Structural and Functional Characterization of Scaffolds Derived from Adult Animals with Different Regenerative Potential: A Comparative Study between Decellularized Axolotl and Mouse Skin.

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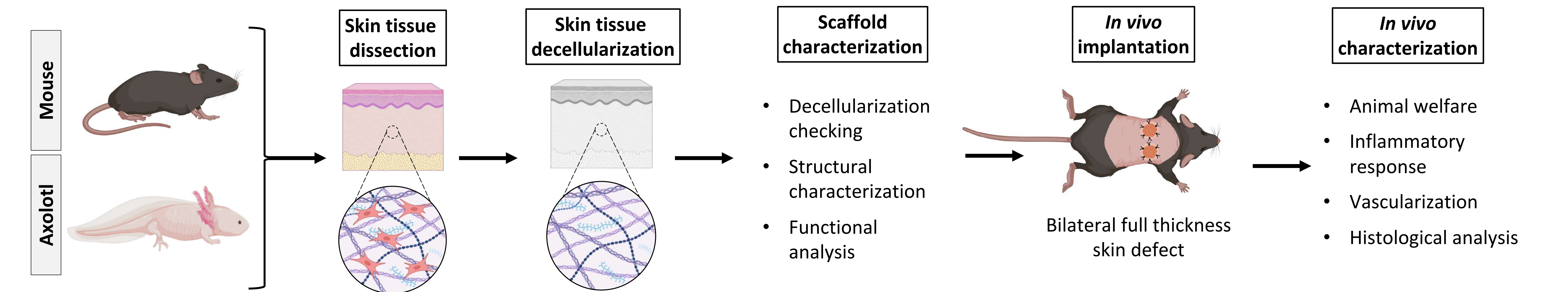[2] Division of Hand, Plastic and Aesthetic Surgery, University Hospital, LMU Munich, Munich, Germany.
