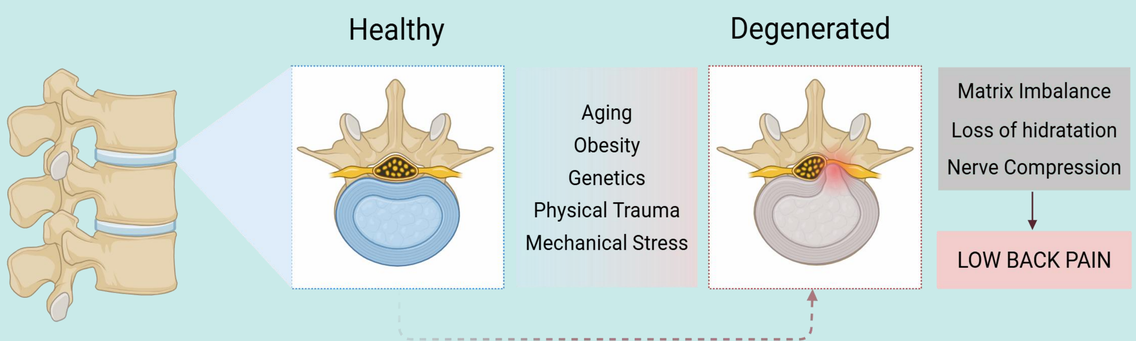
A Dual Rat Model Pipeline for Intervertebral Disc Degeneration Studies

Maria Leonor Moura [1,2 #], **Catarina Milheiro**[1,2,3], **Carla Cunha**[1 *], **Raquel M Gonçalves**[1,2,3 *] **Joana Caldeira** [1,2]

[1]i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, 4200-135 Porto, Portugal; [2]Fetalix (FetalDisc, Lda), Rua Senhora do Porto, N.º 299, 2.º A 4250-455 Porto; [3]ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto 4050-313, Portugal; [*]equal contribution; [#]lmoura@i3s.up.pt

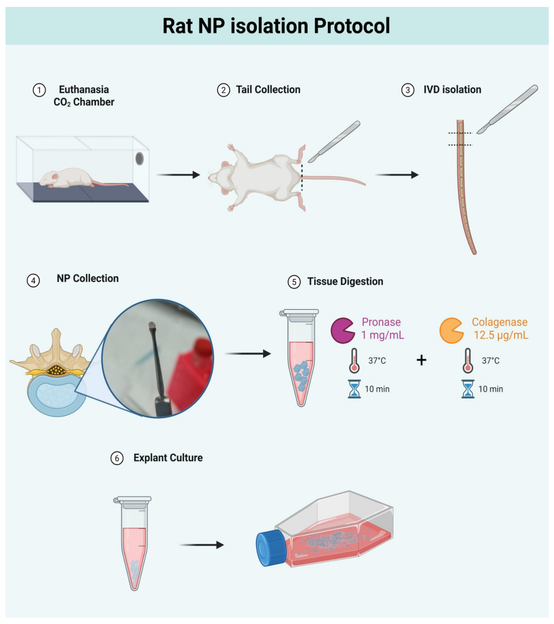
BACKGROUND



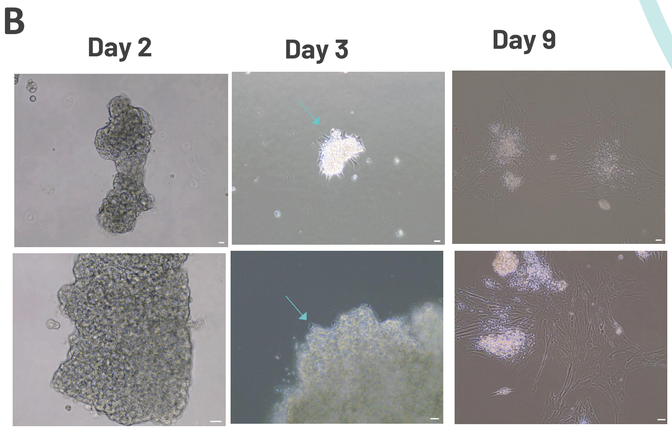
Intervertebral disc (IVD) **degeneration** is a major contributor to chronic low back pain and disability. Degeneration is characterized by loss of proteoglycans and water content in the nucleus pulposus (NP), structural disruption of the annulus fibrosus, and reduced disc height, ultimately impairing spinal function. Understanding these processes is critical for developing effective therapies. *Rattus norvegicus* offers a valuable translational model due to similarities in disc biology and the ability to study degeneration in a controlled environment.

