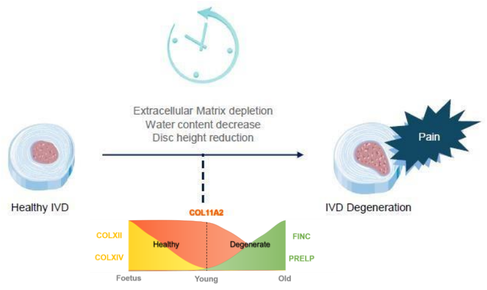


Developmentally inspired strategies for Intervertebral Disc regeneration

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BACKGROUND



***HYPOTHESIS**: fetal-inspired decellularized matrices will recapitulate early developmental stage microenvironments, thus promoting IVD regeneration*

RESULTS PART 1

Optimization of the decellularization procedure



Figure 1. Decellularization efficiency with the optimal protocol. Left - Quantification of DNA by PicoGreen in native and decellularized fetus (F) and young (Y) NPs. Error bars represent mean ± SEM (n=3). Kruskal-Wallis test followed by Dunn's multiple comparison test. **p<0.01. **Right -** Alcian Blue and Picro-Sirius Red staining of tissue sections from native and decellularized Fetal (F) and Young (Y) NPs. Representative images of three biological replicates. Red: collagen; blue: GAGs. 5X magnification.

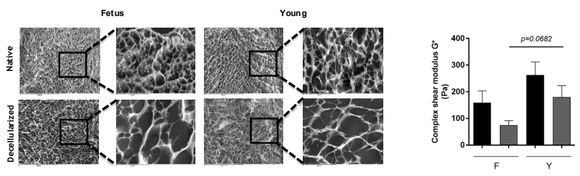


Figure 2. Structural and biomechanical characterization. Left - Representative images of collagen fibers network of native and decellularized fetus (F), young (Y) NPs, obtained by Cryo-SEM. 1000X magnification 1000X (left images); 5000X magnification (right images). **Right -** Complex shear modulus (G*) values of Fetal (F) and Young (Y) decellularized NPs, compared to the correspondent native tissues (n=3). Error bars show mean ± SEM. Kruskal-Wallis test followed by Dunn's multiple comparison test.

Scaffold repopulation, ECM synthesis and angiogenic potential

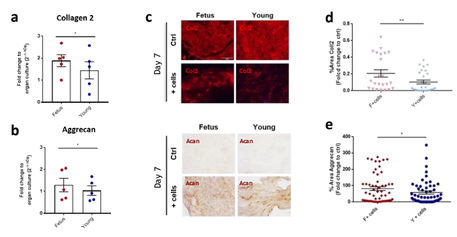


Figure 3. Extracellular matrix *de novo* synthesis of repopulated NPs after 7 days of *ex vivo* culture. a) mRNA expression level of *ACAN* and **b)** *COL2A1*. Fold change to organ culture (native NPs cultured for 7 days). Error bars represent mean ± SEM (n=5). Friedman test followed by Dunn's multiple comparison. *p<0.05. **c)** Representative images of ACAN immunohistochemistry and COL2 immunofluorescence of F and Y repopulated matrices. 20X magnification. **d)** ACAN and **e)** COL2 percentage area quantification obtained by ImageJ. Fold change to the control (decellularized matrices). ACAN: n=6-8. COL2A1: n=4-6. Kruskal-Wallis followed by Dunn's multiple comparison. *p<0.05; **p< 0.01.

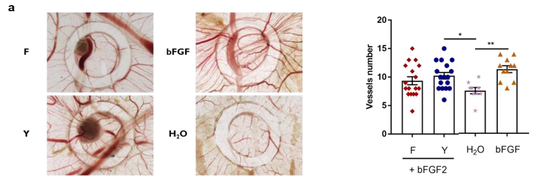
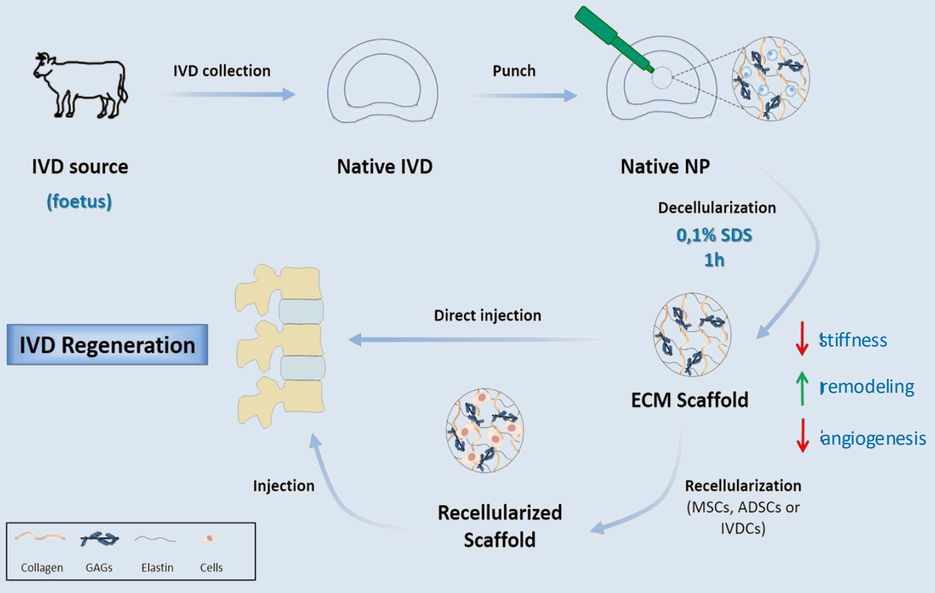


