellularization protocols produced dRDMs with distinct biological profiles. Although the SDC 3H protocol achieved DNA levels below the recommended threshold, it was associated with greater collagen degradation, whereas the SDC 30 min protocol better preserved native dermal collagen. Both matrices were sterile, endotoxin-free, and did not induce ROS production in PBMCs. However, dry dRDMs and SDC 3H-treated matrices promoted complement activation and increased PBMC necrosis. Overall, despite not meeting the recommended DNA threshold, the SDC 30 min protocol demonstrated superior immunocompatibility and collagen preservation, highlighting that DNA content alone is not a reliable predictor of decellularization efficiency. Instead, immunocompatibility, collagen integrity, physical cell–matrix interactions, and potential detergent residues play a critical role in defining matrix performance.

REFERENCES

1. Rosadas M et al., 2024, Frontiers in Biomaterials and Bio-Inspired Materials

2. Kasravi M et al., 2023, Biomaterials Research

3. Cheng W et al., 2025, Acta Biomaterialia.

ACKNOWLEDGEMENTS

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