Decellularized Matrices with Inductive Cues and Structural Integrity for Functionalized Scaffolding and Bioprinting

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BACKGROUND: In regenerative medicine, two important aspects for grafting materials are biological functionality and mechanical strength. Extracellular matrices (ECM) provide the biological, physical, and mechanical properties essential for cell proliferation and differentiation. However, decellularization processes are often extremely abrasive, damaging the 3D structure and the native growth factors of the tissue. In the present study, a novel method for decellularization has been proposed, with promising results in structure preservation and cell signalization. This process would allow using decellularized matrices (dECM) to fabricate more biocompatible and functional scaffolds and bioinks for regenerative medicine.

HYPOTHESIS: This work has the purpose to demonstrate the suitability of hypotonic treatment for tissue decellularization. The gradual use of hypotonic solutions represents a gentler method for decellularizing tissues, providing matrices with an efficient preservation of structure and inductive cues.

METHODS: Native skin (S), dermis (D) and blood vessels (BV) were decellularized (dS, dD and dBV). Tissue samples were submerged in a continuous hypotonic solutions gradient (80-0 mM), in nuclease solution, and incubated (37°C, 5% CO2). Subsequently, DNA was extracted from samples, and visualized on an agarose gel electrophoresis (GE); DNA concentration was quantified using Qubit™. Additionally, a histological examination was performed with hematoxylin and eosin staining (H&E). Finally, human fibroblasts were cultivated on the dECMs and stained with DAPI for nuclei visualization.

RESULTS: Both quantitative and qualitative confirmation of decellularization were obtained from DNA quantification and GE visualization. Figure 1A presents a decrease on DNA concentration between ECM samples and dECMs; we correlated such difference as the percentage of decellularization achieved on each tissue (dS: 98.82%; dD:72.43%; dBV: 98.49%). Correspondingly, there is no band visualization on dECM samples on GE, differing from ECMs lanes (Fig. 1B and 1C). H&E examination for S shows presence of cell nuclei; epidermis exhibits orthokeratotic keratinization, while there is minor edema and preserved adnexa in the dermis. Whereas, in H&E for dS, there is absence of cells (Fig. 2). Similar results can be observed for D and dD, and BV and BV, where D also presents edema, and BV shows an arterial wall in which the tunica intima, media and adventitia can be observed. Finally, cytochemical staining with DAPI, showed cell migration of human fibroblasts throughout dECMs structure (Fig. 3), demonstrating preservation of structural integrity and functionality to support cell growth and adhesion.

CONCLUSIONS: Skin, dermis and blood vessels were decellularized implementing a hypotonic process that allowed for effective cell removal and successful preservation of physical structure and biological functionality. These results indicate a promising use of decellularized matrices in regenerative medicine as scaffolding and bioprinting materials.

