Developing representative in-vitro models of the human testis

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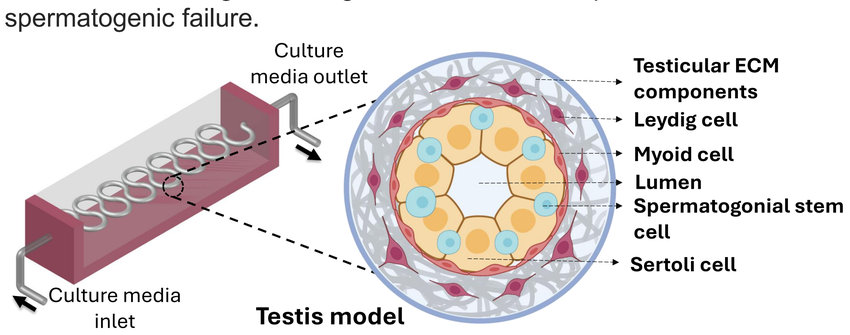
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STATE-OF-THE-ART

- Male infertility drives 50% of cases among infertile couples; however, a 50-year, 50% global decline in sperm counts reveals critical gaps that remain critically under explored.
- Treatment relies on hormonal therapies, surgery, and ART, while research uses animal models and 2D cultures, lacking physiologically relevant human tissue models of spermatogenesis. These models fail to replicate the 3D, multicellular seminiferous tubule microenvironment, leaving the root causes of spermatogenic failure uninvestigated.
- Our biomimetic hydrogel-based 3D in vitro testis model reconstructs the seminiferous tubule niche, enabling discovery of underexplored infertility causes and diagnostic biomarkers.

HYPOTHESIS

- Testicular extracellular matrix (ECM) / methacrylated gelatin (GelMA) blended hydrogels replicating the geometry, stiffness, and biochemical composition of seminiferous tubules will support superior germ cell attachment, proliferation, and spatial organization compared to 2D cultures, enabling investigation of underexplored causes of spermatogenic failure.



OBJECTIVES

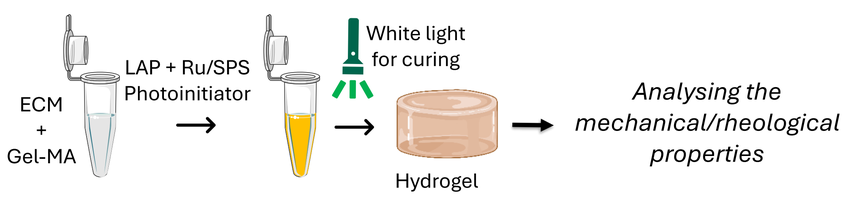
1. Fabrication of testicular ECM and Gel-Ma blended hydrogels matching the viscoelastic and mechanical features of testis tissue.

2. Evaluation of testicular cell biocompatibility within biopolymer-blended hydrogel scaffolds and the influence of varied mechanical stiffness on cell behaviour and function.

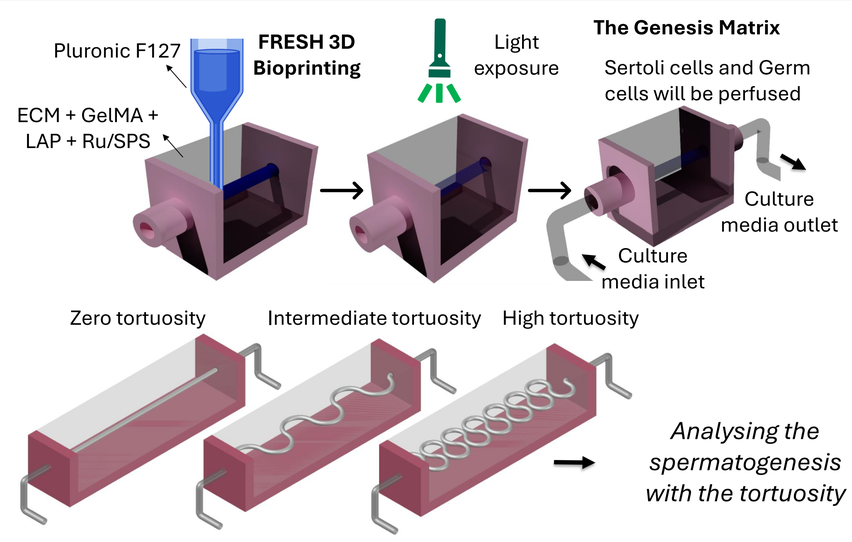
3. Assess the effect of tubule-inspired tortuosity and surface topography on Sertoli cell arrangement and sperm production.

