Biofabrication of a cell-laden intervertebral disc model using tissue-specific extracellular matrix bioinks

Valerie Kersten[1,2], Jijo Thomas[1,2], Tugdual Haffner[1,2], and Conor T. Buckley[1,2,3,4]

[1] Trinity Centre for Biomedical Engineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland; [2] Discipline of Mechanical, Manufacturing and Biomedical Engineering, School of Engineering, Trinity College Dublin, Dublin, Ireland; [3] Department of Anatomy and Regenerative Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland; [4] Advanced Materials and Bioengineering Research Centre (AMBER), Royal College of Surgeons in Ireland and Trinity College Dublin, Dublin, Ireland.

Introduction & Methods

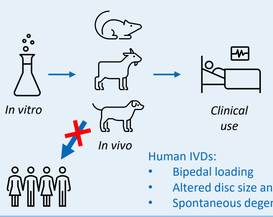
Disc degeneration



ECM catabolism → GAG loss → Reduced hydration

- Disrupted mechanics in load-bearing IVD
- Associated with lower back pain (80% global prevalence) [2]

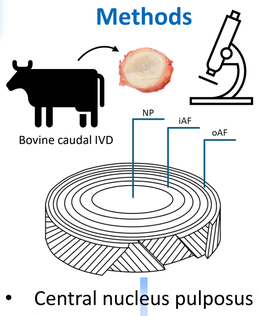
Disc model limitations



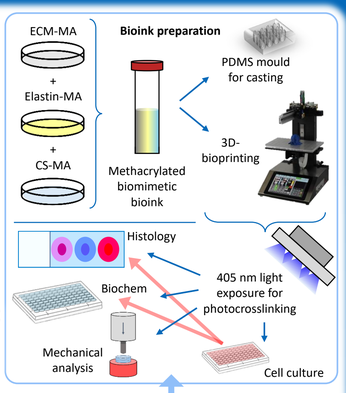
Differences between animal and human IVDs limit translation, necessitating more human-relevant research models

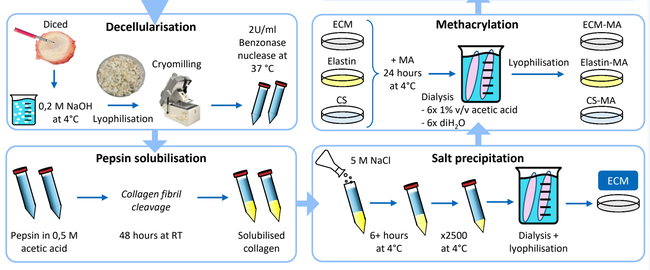
Human IVDs:
- Bipedal loading
- Altered disc size and microenvironment
- Spontaneous degeneration

Methods



- Central nucleus pulposus (NP)
- Lamellar inner and outer annulus fibrosus (AF)



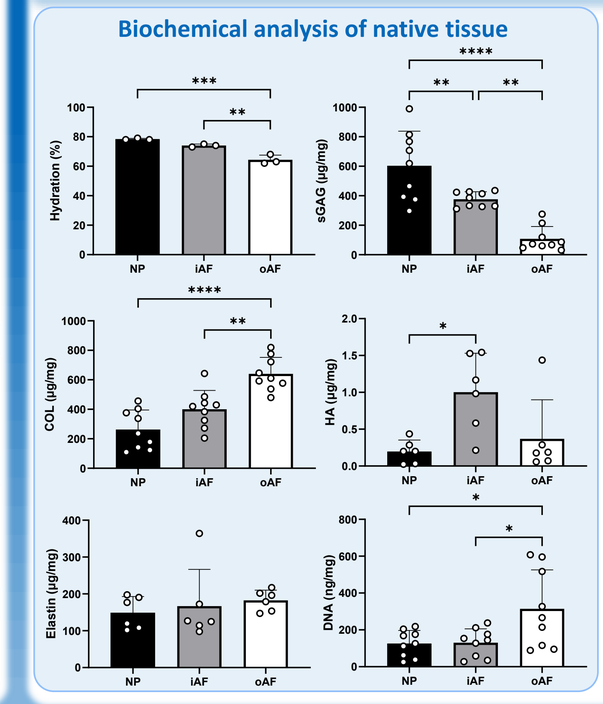


Aim: Engineer biologically representative IVD models to enable human-relevant research