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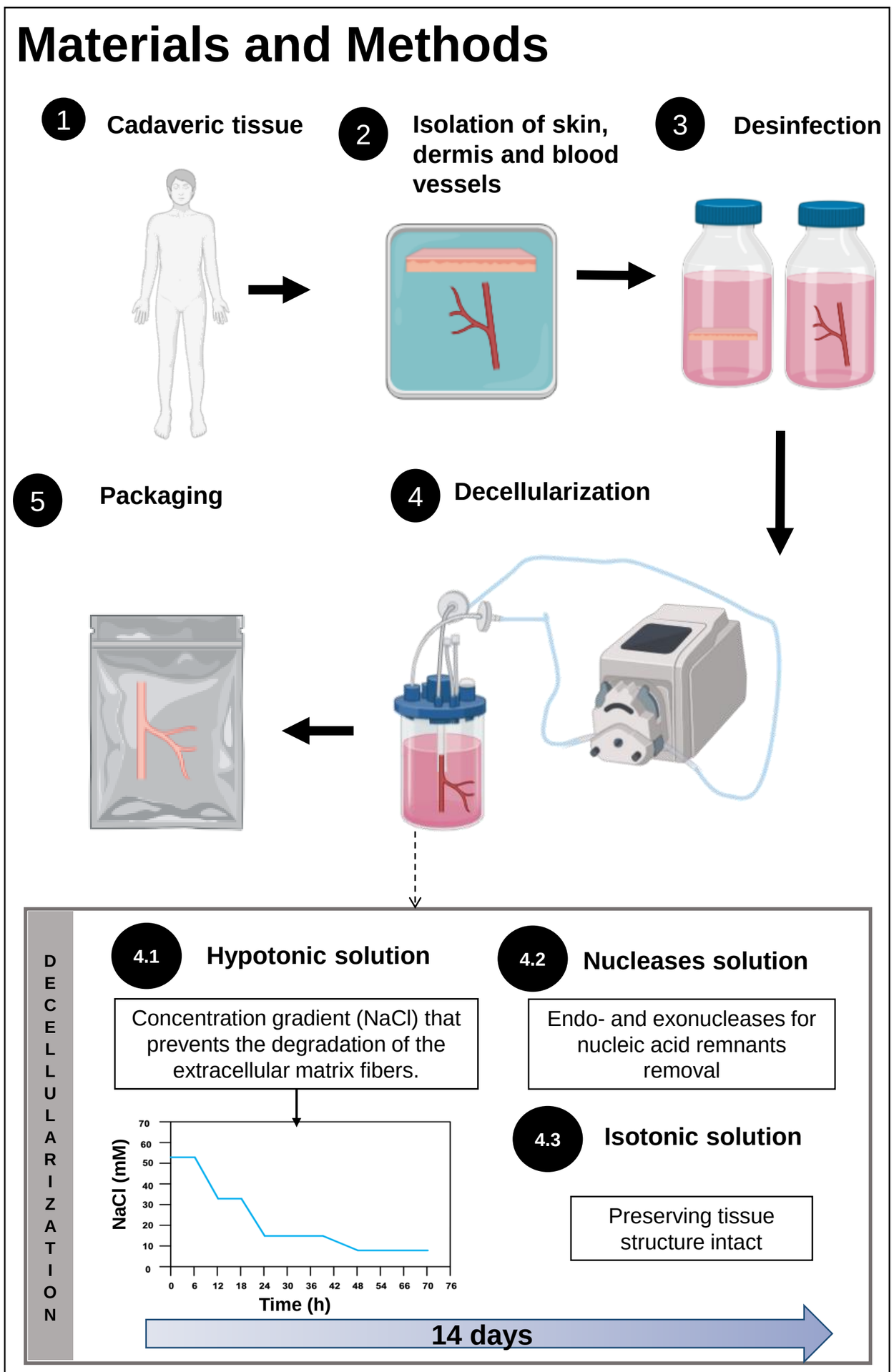
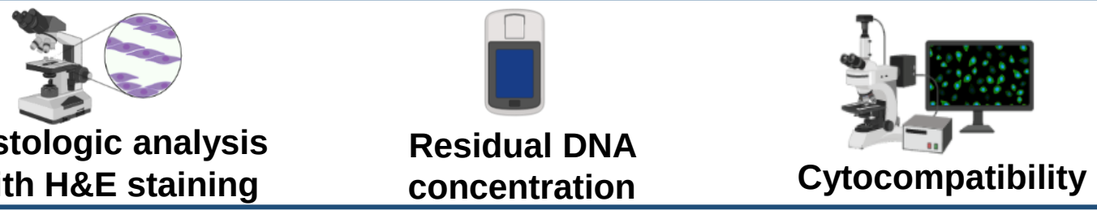
Introduction

In regenerative medicine, two important aspects for grafting materials are biological functionality and mechanical strength. Extracellular matrices (ECM) provide the biological, physical, and mechanical properties essential for cell proliferation and differentiation. However, decellularization processes are often extremely abrasive, damaging the 3D structure and the native growth factors of the tissue. In the present study, a novel method for decellularization has been proposed, with promising results in structure preservation and cell signalization. This process would allow using decellularized matrices (dECM) to fabricate more biocompatible and functional scaffolds and bioinks for regenerative medicine.

Materials and Methods



Evaluation of decellularized extracellular matrix its integrity and functionality after 21 days of preservation