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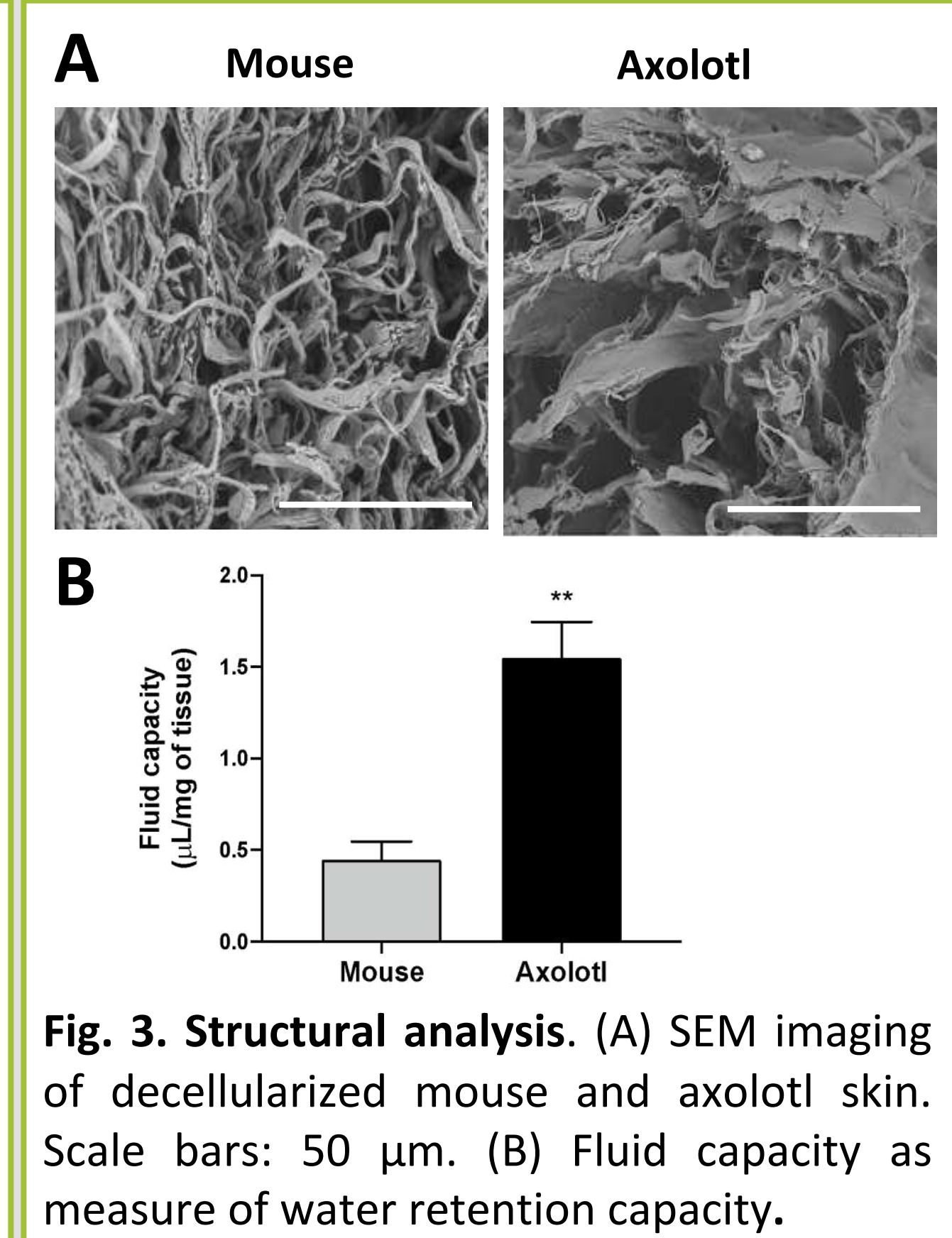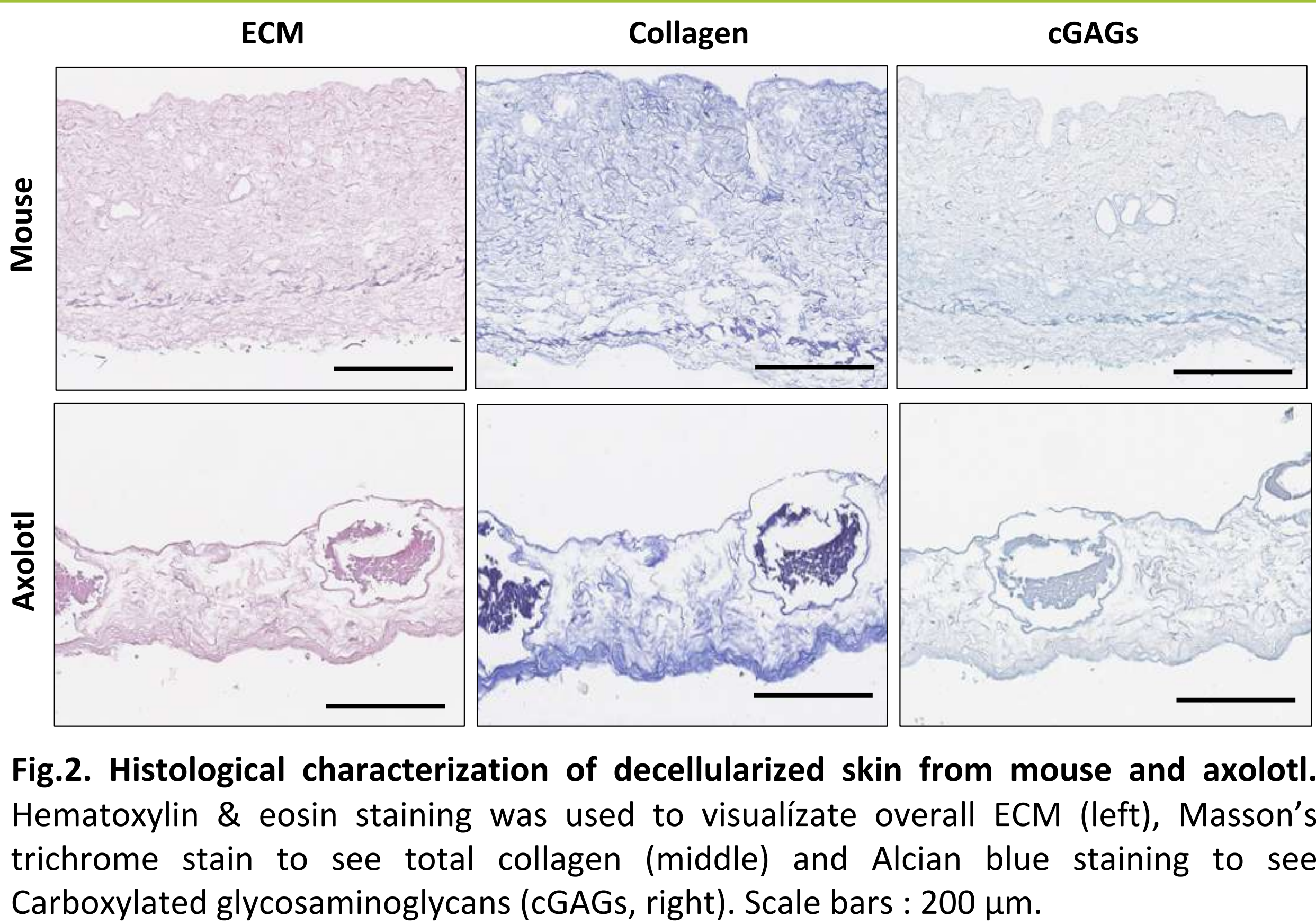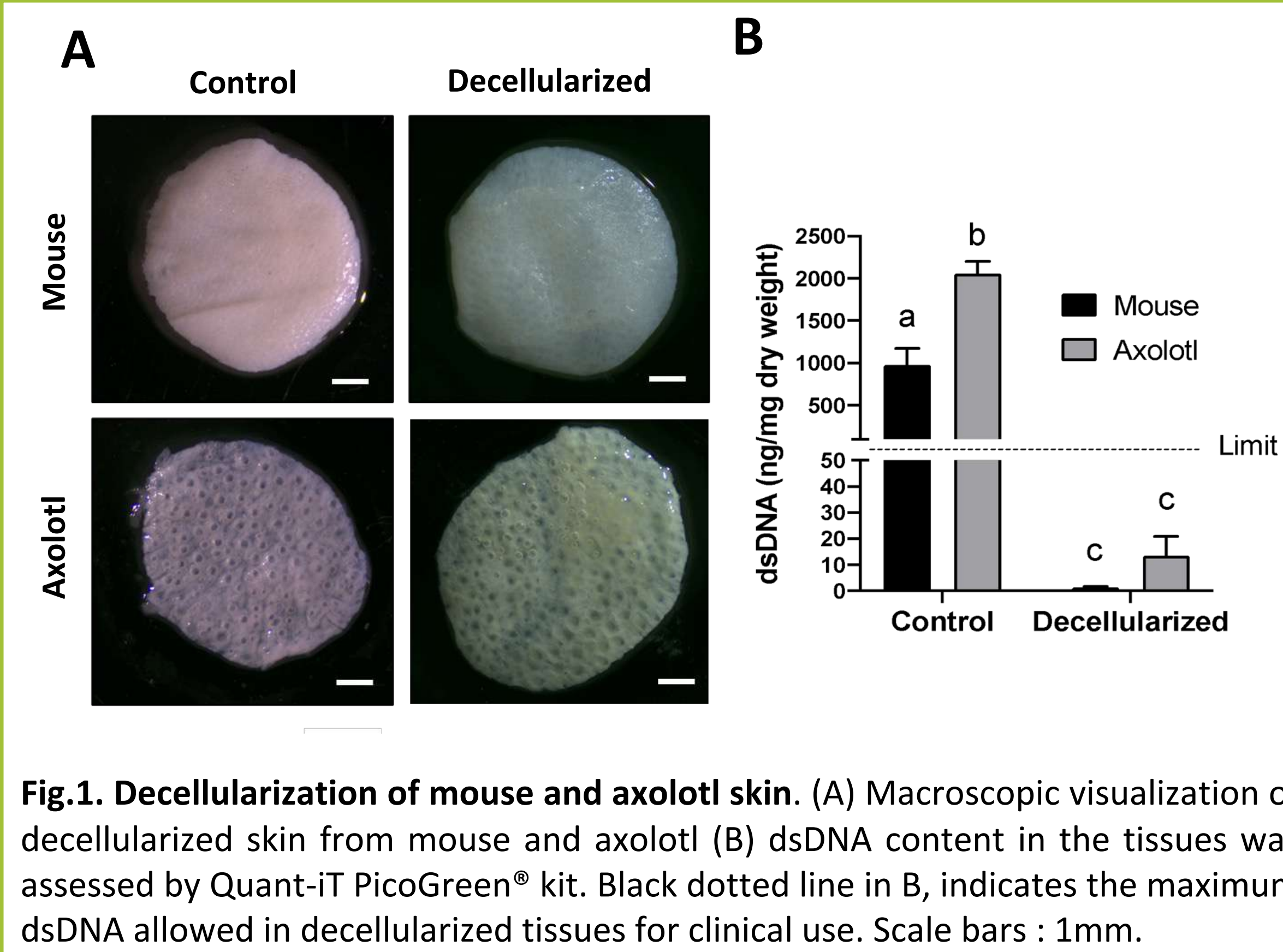
INTRODUCTION