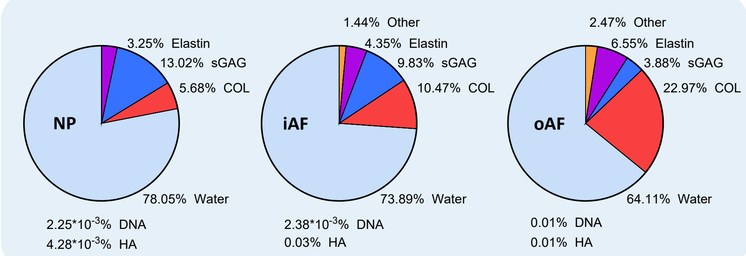
- Biomimetic ECM-environment
- Mechanical competence
- Cytocompatible
- Spatially-defined architecture

Results - Native tissue composition and bioink design

Biochemical analysis of native tissue

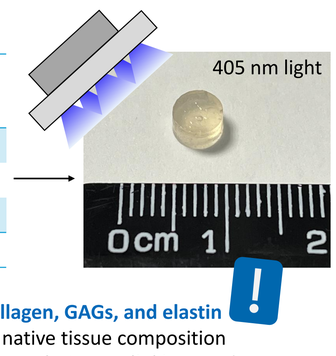


Native tissue composition



Rational bioink design

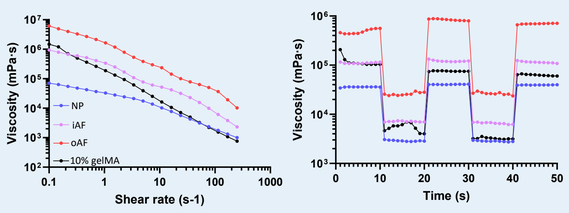
Bioink formulation (mg/ml)	NP	iAF	oAF
ECM-MA	70	140	360
CS-MA	170	130	60
Elastin-MA	40	60	100
Total density	**280**	**330**	**520**



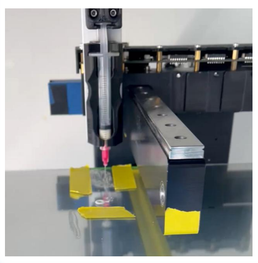
- Native NP, iAF, and oAF tissue consists of **collagen, GAGs, and elastin**
- 3 **engineered biomimetic bioinks** based on native tissue composition
- **Methacrylated ECM, CS, and elastin** for photocrosslinking and tuneable mechanical properties

Results - Characterisation of bioinks

Rheology



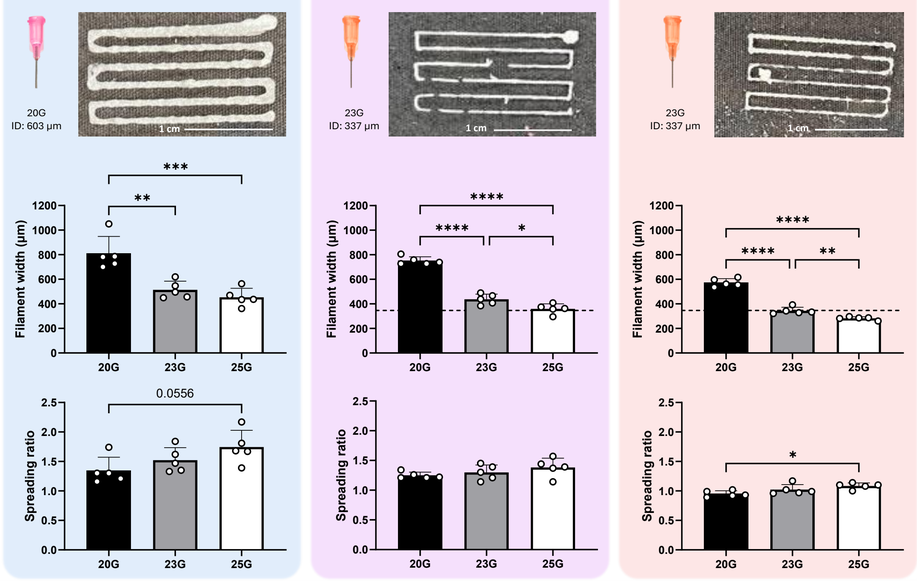
- NP, iAF, and oAF biomimetic bioinks showed similar viscosity to control 10% gelMA
- All bioinks exhibited **shear-thinning behaviour**



