

Introduction

Total-partial gastrectomy, congenital gastric malformations and other causes of permanent gastric size reduction may result in severe side effects and poor quality of life. A bioengineered stomach transplant could thus restore a physiological transit and a digestive function, while being fully compatible.

Although, the clinical applications are limited, a bioengineered stomach could be an excellent 3D study model of composite tissue engineering. Our previously reported technique, of vascularized extra-cellular matrix (ECM) production from a porcine auricular subunit graft, has been translated to a pig stomach model.

Materials and methods

Eleven neonatal porcine stomachs were surgically harvested along with their vascular pedicle and decellularized by sequential perfusion of demineralized water, sodium dodecyl sulfate, Triton X-100, finalized by DNase and PBS solutions. Cell clearance was evaluated by H&E and DAPI staining, and DNA quantification. Extracellular matrix preservation was assessed by Masson's Trichrome (MT), type I and type IV collagens, and laminin stainings, elastin and GAGs quantification.

The vascular network was evaluated with angio-CT. Native and decellularized stomachs were perfused through the esophagus with air to assess the preservation of the tightness of the gastric wall and then with saline serum to evaluate the mechanical properties during the filling.

Finally, matrix samples were sterilized and cultured with a fibroblastic cell line and analyzed by H&E and Live/dead stainings.

Results

Scaffold production – In vitro and in vivo vasculature assessment

Decellularization was successful in all stomach grafts within 8 days. The acellular scaffold is white with a preservation of the initial morphology and also the inner villi. CBCT showed a preservation of the entire vascular tree could allow a satisfying future reperfusion (**Fig 1**).