METHODOLOGY

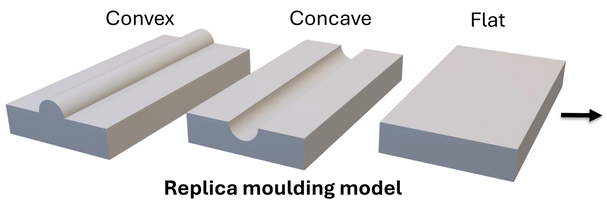
Fabrication of testicular ECM-GelMA hydrogel



Fabrication of testis model with different tortuosity



Fabrication of scaffolds with different surface topography and stiffness



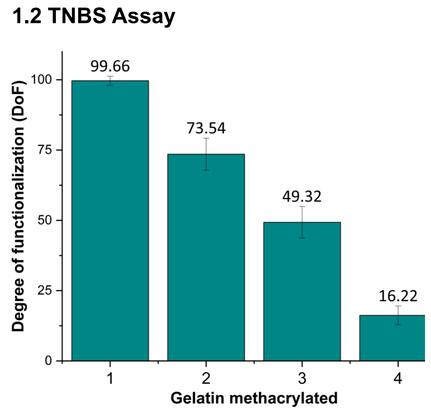
RESULTS

- Decellularized testicular ECM was blended with GelMA at four degrees of methacrylation confirmed by TNBSA assay and crosslinked with photoinitiators achieving gelation within 10–15 seconds.
- Polymer leach-out analysis revealed a burst release within the first hour, correlating with scaffold swelling behaviour.