Results

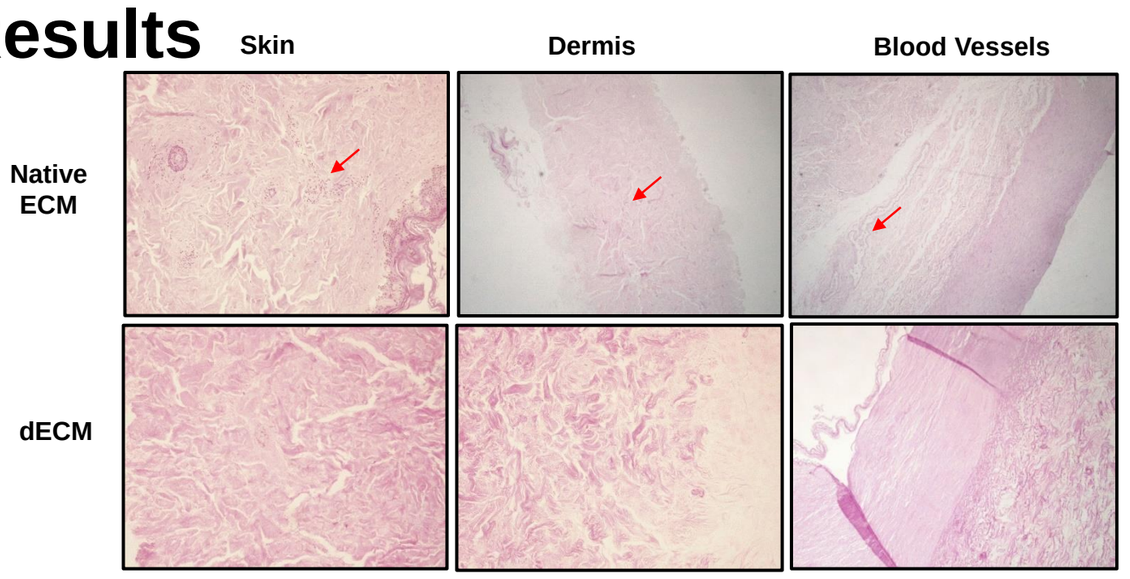


Figure 1. Histological examination of tissue samples, stained with hematoxylin and eosin staining (H&E). Red arrows signaling cell nuclei. ECM: Extracellular matrix. dECM: Decellularized matrix.

A

Sample	Removal of DNA (%)
Skin	98.82
Dermis	72.43
Blood vessels	98.49

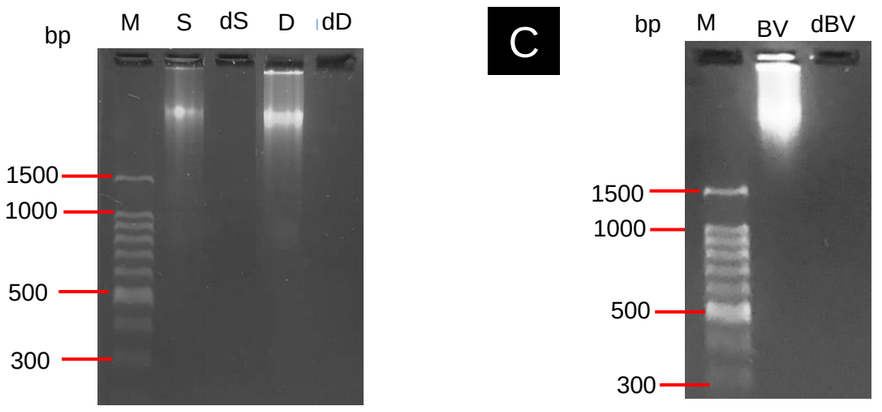


Figure 2. A) DNA concentration on tissue samples, quantified with Qubit™. dECM: Decellularized matrix. **B)** and **C)** DNA visualization on agarose gel electrophoresis (GE) of tissue samples. bp: Base pair. M: Molecular weight marker. S: Native skin. dS: Decellularized skin. D: Native Dermis. dD: Decellularized dermis. BV: Native blood vessels. dBV: Decellularized blood vessels.

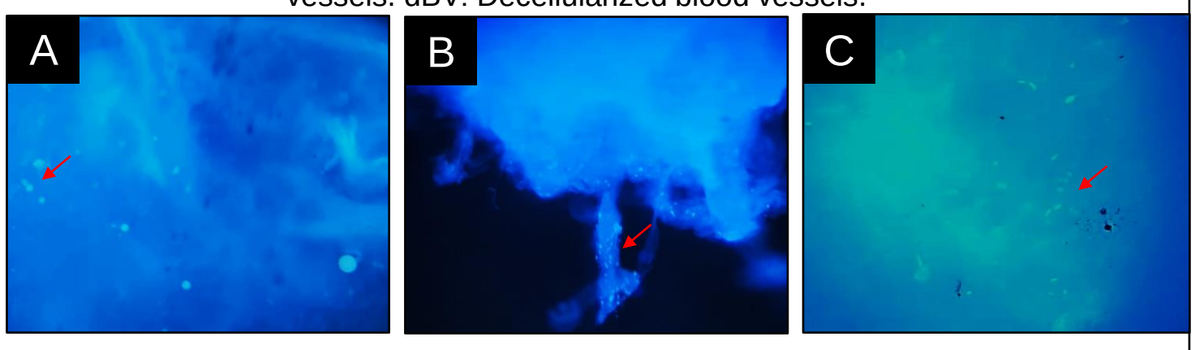


Figure 3. Cytochemical staining with DAPI. **A)** Decellularized skin. **B)** Decellularized dermis. **C)** Decellularized blood vessels. Red arrows signaling cell nuclei.

Discussion and Conclusions

Skin, dermis and blood vessels were decellularized implementing a hypotonic process that allowed for effective cell removal and successful preservation of physical structure and biological functionality. These results indicate a promising use of decellularized matrices in regenerative medicine as scaffolding and bioprinting materials

References

[1]Gilbert TW, Sellaro TL. Badylak SF. Biomaterials, 27(19), 3675-3683, 2006;
[2]Crapo PM, Gilbert TW, Badylak SF. Biomaterials, 32(12), 3233-3243,2011.