Tuneable Hydrogels for Neural Modelling

P. Walczak[1], E. J. Hill[2], and R. Parri[1]

[1] *College of Health & Life Sciences, School of Biosciences, Aston University, Birmingham, U.K.*

[2] *Department of Chemistry, Loughborough University, Loughborough, U.K.*

Keywords— Tissue Engineering, Hyaluronic acid, Bioprinting

INTRODUCTION

Diseases of the Central Nervous System (CNS) are incurable and debilitating, incurring high socioeconomic cost [1]. *In vitro* models of the CNS support the search for treatments by circumventing issues associated with *in vivo* models. Unfortunately, *in vitro* models often lack the necessary biophysical features to mimic living tissue. Tissue engineering of biomaterials enables production of advanced 3D culture systems, equipping models with an additional dimension of biophysical information to guide cell fates. Hydrogels are favoured for CNS modelling due to their customisable nature [2], with modulation of stiffness noted as a powerful tissue engineering tool [3]. Hydrogels lend readily for use when bioprinting due to their biocompatibility and low-viscosity of ungelated precursor [4], with light-based crosslinking approaches permitting fine-tuning of crosslinking kinetics and therefore mechanical properties.

MATERIALS AND METHODS

Chemical modification of Hyaluronic Acid (HA) to form Hyaluronic Acid Methacrylate (HAMA) enabled Ultraviolet (UV) crosslinking of HAMA hydrogels at 365nm utilising LAP (Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (Sigma-Aldrich, UK, 900889)) photoinitiator' for free radical photopolymerisation. Final formulations were made to 2% (w/v) HAMA, 10% Extralink PEGDA (Mattek, Slovakia) in PBS containing varying concentrations of LAP. Mechanical properties were then assessed by the Kinexus Ultra+ rheometer (Malvern Panalytical, UK).

Extrusion printing (Inkredible+, Cellink, USA) facilitated generation of single layer 3D lattice constructs comprised of an alginate & cellulose composite (CELLINK Bioink, Cellink, USA). Printing conditions were as follows: 1) SHSY-5Y neuroblastoma cells previously transfected with mCherry fluorescent protein were loaded into the bioink at $2x10^6$ per ml. 2) Pressure of extrusion was 12kPa with a 22G print nozzle. 3) Constructs were gelated via immersion in $CaCl^2$ for 10 min, followed by a 5min RPMI wash. Bioprinted constructs were then fed with RPMI 1640 medium containing 10% (v/v) FBS, 1% L-Glutathione and 2% Penicillin/Streptomycin (All available from Sigma). Gentle half media changes were performed every other day. Cell viability was evaluated via the EVOS XL Core Imaging System (Life Technologies).

RESULTS AND DISCUSSION

Results demonstrate that stiffness or elastic moduli (here quantified as G') of HAMA hydrogels is highly dependent upon concentration of crosslinker; with a 10-fold decrease in LAP crosslinker (from 17mM to 1.7mM) presenting with an over 100-fold decrease in elastic moduli. Fluorescence imaging demonstrates biocompatibility of CELLINK Bioink to D28 in vitro, however cell spreading within the 3D hydrogel was limited, with SH-SY5Ys preferentially migrating to the tissue culture treated plastic underneath to facilitate spreading.

CONCLUSIONS

This work highlights the ease with which mechanical properties of UV-cured hydrogels can be fine-tuned. HAMA hydrogels containing 3.4mM LAP displaying the most biologically relevant stiffness of ~1kPa. Results also demonstrate compatibility of the CELLINK for long-term cell viability. Future work will look to integrate HAMA biomaterials into the Inkredible+ printing system.

REFERENCES

1. Matthews, F.E. et al. Nat. Commun. 7:11398, 2016
2. George, J. et al. Biotechnol. Adv. 42:107370, 2020
3. Baruffaldi, D. et al. ACS Appl. Bio Mater. 4:2233–2250, 2021
4. Chimene, D. et al. Adv. Mat. 32:1902026, 2020

ACKNOWLEDGEMENTS (IF ANY)

We thank EPSRC and SFI for funding the lifETIME Centre for Doctoral Training (EP/S02347X/1).