3D manufactured islet micro-tissues for the treatment of type-1 diabetes

AA Popov[1,2], G Montalbano[1], WE Scott[2], J Chen[1], JAM Shaw[2], K Dalgarno[1], AM Ferreira-Duarte[1]

[1] *Mechanical and Systems Engineering, Newcastle University, UK.* [2] *Institute of Cellular Medicine, Newcastle University, UK.*

INTRODUCTION: Type-1 *diabetes mellitus* is defined by the inability to control systemic glucose levels, due to an autoimmune destruction of the insulin-producing β-cells. Treatment involves routine glucose monitoring and insulin infusion. However, some patients with hypoglycaemia unawareness may require pancreas or islet transplantation [1]. Islet transplantation is a minimally invasive 'micro-tissue' therapy aimed at reducing hyperglycaemia and eliminating severe hypoglycaemia. This is achieved by infusing islets of Langerhans into the portal vein; where they engraft in the liver. Major limitations of islet transplantation include the need for life-long immunosuppression and a high tissue demand [2]. This work aims to overcome these restrictions and improve engraftment by applying 3D bioprinting to standardise islet size and ECM composition with the goal to reduce tissue loss immediately post transplantation and improve engraftment. A tri-component 'bioink' hydrogel was developed and investigated using collagen, alginate and fibrin (CAF), at three collagen concentrations (0.5%, 1% and 2.5%), for β-cell replacement therapy to treat type-1 diabetes.

METHODS: CAF hydrogels were produced +/- MIN6 β-cells. Morphology was characterised using SEM and TEM, while chemical composition was evaluated using XRD and FTIR. Mechanical properties, viscosity and biodegradation of the hydrogel material was also analysed. CAF hydrogels seeded with MIN6 β-cells were cultured for 7 days, to assess biocompatibility. Micro-tissues <150 μm were manufactured using an *nScrypt* 3Dn printer and evaluated for viability, metabolic activity, and functionality via immunostaining for insulin and E-cadherin.

RESULTS: CAF hydrogels with 0.5%, 1% and 2.5% collagen demonstrated pore size in the 40-200 μm range (Fig. 1A). 0.5% and 1% CAF hydrogels had highly inter-connected porosity, which was not present in 2.5% gels. TEM showed collagen fibre formation in hydrogels made with 1 % collagen, but not in 0.5 and 2.5% gels (Fig. 1B). Rheological testing demonstrated that all CAF hydrogels had a G' modulus of ~1000 Pa, similar to human pancreatic tissue [3]. 1 and 2.5% CAF hydrogels were stable for >15 days of biodegradation, while 0.5% hydrogels disintegrated after 5 days. Biocompatibility assessed by cell viability and metabolic activity was inversely proportional to collagen concentration, with the best results found for 0.5% collagen. Furthermore, bioink extrusion became more difficult as collagen concentration increased and 2.5% CAF hydrogels could not be accurately extruded. Insulin and E-cadherin immunostaining demonstrated similar functionality of MIN6 β-cells before and after bioprinting.

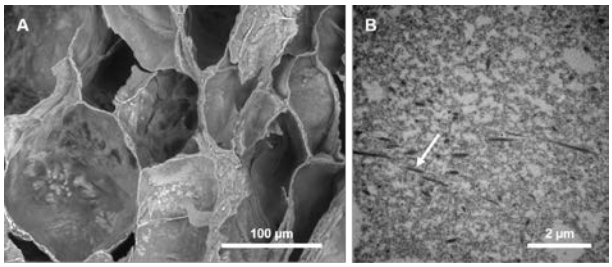

Fig. 1: SEM (A) of hydrogel architecture and TEM (B) of collagen fibre formation (indicated by arrow), in CAF hydrogels made with 1% collagen.

DISCUSSION & CONCLUSIONS: A novel collagen, fibrin and alginate hydrogel (CAF) was developed for use as a bioink substrate in the manufacture of islet micro-tissues. A general enhancement in mechanical properties, porosity and resistance to biodegradation was observed with increasing collagen content. This was offset by reduced biocompatibility and an increased difficulty to extrude the bioinks, which may make printing of CAF hydrogels impossible. 7 day culture of micro-tissues highlighted the influence of hydrogel structure and composition on cell compatibility in 3D – demonstrating the necessity for appropriate bioink formulation as a substitute for native ECM in manufactured micro-tissues.

REFERENCES: [1] A.M. Shapiro, J.R. Lakey, E.A. Ryan, et al (2000) *N Engl J Med* **343**: 230-8. [2] K.K. Papas, C.K. Colton, R.A. Nelson, et al (2007) *Am J Transplant* 7: 707-13. [3] C. Wex, M. Fröhlich, K. Brandstädter, et al (2015) *J Mech Behav Biomed Mater* **41**: 199-207.

ACKNOWLEDGEMENTS: The authors would like to thank C Olivieri, N Al Jahdhami and M Honkanen-Scott.