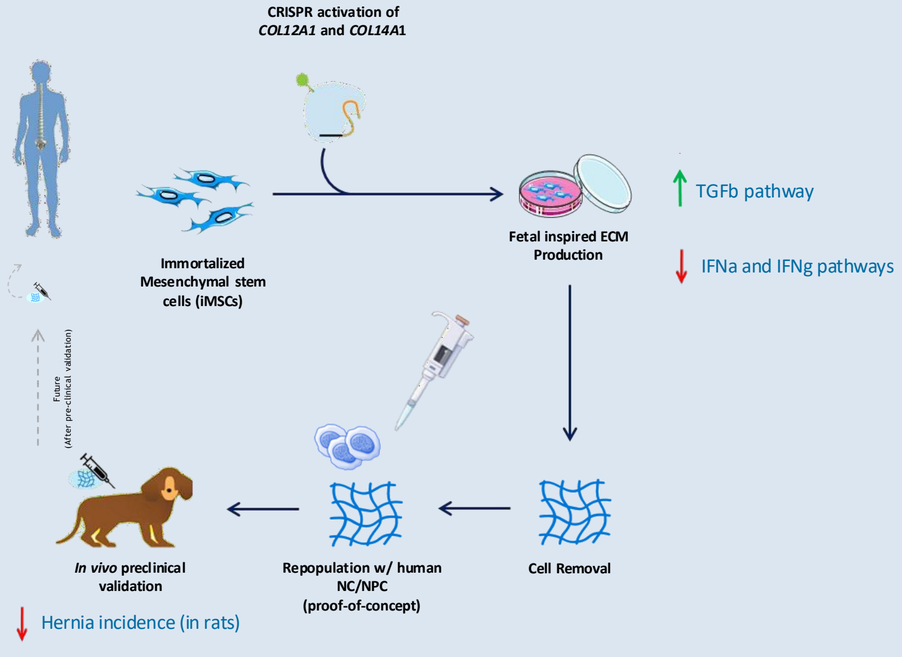
Figure 4. *In vivo* anti-angiogenic potential of decellularized NPs. a) Representative images of chorioallantoic membranes (CAMs) treated with basic fibroblast growth factor-2 (bFGF2) and implanted with Fetal (F) and Young (Y) decellularized NP matrices. **b)** Neovessel quantification. Error bars represent mean ± SEM (n=9-17). One-Way Anova followed by Dunnett's multiple comparison test was used for statistical analysis. *p<0.05 and **p<0.01.

Decellularized ECM-based strategies that mimick the pre-natal microenvironment

SOLUTION 1



SOLUTION 2 *(P-747 & P-767)*



RESULTS (CONT.)

Particle characterization and preclinical validation of the fetal decellularized material

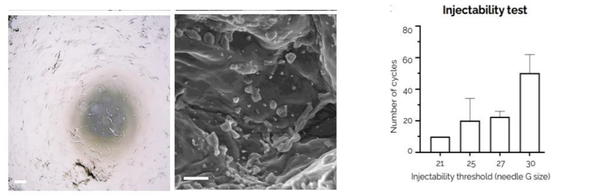


Figure 5. Particle characterization and injectability. (A) Morphologic and topographic analysis by brightfield (left) and SEM (right). Magnification 4x and scale bar 100 µm (left). Magnification 5000x and scale bar 10 µm (right). (B) Injectability test. The biomaterial was injectable in a 30G needle within 50 cycles.

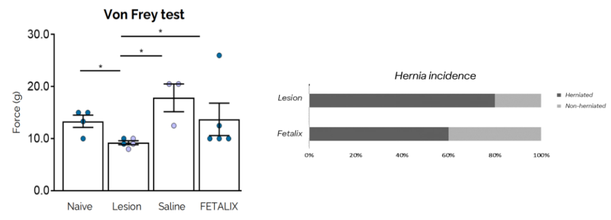


Figure 6. *In vivo* assessment of the fetal decellularized matrix biological effect. (A) Von Frey Assay. Von Frey assay. FETALIX presents pain sensitivity similar to naïve, thus suggesting a positive therapeutic outcome. Mann Whitney test (one-tailed). *p< 0.05. (B) Hernia incidence decreased after fetal-inspired biomaterial administration in a rat model of disc degeneration.

CONCLUSIONS

- A **0.1% 1h SDS-based protocol** was more effective in removing cells and preserving ECM composition and architecture from different aged NPs.
- **Fetal** scaffolds promoted **increased Aggrecan and Collagen type II** expression **by disc cells**; and were more potent at **inhibiting angiogenesis**.
- Smaller and porous **injectable particles** were produced after 50 milling cycles.
- **Particle administration *in vivo***, in a rat model of disc degeneration, **restored sensitivity** to naïve levels and **reduced hernia incidence**.

Injection of the fetal-inspired scaffolds constitutes a promising minimally invasive strategy to treat IVD degeneration and Low Back Pain.

ACKNOWLEDGEMENTS

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