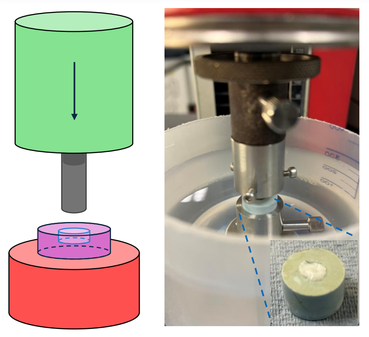
- All bioinks **extruded successfully**
- iAF and oAF bioinks filament width was **similar to lamella target**

Bioink	Nozzle
NP bioink	20G
iAF bioink	23G
oAF bioink	23G

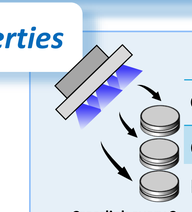
Filament extrusion



Results - Compressive properties

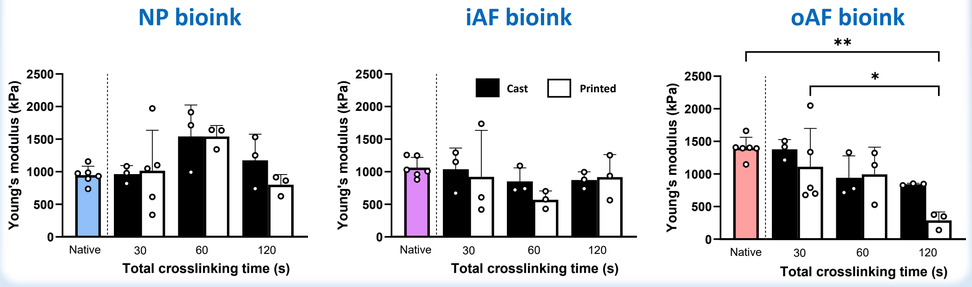


Photocrosslinking

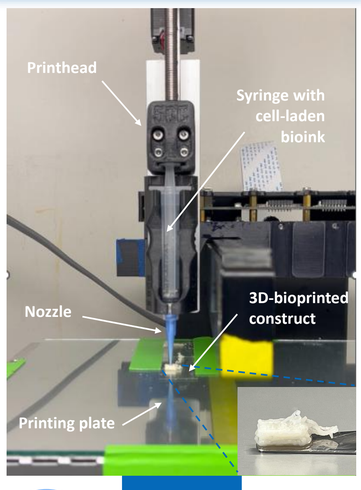


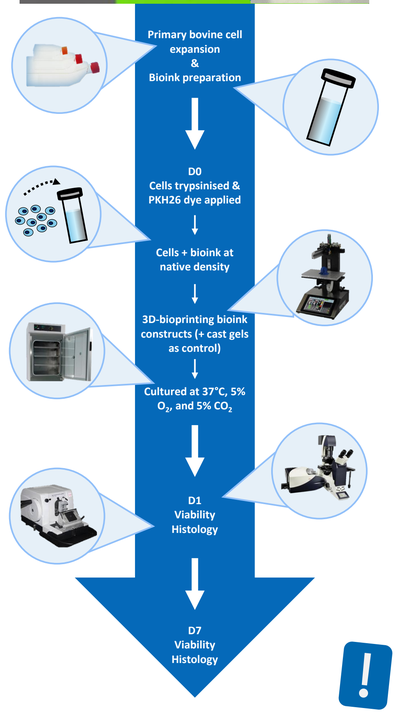
Gel type	Protocol
Cast	Single exposure (30, 60, 120 s)
Printed	3 * (2 layers + X s exposure), where X is 10, 20, or 40

