A Biomimetic Injectable Extracellular Matrix Self-Healing Hydrogel for Nucleus Pulposus Replacement and Intervertebral Disc Repair

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Engineering Solutions to Regenerate

Introduction

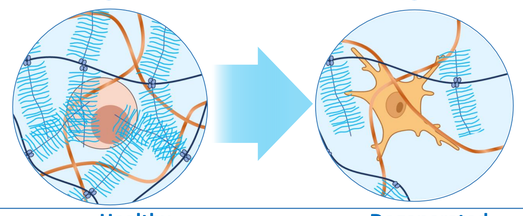
Back Pain- A Global Issue
600 million people worldwide
- Single leading cause of disability in people under 45 years of age

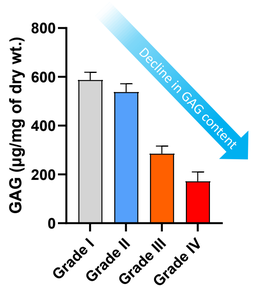


Socioeconomic burden €240 billion annually in the EU

Healthy NP ECM is rich in collagen II, GAGs, and structural proteins

ECM degeneration reduces collagen II and GAGs, disrupts disc homeostasis[1,2]





Current treatment options

Non-surgical & surgical approaches
- Symptomatic redemption, spinal fusion, Total Disc Replacement

Emerging regenerative approaches
Cell/materials based therapies
- Injectable Disc Cell Therapy : allogenic progenitor cells
- BioRestorative (BRTX-100) : stem cells
- DiscSeel® : fibrin with or without cells

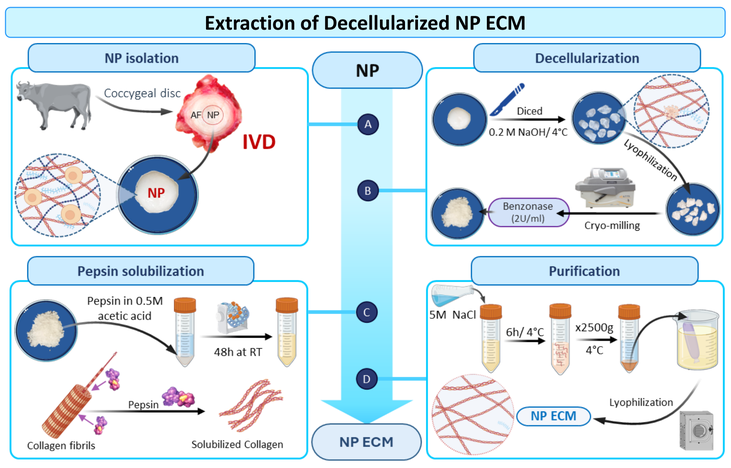


These approaches have drawbacks
- Cells takes very long time to restore matrix due to limited matrix production
- The biomaterials like fibrin mechanically inferior to tissue to be a replacement

Aim : Develop an injectable hydrogel for IVD regeneration with
- **Ideal mechanical properties:**
  - Similar native tissue mechanics
- **Biochemical cues for IVD repair**
  - Employing Native ECM and GAG

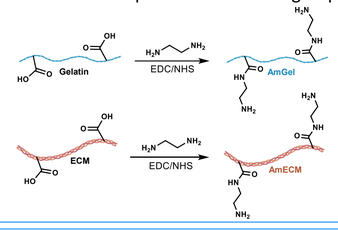
Hydrogel design

Extraction of Decellularized NP ECM

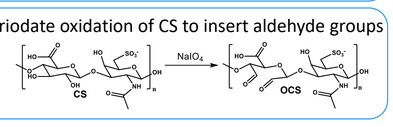


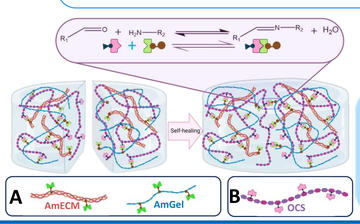
Hydrogel is prepared by in situ crosslinking of ED modified Gelatin (AmGel) and ED modified ECM (AmECM) with Oxidized chondroitin sulphate (OCS)

