Comparative analysis of skin extracellular matrix derived from mice in regenerative and reparative developmental stages

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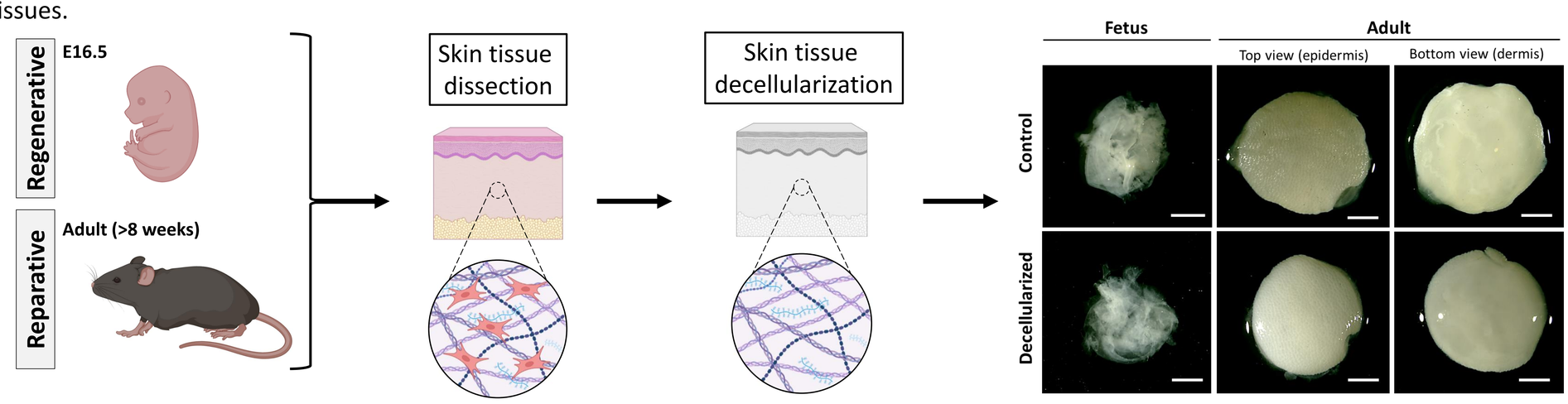
INTRODUCTION

Tissue regeneration capacities vary significantly among different organisms of the animal kingdom. Fetal mammals, in contrast to adults, are capable of regenerating certain tissues such as skin. In fact, tissues derived from animal models with high regenerative capacity, have been described to have intrinsic mechanisms that allow for scarless wound healing. One of the most promising approaches to generate scaffolds is the use of decellularized animal tissues which, compared to artificial biomaterials, have the advantage of retaining the native ECM along with its cell adhesion ligands and their structural features, resulting in increased cell infiltration, higher functional value, mechanical strength and lower inflammatory response. In this project we hypothesize that the extracellular matrix (ECM) derived from the skin of animals with greater regenerative capacities will have a greater regenerative potential when used as scaffold for tissue regeneration.

METHODOLOGY

In this work we optimized a skin decellularization protocol to further characterize and compare the ECM derived from fetus and adult mice, which present regenerative and reparative capacities respectively. First, structural, biomechanical and functional analysis of the decellularized tissues was performed by different techniques, including histology, SEM imaging and texture analysis. Moreover, different techniques were employed in order to assess the *in vitro* biocompatibility of scaffolds derived from fetus or adult mice skin. First, cells were seeded directly on decellularized skin tissues. Secondly, commercial collagen scaffolds were supplemented with ECM-based hydrogels generated with freeze dried and milled decellularized tissues.



Skin characterization:

1. Descellularization checking.
2. Structural characterization.
3. Biomechanical and functional characterization.
4. *In vitro* biocompatibility.