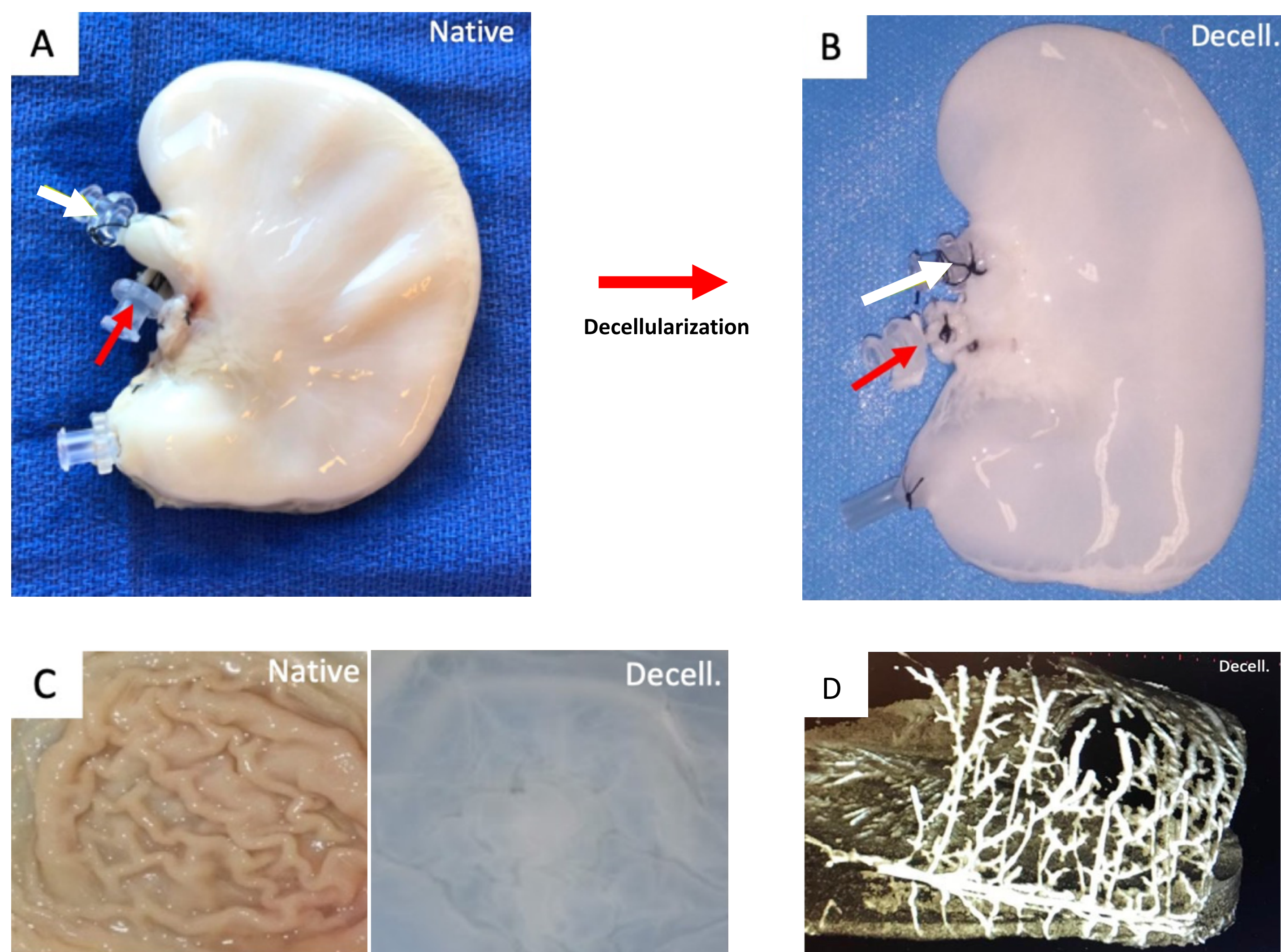


Fig 1: Native (A) and decellularized (B) stomach graft; red arrow = celiac trunk, white arrow : esophagus. (C) Inner side before (up) and after decellularization (low) show that the villi are preserved. (D) CBCT of the decellularized stomach after injection of barium sulfate in celiac trunk.

Cell clearance and ECM preservation

Cell removal was confirmed by H&E and PAS stainings. The DAPI staining confirmed the nucleus clearance and the quantification of the DNA after the decellularization process showed a reduction of 99% of the DNA levels (**Fig 1**).

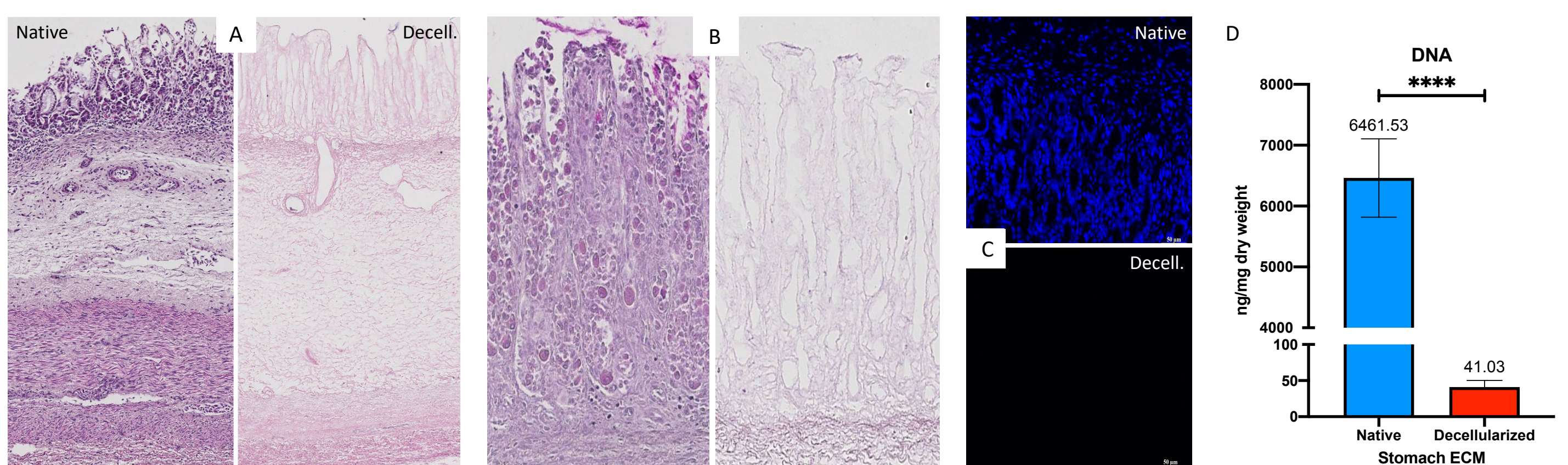


Fig 2: H&E with 4x gross magnification (A) and PAS staining with 20x gross magnification (B) of native and decellularized stomachs. (C) DAPI staining of native and decellularized stomachs (Scale bare 50 µm). (D) DNA quantification in native and decellularized samples.