Ethylene diamine (ED) modification of ECM and Gelatin to Incorporate more amine groups



Periodate oxidation of CS to insert aldehyde groups

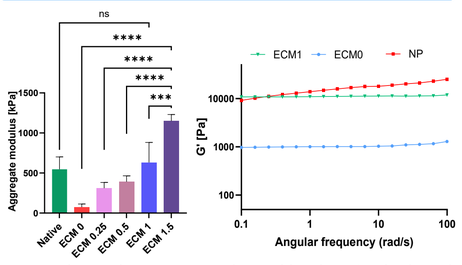




AmECM/AmGel crosslinks with OCS by Schiff base to form a self-healing hydrogel

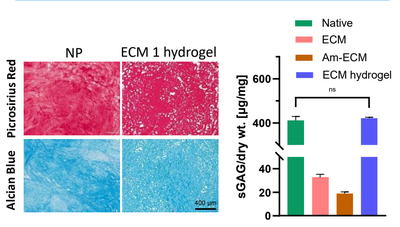
Physiochemical characterization

Mechanical properties



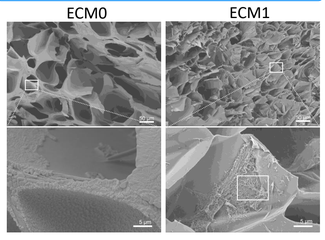
NP mechanical properties achieved by the ECM hydrogel

Biochemical analysis



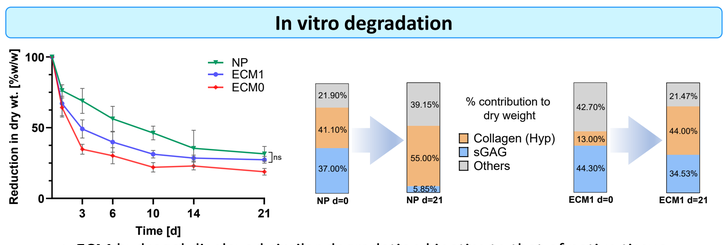
ECM hydrogel exhibited similar biochemical properties to that of native tissue

SEM Imaging



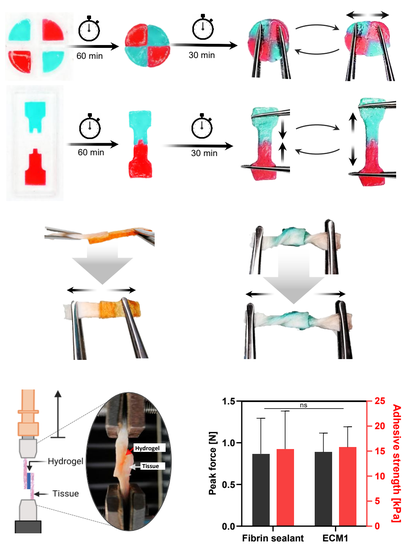
FESEM images of freeze-dried ECM hydrogel displayed IPN of collagen fibres

In vitro degradation



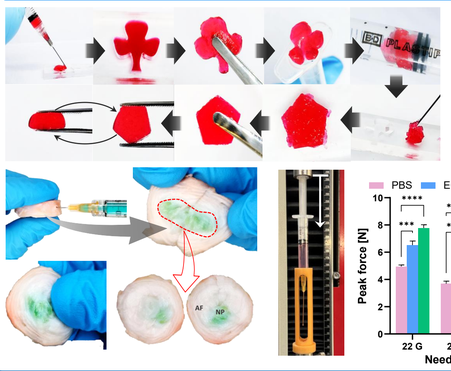
- ECM hydrogel displayed similar degradation kinetics to that of native tissue
- The hydrogel retains more CS via chemically crosslinked network

Self healing and Bioadhesive property



Schiff base crosslinking and ionic interactions supports self-healing of the hydrogel