In vitro trial using primary rat NP cells

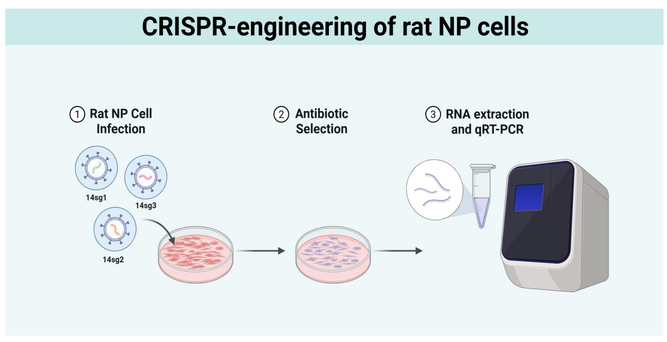
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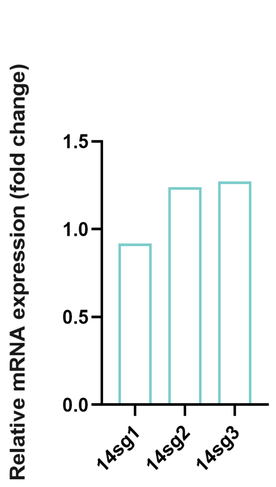
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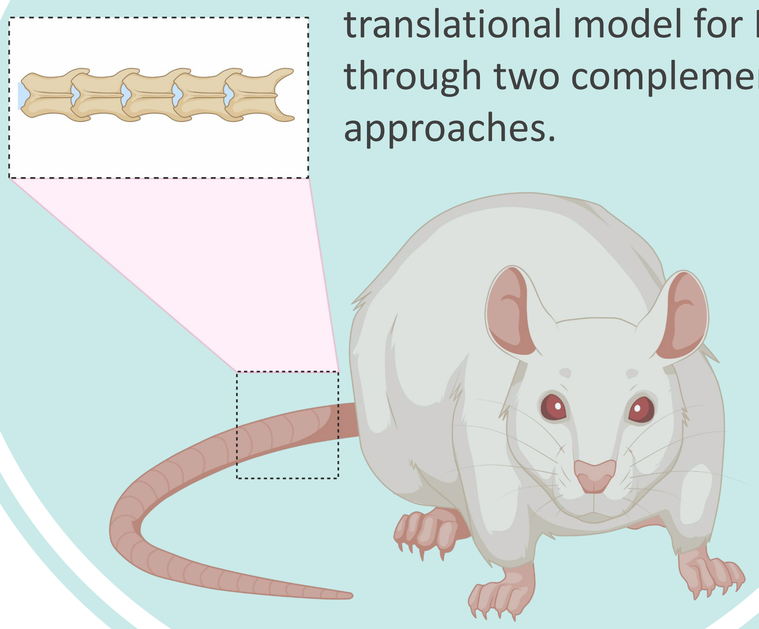
D



A. Schematic representation of the optimized protocol for rat NP isolation and cell culture.
B. Representative images of the explant culture over time. At Day 2, the explant is attached to the bottom of the flask; by Day 3, cells are visible at the explant periphery (indicated by the blue arrow); and by Day 9, distinct cell colonies have formed. The left images were acquired at 10× magnification, while the right images were acquired at 20× magnification. Scale bar = 100 µm.
C. Schematic representation of the CRISPRa workflow applied to primary rat NP cells isolated using the optimized protocol shown in panels A and B. sgRNAs targeting the Col14a1 promoter (14sg1, 14sg2, 14sg3) were designed and cloned into the lentiSAMv2 vector (enhanced dCas9-VP64 system). Viral particles produced in HEK293T cells were used to transduce rat NP cells. Antibiotic kill-curve assays defined the optimal selection concentration, after which clonal populations were expanded. RNA was extracted from selected clones and Col14a1 expression levels were quantified by qRT-PCR.
D. Quantification of *Col14a1* expression was evaluated by qRT-PCR. (n=1)