ECM preservation

Histological evaluation showed a preservation of the ECM components, mostly type I and IV collagen (**Fig 3-4**). Matrix proteins quantification revealed an increase in collagen but a decrease in elastin and GAGs levels (**Fig 3**).

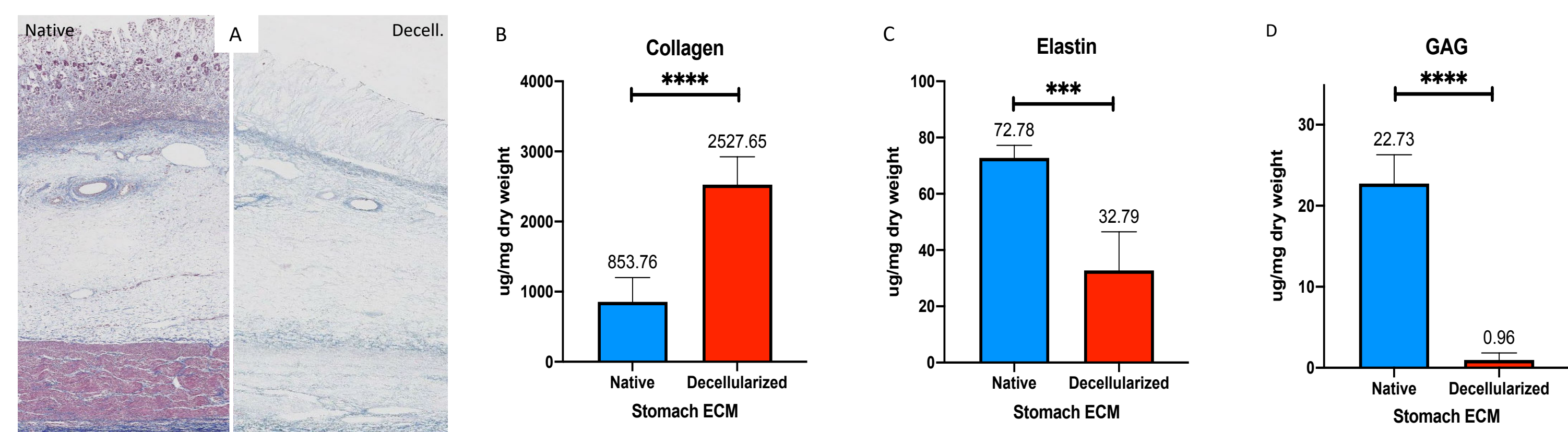


Fig 3: Masson Trichrome staining (A) of native and decellularized stomachs. Quantification of Collagen (B), Elastin (C), GAG (D) before and after decellularization.

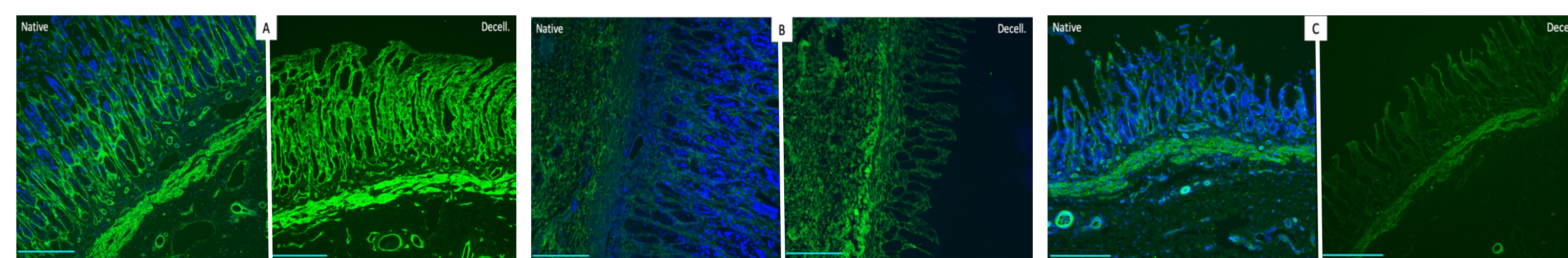


Fig 4: Type IV (A) and type I (B) collagen and laminin (C) immunofluorescence of native and decellularized stomachs. Scale bar = 200 µm