Injectability



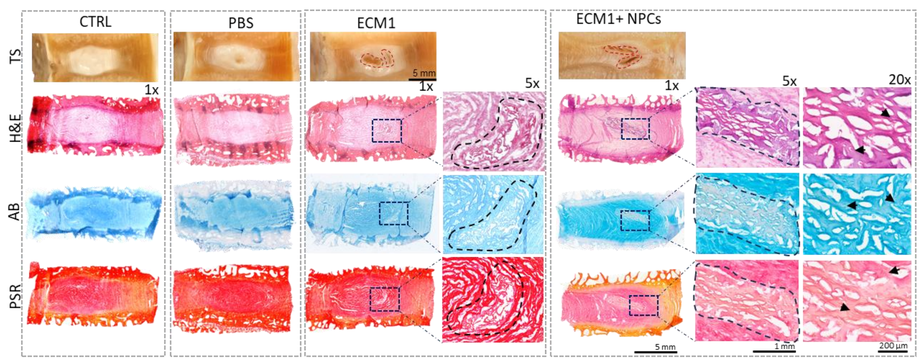
The ECM hydrogel exhibited thixotropic injectability aided by dynamic crosslinking

The hydrogel can be injected with minimal force supporting minimally invasive delivery

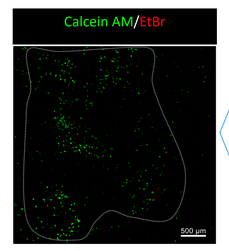
Ex vivo assessment in degenerated IVD organ model



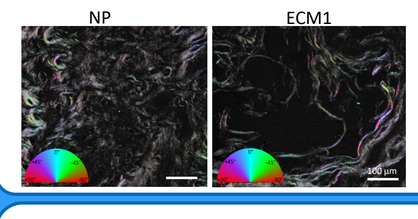
Ex vivo degenerated disc model was created with 4U chondroitinase ABC and treated with ECM hydrogel or ECM hydrogel + NP Cells



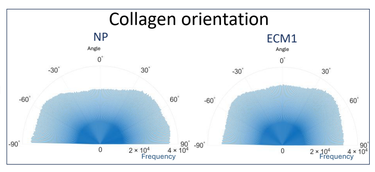
- ECM hydrogel was well integrated with native tissue
- Cells remained viable



Viability of injected cells

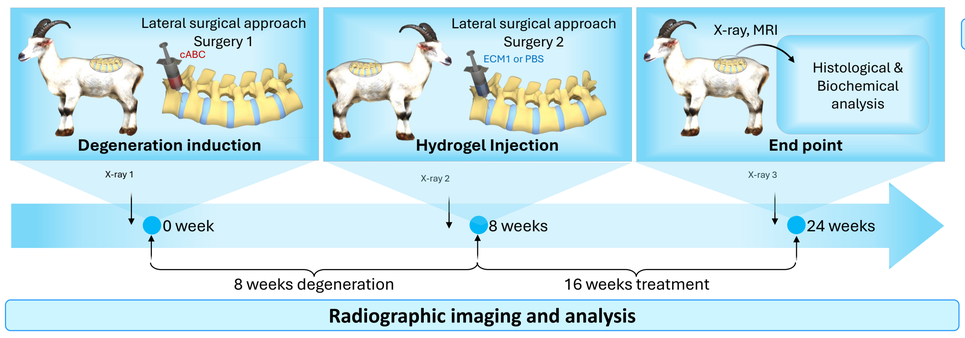


ECM hydrogel displayed random collagen distribution similar to that of native NP tissue

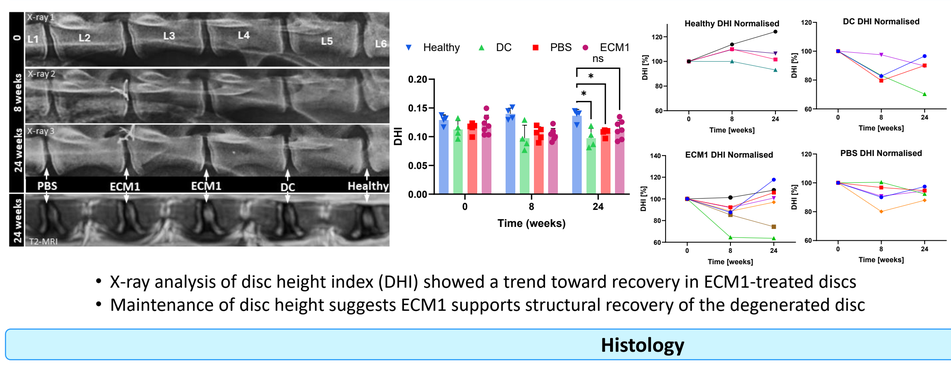


- The ECM hydrogel can be effectively delivered by needle injection
- The hydrogel was successfully integrated with native tissue.
- Hydrogel supported cell delivery ex vivo