RESULTS

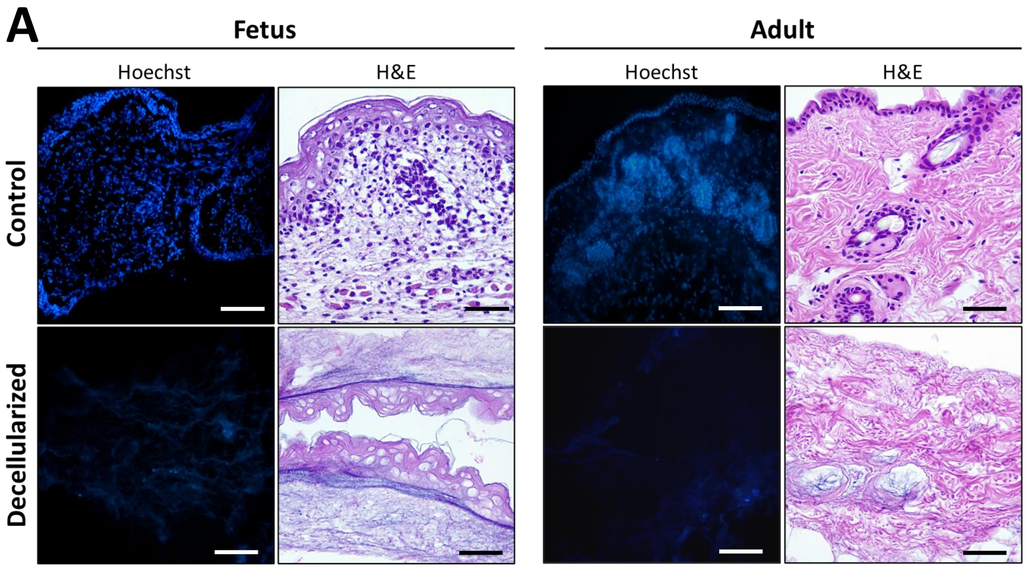


Fig. 1: Decellularization of fetus and adult skin. (A) Decellularization was confirmed by Hoechst nuclei staining and H&E. (B) Moreover, absence of DNA content in the decellularized tissues was assessed by electrophoresis in agarose gel (upper) and using dsDNA Quant-iT PicoGreen® kit (lower). Arrow in B, upper, indicates 200 Bp. Red dotted line in B, lower, indicates the maximum DNA allowed in decellularized tissues for clinical use. Scale bars in A represent 250 µm (Hoechst) and 50 µm (H&E).

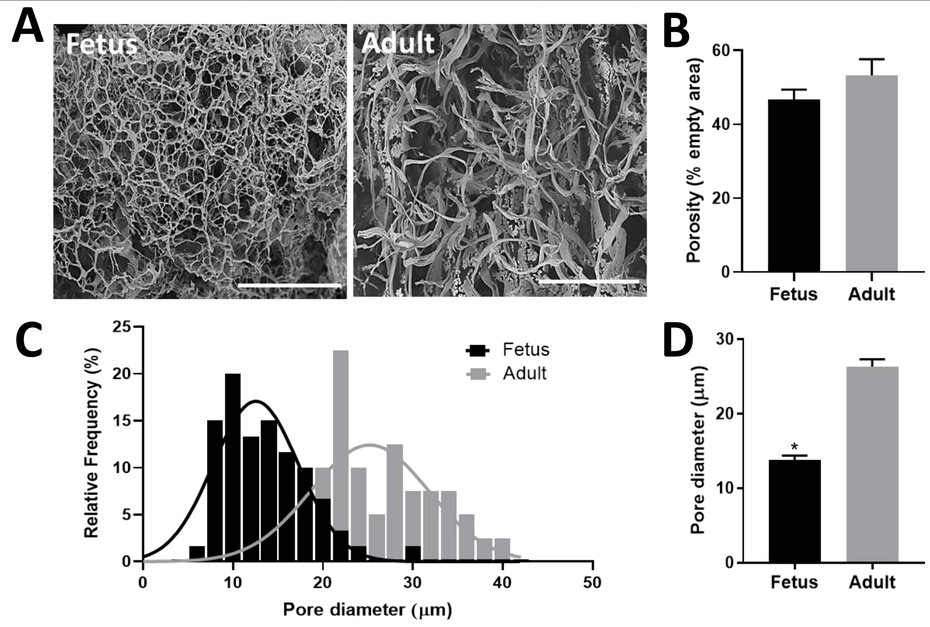


Fig. 2: Structural analysis. (A) SEM imaging of decellularized fetus and adult skin was performed. (B) Porosity, (C) pore distribution and (D) pore diameter of both tissues was studied. Scale bars represent 50 µm.