Mechanical Tests

After and before the process, air injection through the gastric lumen showed a preservation of the tightness by the absence of bubbles during the injection (**Fig 5 A-B**). The water perfusion showed a pressure-volume curve with a similar aspect after decellularization but an increase of 1,4-2,1x of the accumulated volume to have the same gastric lumen pressure after decellularization (**Fig 5 C-D**)

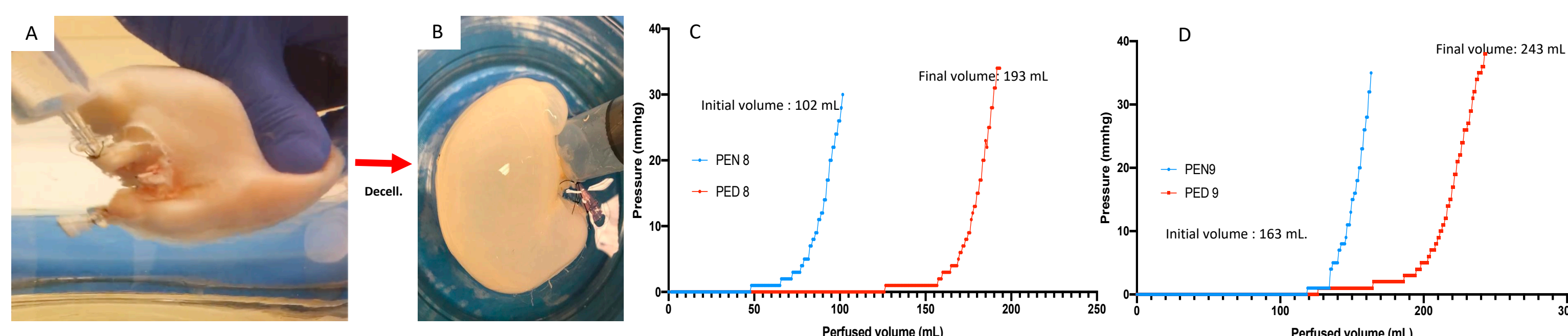


Fig 5: (A) Before and (B) after air injection showed the absence of leakages. (C) Pressure-Volume curve before (blue) and after (red) decellularization.

ECM recellularization

Seeded cells attest to biocompatibility of the stomach matrix and evident cell viability after 7 days (**Fig 6**).

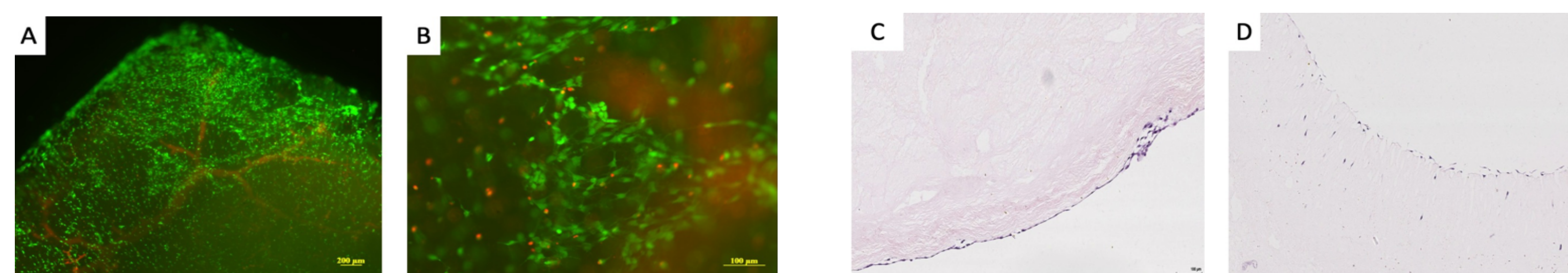


Fig 6: Live/Dead staining at 10x (A) et 20x (B) at day 15. H&E staining at J7 of the serosa surface (C) and the mucosa surface (D).

Conclusion

Full stomach acellular graft can be obtained using our “perfusion-decellularization” procedure while preserving the ECM architecture, a perfusable vascular tree, a gastric tightness and a cellular growth potential.

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References