In vivo efficacy study

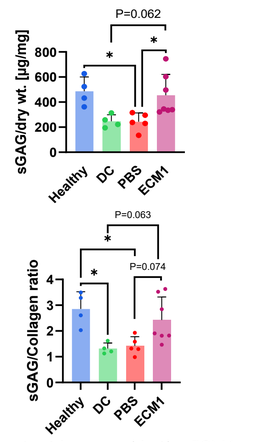


Radiographic imaging and analysis



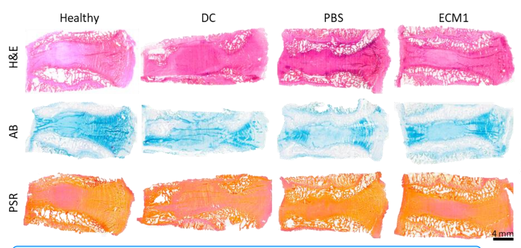
- X-ray analysis of disc height index (DHI) showed a trend toward recovery in ECM1-treated discs
- Maintenance of disc height suggests ECM1 supports structural recovery of the degenerated disc

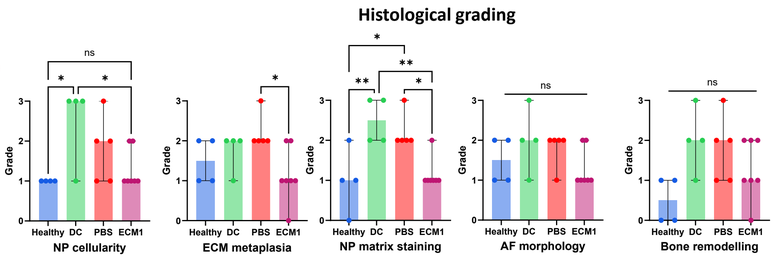
Biochemical analysis



- GAG content increased in the ECM1 group, suggesting improved proteoglycan-rich matrix regeneration
- The GAG/Collagen ratio was higher following ECM1 treatment, indicating restoration toward a more NP-like matrix composition

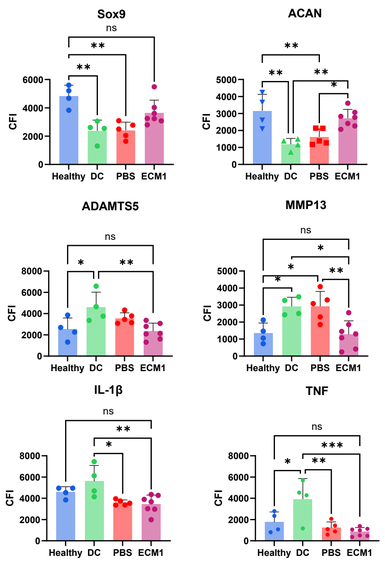
Histology





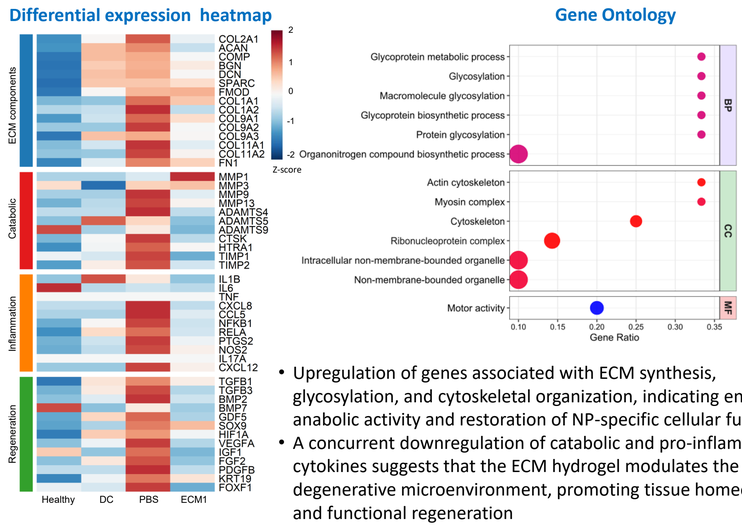
- ECM1 treatment significantly improved NP-specific histological features, including NP cellularity, ECM metaplasia, and NP matrix staining comparable with healthy discs.
- ECM1 preferentially preserved and restored the GAG-rich NP matrix, indicating effective repair of key native tissue characteristics.