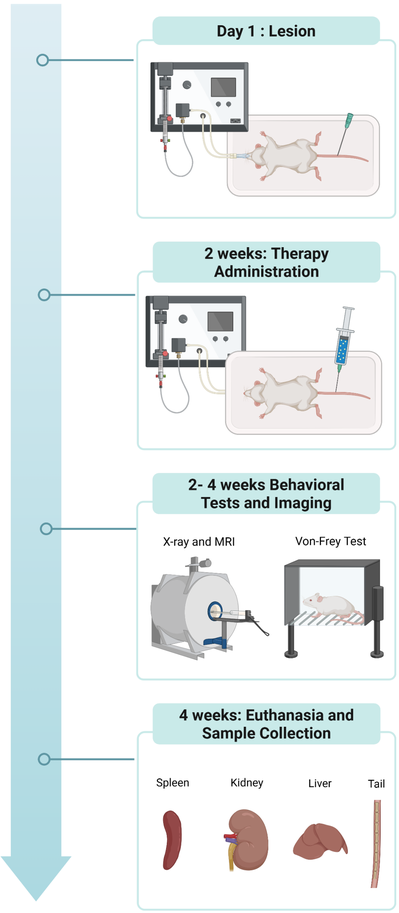
AIM

Use *Rattus norvegicus* as a translational model for IVDD through two complementary approaches.

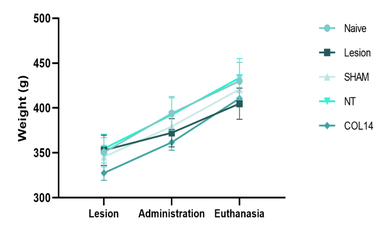


In vivo trial using a rat IVDD model

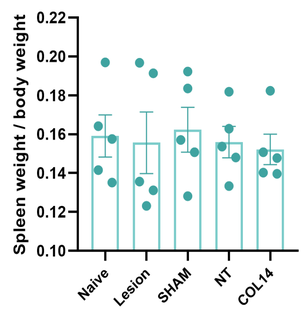
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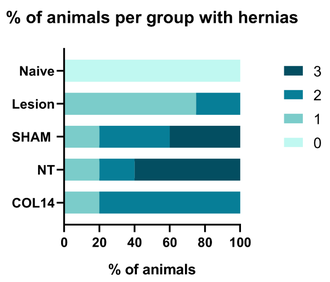
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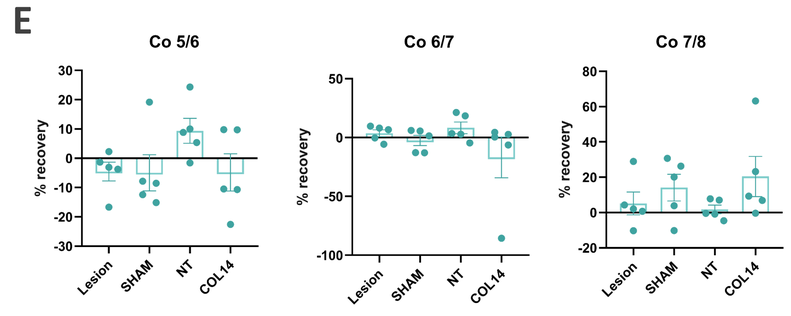
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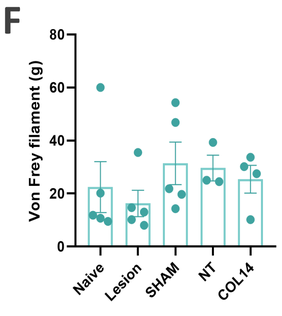
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E



F



A. Schematic representation of the *in vivo* trial. **B-F.** Preliminary results assessing the safety of the injectable therapy Col14 engineered matrices. **B.** Animal weight (g) Mean ± SEM. **C.** Spleen Weight normalized by animal body weight. Mean ± **D.** % of animals per group with different number of hernias. **E,** % of disc height recovery in consecutive coccygeal IVDs (Co5/6, Co6/7, and Co7/8). Mean ± SEM. **E.** Behavioral assay by Von Frey.
A total of 25 animals were used (n = 5 per group). Statistical analysis was performed using one-way ANOVA. No significant differences were observed.

CONCLUSION

We optimized a reliable protocol for isolating and culturing primary rat NP cells and demonstrated that CRISPRa can be used to achieve preliminary activation of *Col14a1* in these cells. In parallel, COL14-enriched matrices were tested in a rat needle-puncture IVDD model and showed a favorable safety profile, with no detectable local or systemic adverse effects. Together, these findings provide a solid foundation for future studies using *Rattus norvegicus* as a relevant translational platform for evaluation of IVDD treatments safety and efficacy.

Contact me using **LinkedIn** or **lmoura@i3s.up.pt**

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