- **Confined compression** under wet conditions mimics the native IVD environment
- 30 seconds of photocrosslinking yielded hydrogels with **compressive strength comparable to native tissue**

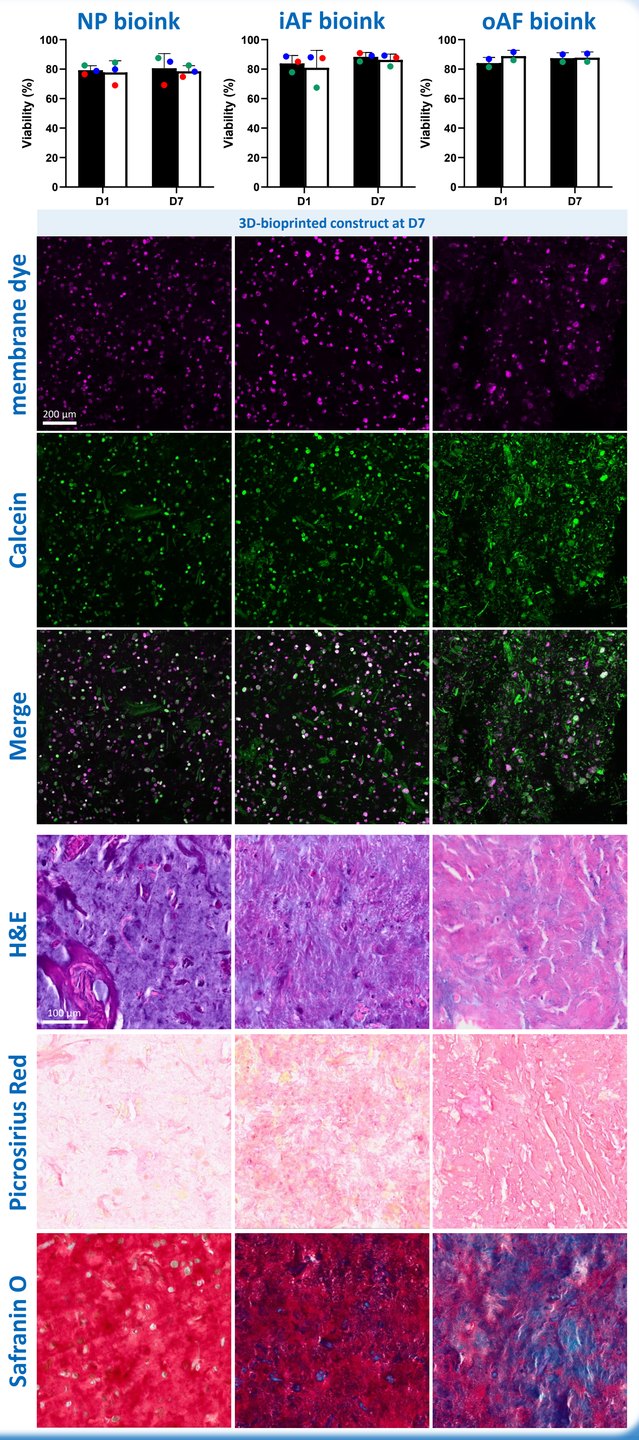


Results - 3D-bioprinting

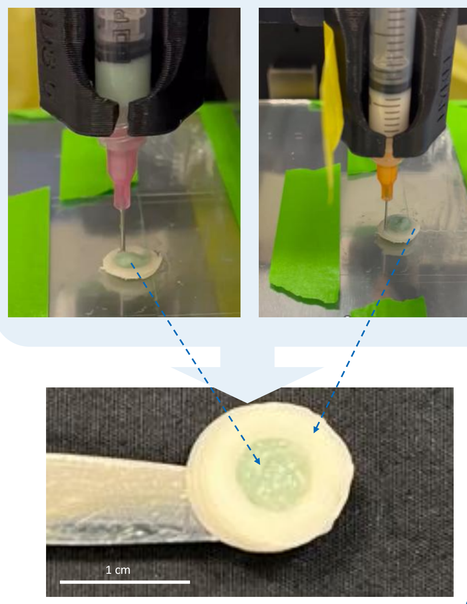




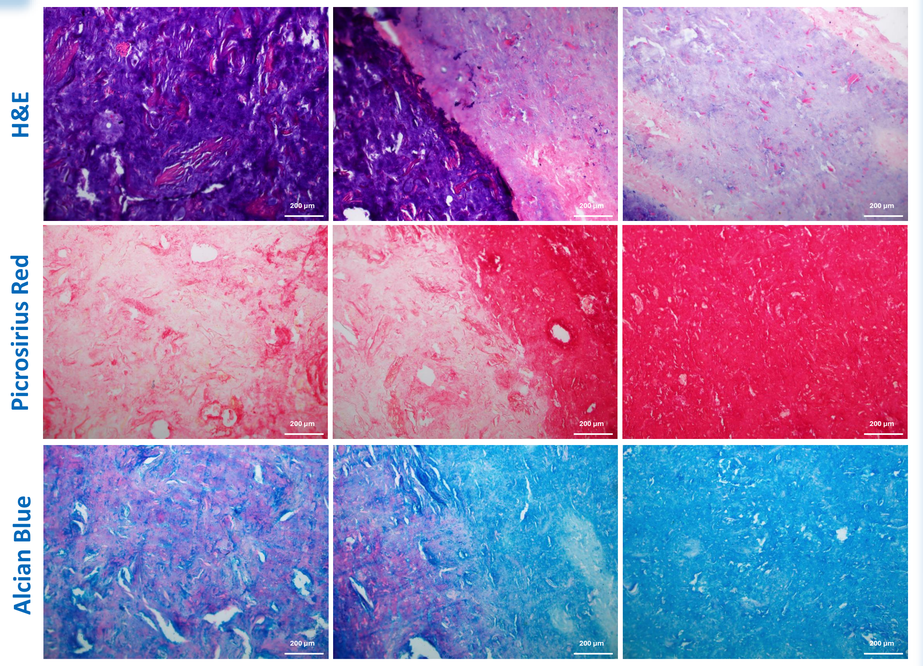
- **High viability** confirms cytocompatibility of 3D-bioprinting
- Histology confirms biomimetic bioinks replicate **region-specific native ECM composition**, consistent with native tissue biochemistry (above)



Results - Biofabrication of IVD model



- **Multi-bioink, multi-printhead** printing enabled region-specific IVD fabrication

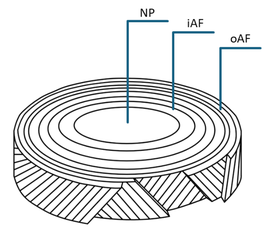


- Histology confirms spatial organisation of **distinct bioink regions**, consistent with region-specific ECM composition
- A **continuous, well-integrated interface** between regions supports 3D-bioprinting as a **robust approach** for multi-region tissue engineering

Discussion & Conclusions

Biologically representative IVD models were successfully engineered to enable human-relevant research

- Biomimetic, region-specific ECM environment achieved using **printable bioinks** that replicate native tissue composition
- Constructs exhibit **native-like mechanical properties**, supporting load-bearing function
- High cell viability confirmed **cytocompatiblity** of the bioinks and 3D-bioprinting process
- Multi-bioink 3D-bioprinting enabled **spatially defined, region-specific IVD architecture**



Acknowledgement

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement ERC-2019-CoG-864104; INTEGRATE).

Contact



Valerie Kersten
PhD candidate
Trinity College Dublin
kerstenm@tcd.ie

www.buckleylab.eu

Abbreviations
IVD – intervertebral disc
ECM – extracellular matrix
NP – nucleus pulposus
AF – annulus fibrosus
iAF – inner annulus fibrosus
oAF – outer annulus fibrosus
(s)GAG – (sulphated) glycosaminoglycan
CS – chondroitin sulphate (a GAG)
MA – methacrylic anhydride, methacrylated
HA – hyaluronic acid

References
1 Galbusera *et al*. Eur. Spine J. (2014)
2 Dieleman *et al*. JAMA (2016)

Trinity Centre for Biomedical Engineering