Tissue regeneration capacities vary significantly among different species within the animal kingdom. For instance, certain invertebrates such as planarians, hydra, and starfish are renowned for their ability to completely regenerate and restore missing parts of their bodies. This capability is not confined to "lower" organisms, as some vertebrates like axolotls (Ambystoma mexicanum) also possess the potential to regenerate complete limbs, heart, brain, muscles, and skin, among others. Interestingly, the regenerative capacity of these organisms persists throughout their entire lifespan and remains unaffected by aging. In contrast to these species, mammals can only regenerate tissues at early stages and lose this ability over time. This work aims to investigate whether scaffolds developed from the extracellular matrix (ECM) of animals with superior regenerative capacities might exhibit enhanced regenerative potential following implantation.
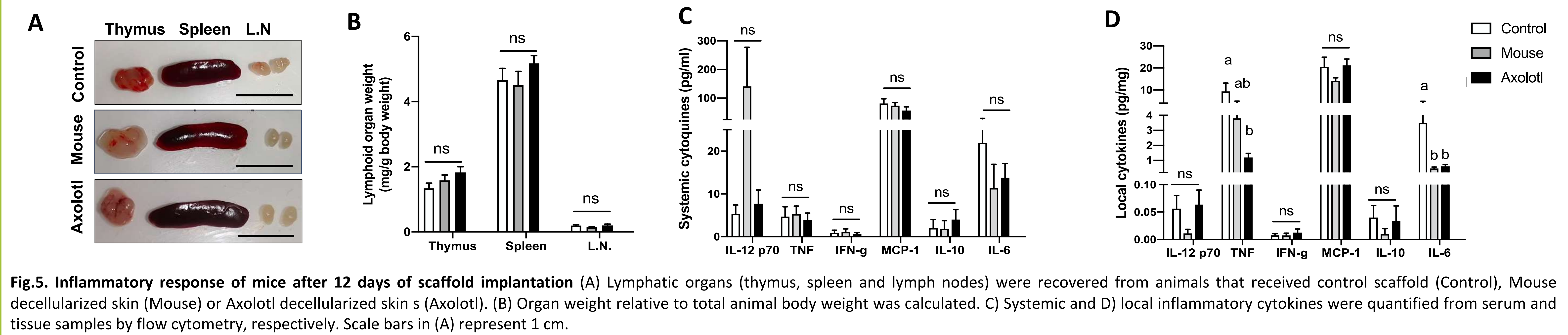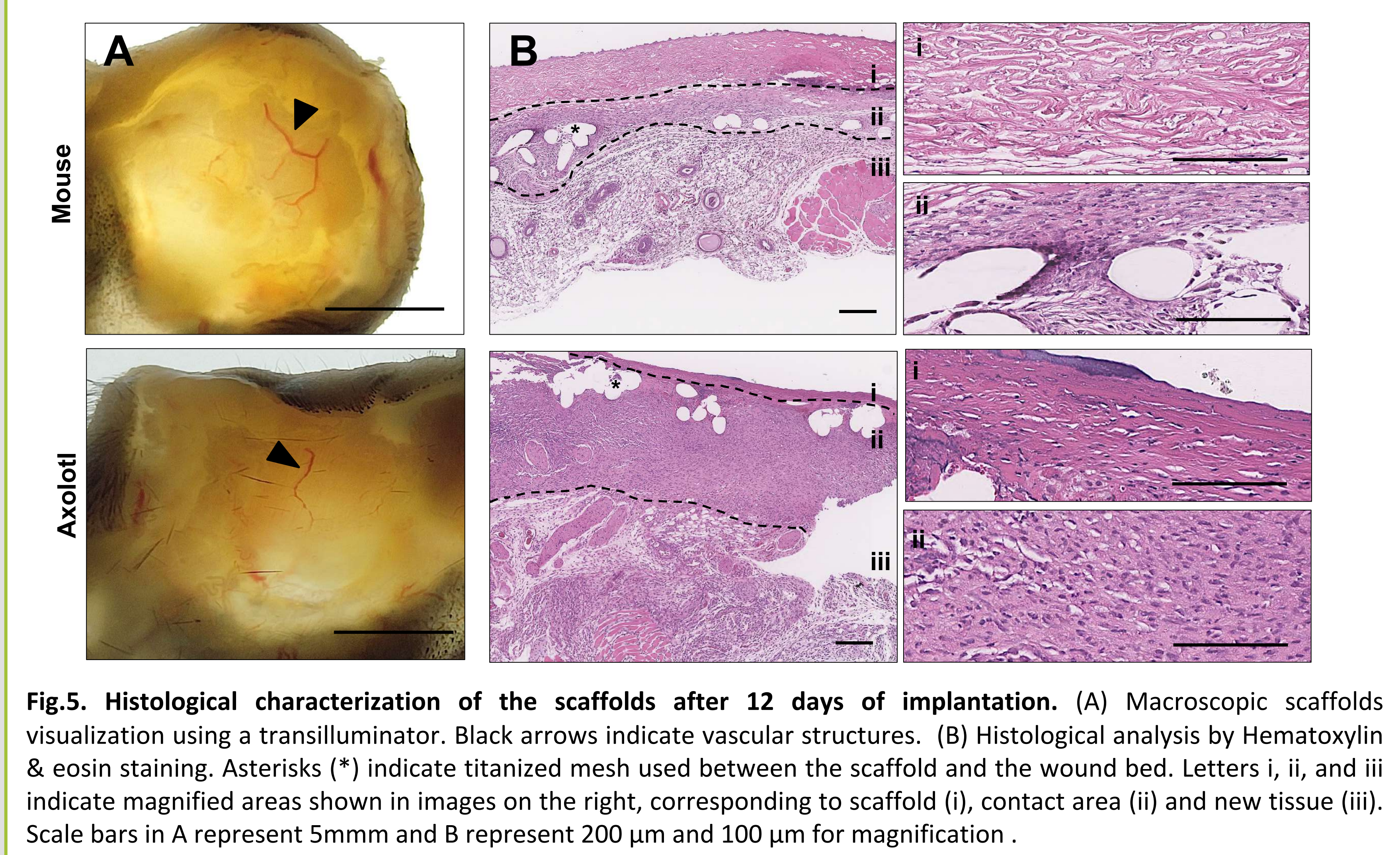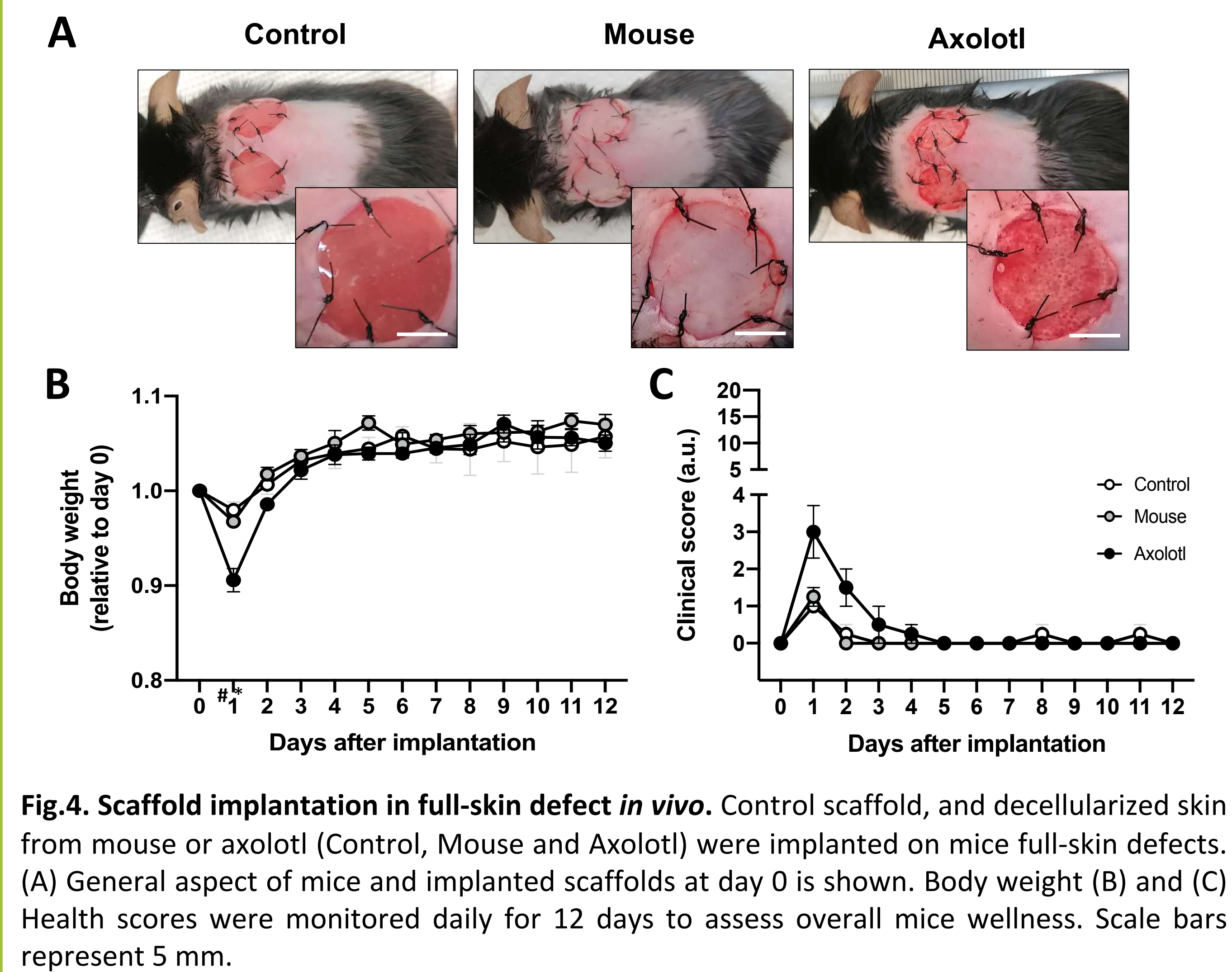
METHODS