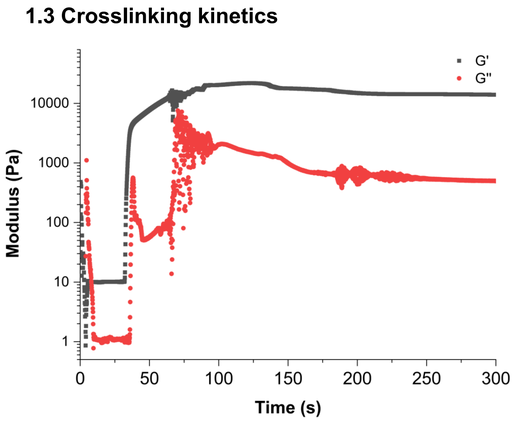
1.1 H&E staining



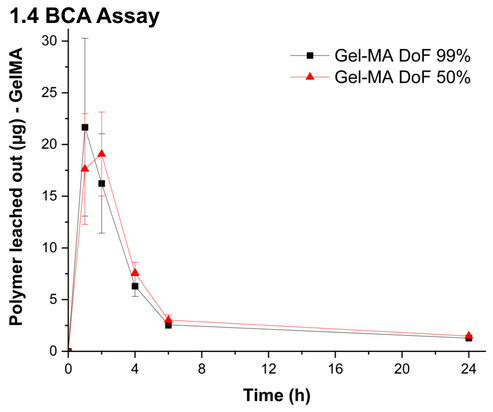
1.2 TNBS Assay



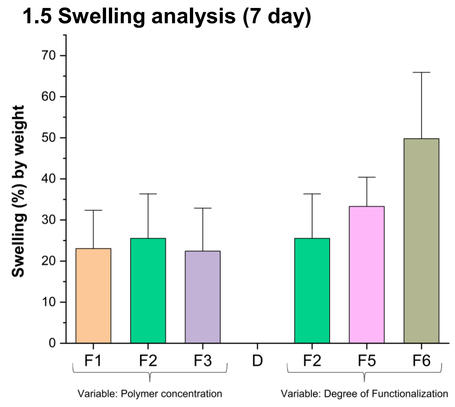
1.3 Crosslinking kinetics



1.4 BCA Assay

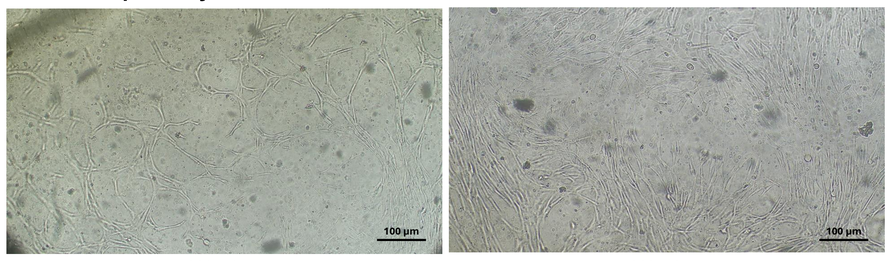


1.5 Swelling analysis (7 day)



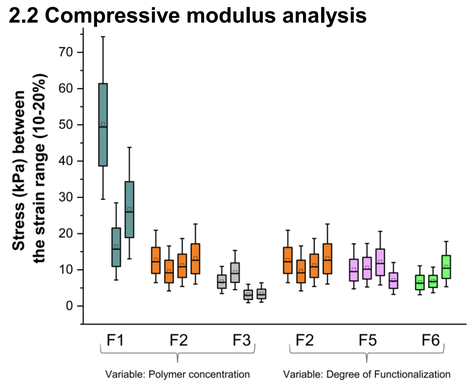
- Compressive mechanical characterisation confirmed tunable stiffness across formulations, enabling scaffolds with distinct mechanical properties for testicular tissue modelling.
- Testicular cells seeded on ECM+GelMA scaffolds demonstrated initial biocompatibility, confirming cellular adhesion and viability across formulations.

2.1 Biocompatibility assessment



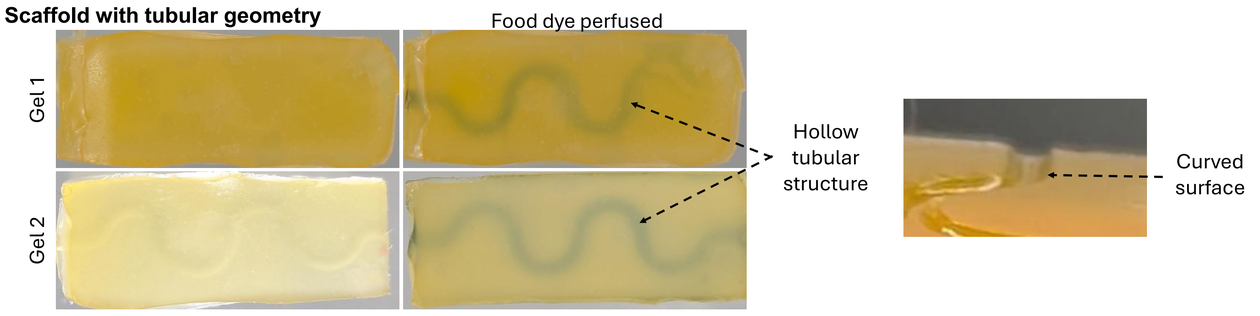
Phase contrast images - Human primary testicular cells seeded on top of the ECM+GelMA hydrogel

2.2 Compressive modulus analysis



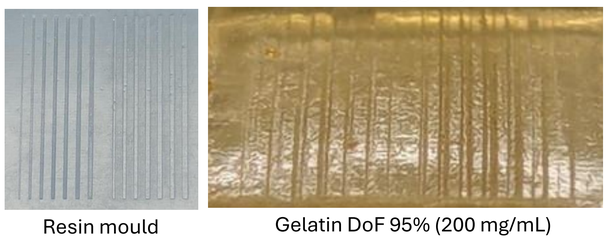
- Hollow tubular hydrogel scaffolds were successfully fabricated and perfused with food dye to visualise and confirm open luminal architecture.
- 3D-printed resin moulds with defined surface topographies were used to fabricate hydrogel scaffolds via replica moulding, enabling systematic investigation of surface topography on testicular cell behaviour.

3.1 Scaffold with tubular geometry



ECM (20 mg/mL) /Gelatin (200 mg/mL) Scaffold

3.2 Scaffold with different surface topography



CONCLUSIONS

- The fabrication of shape-mimicking scaffolds using testicular tissue-derived ECM provides a representative platform for studying the processes of spermatogenesis, including the structural changes and tortuosity that occur during puberty.
- Insights gained from a deeper understanding of the influences of tissue architecture and microenvironment are crucial for achieving complete spermatogenesis in vitro and will lead to new strategies for tackling male infertility.

ACKNOWLEDGEMENTS

- The lifETIME CDT was supported by Research Ireland 18/EPSRC-CDT/3583 and the Engineering and Physical Sciences Research Council EP/S02347X/1. This work emanated from research supported in part by a grant from Research Ireland and the European Regional Development Fund (ERDF) under grant number 13/RC/2073_P2.

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