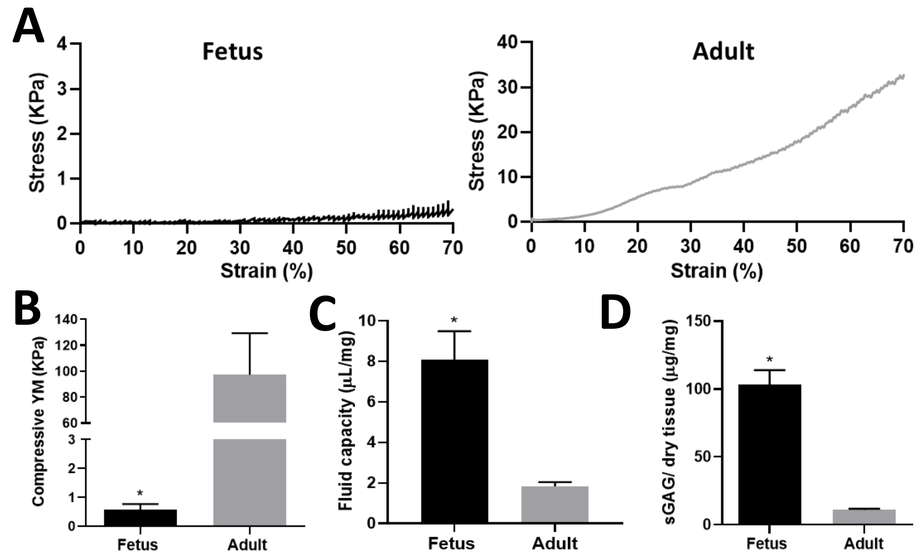


Fig. 3: Mechanical and functional characterization. (A) Compressive tests using a texture analyzer were performed on both fetus and adult decellularized skin tissues. (B) Compressive Young Modulus (YM) was calculated from the linear region of the stress-strain curves. (C) Fluid capacity of fetus and adult skin was quantified, correlating as well with GAG content (D).

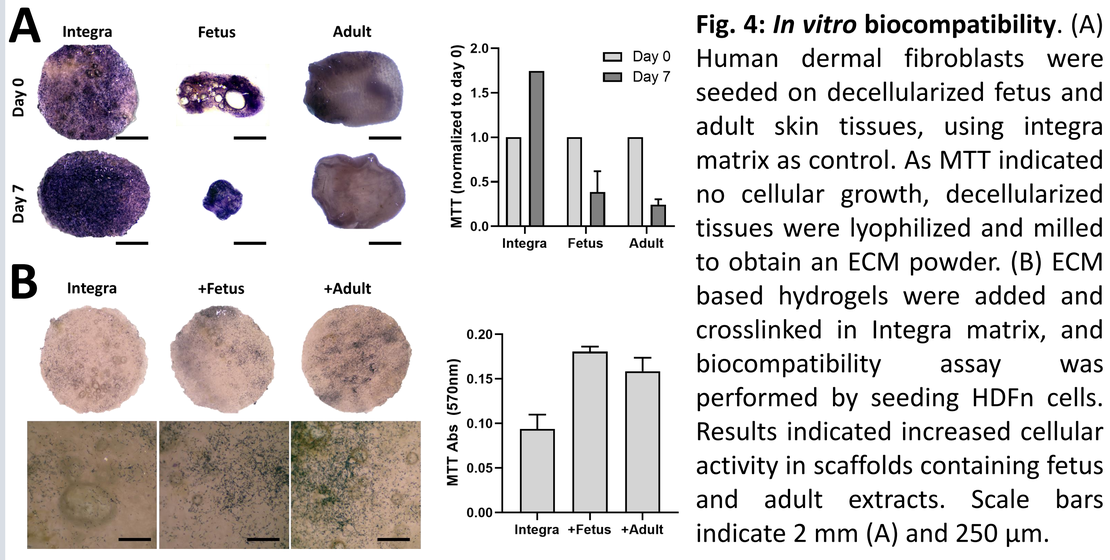


Fig. 4: *In vitro* biocompatibility. (A) Human dermal fibroblasts were seeded on decellularized fetus and adult skin tissues, using integra matrix as control. As MTT indicated no cellular growth, decellularized tissues were lyophilized and milled to obtain an ECM powder. (B) ECM based hydrogels were added and crosslinked in Integra matrix, and biocompatibility assay was performed by seeding HDFn cells. Results indicated increased cellular activity in scaffolds containing fetus and adult extracts. Scale bars indicate 2 mm (A) and 250 µm.

CONCLUSIONS AND FUTURE WORK

Decellularized fetus and adult skin tissues were successfully obtained. Structural, mechanical and functional properties differed significantly among both decellularized fetus and adult tissues, which could potentially influence the biocompatibility and regenerative potential of these tissues used as scaffolds. However, further characterization has to be performed, including quantification of ECM components such as collagen or elastic fibers. *In vitro* assays are being optimized to effectively assay the biocompatibility and regenerative potential of the scaffolds. Finally, *in vivo* models could give further insights of the regenerative potential of the scaffolds previously obtained from regenerative vs reparative developmental stages.

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