RESULTS

Scaffold characterization

Fig.1. Decellularization of mouse and axolotl skin. (A) Macroscopic visualization of decellularized skin from mouse and axolotl (B) dsDNA content in the tissues was assessed by Quant-iT PicoGreen® kit. Black dotted line in B, indicates the maximum dsDNA allowed in decellularized tissues for clinical use. Scale bars : 1mm.

Fig.2. Histological characterization of decellularized skin from mouse and axolotl. Hematoxylin & eosin staining was used to visualíze overall ECM (left), Masson's trichrome stain to see total collagen (middle) and Alcian blue staining to see Carboxylated glycosaminoglycans (cGAGs, right). Scale bars : 200 µm.

Fig. 3. Structural analysis. (A) SEM imaging of decellularized mouse and axolotl skin. Scale bars: 50 µm. (B) Fluid capacity as measure of water retention capacity.

In vivo implantation

Fig.4. Scaffold implantation in full-skin defect in vivo. Control scaffold, and decellularized skin from mouse or axolotl (Control, Mouse and Axolotl) were implanted on mice full-skin defects. (A) General aspect of mice and implanted scaffolds at day 0 is shown. Body weight (B) and (C) Health scores were monitored daily for 12 days to assess overall mice wellness. Scale bars represent 5 mm.

Fig.5. Histological characterization of the scaffolds after 12 days of implantation. (A) Macroscopic scaffolds visualization using a transilluminator. Black arrows indicate vascular structures. (B) Histological analysis by Hematoxylin & eosin staining. Asterisks (*) indicate titanized mesh used between the scaffold and the wound bed. Letters i, ii, and iii indicate magnified areas shown in images on the right, corresponding to scaffold (i), contact area (ii) and new tissue (iii). Scale bars in A represent 5mmm and B represent 200 µm and 100 µm for magnification .

Fig.5. Inflammatory response of mice after 12 days of scaffold implantation (A) Lymphatic organs (thymus, spleen and lymph nodes) were recovered from animals that received control scaffold (Control), Mouse decellularized skin (Mouse) or Axolotl decellularized skin s (Axolotl). (B) Organ weight relative to total animal body weight was calculated. C) Systemic and D) local inflammatory cytokines were quantified from serum and tissue samples by flow cytometry, respectively. Scale bars in (A) represent 1 cm.

CONCLUSIONS AND PROJECTIONS

This study provides important insights into the role of ECM in the tissue regeneration process of animals with high regenerative capacity, as well as a direct impact on developing new biomaterials for tissue engineering and regeneration.

FUNDING