Immunofluorescence



- Upregulation of pro-regenerative ECM-related genes in the ECM1-treated group
- Catabolic markers associated with DDD were reduced.
- Inflammatory markers linked to DDD were also downregulated

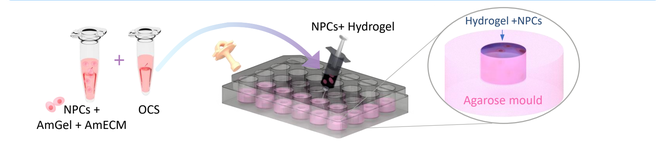
Transcriptomic analysis

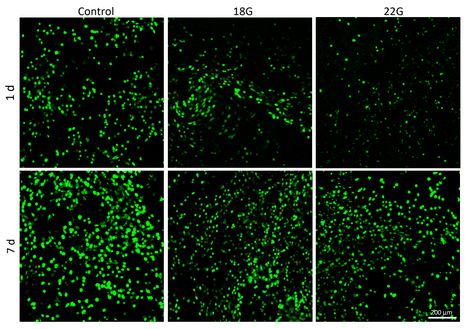


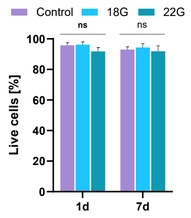
- Upregulation of genes associated with ECM synthesis, glycosylation, and cytoskeletal organization, indicating enhanced anabolic activity and restoration of NP-specific cellular function
- A concurrent downregulation of catabolic and pro-inflammatory cytokines suggests that the ECM hydrogel modulates the degenerative microenvironment, promoting tissue homeostasis and functional regeneration

In vitro viability and bioactivity of ECM hydrogel

Post injection viability



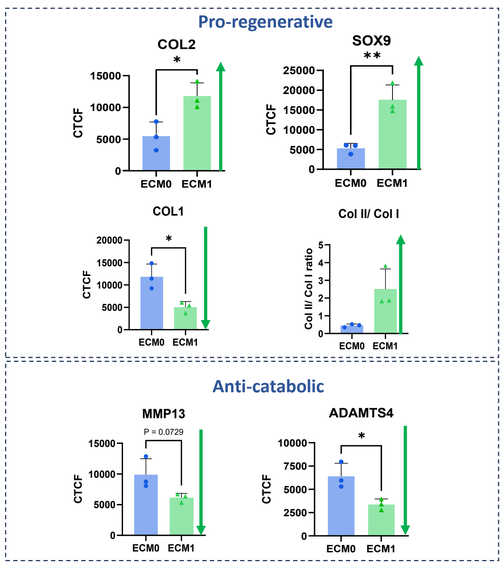




- Hydrogel supported excellent post injection viability of encapsulated cells

- The ECM hydrogel supported cell viability and production of NP specific markers by encapsulated cells
- This establishes the developed hydrogel as a platform for regeneration and cell delivery

Immunofluorescence



NP cells encapsulated in ECM hydrogels demonstrated pro-regenerative and anti-catabolic properties

Conclusion

- **Injectable biomimetic hydrogel** successfully developed using NP ECM components
- **Replicates biochemical and biomechanical properties of NP tissue**
- **The developed hydrogel supports cell delivery** and **promotes regeneration**
- **ECM hydrogel creates a protective, restorative niche** for disc repair
- ECM hydrogel demonstrated **functional disc restoration in a caprine degenerated disc model**
- Similar approaches can be extended to other musculoskeletal tissues



References

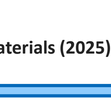
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Acknowledgements

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