3D-Bioprinting of functional β-cell spheroids for Type 1 Diabetes treatment

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INTRODUCTION

Type 1 Diabetes Mellitus (T1DM) results from autoimmune destruction of pancreatic β-cells, causing absolute insulin deficiency. Current insulin therapies, such as the injection of insulin, fails to replicate physiological insulin secretion, highlighting the need for alternative treatments. In our laboratory we developed UNIINK, a platform enabling rapid, sterile, and operator-independent fabrication of 3D-bioprinted micro-spheroids using collagen-I crosslinked with tannic acid (ColTA). These spheroids provide structural stability, support β-cell viability and allow nutrient diffusion while offering immune protection. ColTA-spheroids maintained insulin-secreting β-cells for up to two months in vitro. In hyperglycemic mice, transplantation of INS1-E β-cell spheroids significantly reduced blood glucose levels, demonstrating the therapeutic potential of UNIINK for T1DM cell therapy.

METHODS

INS1-E cells (β-cell line from rat insulinoma) were encapsulated in collagen 4 mg/mL, thermally crosslinked at 37 ºC for 10 minutes and submerged in a solution of tannic acid 1%, ColTA for 1 minute. The encapsulations were done either by bioprinting (BioX, Cellink) in a cell density of 7×10^6 cells·mL^{-1}.

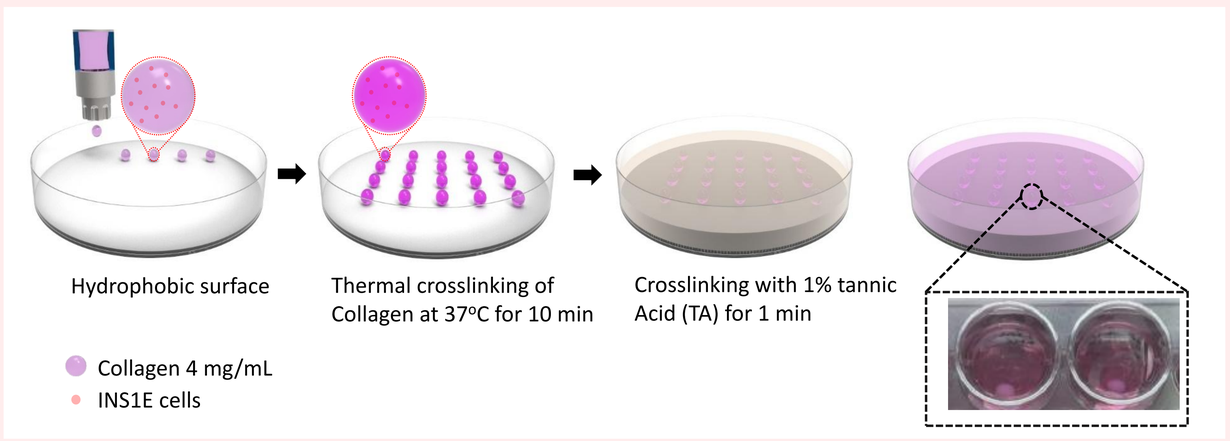


Figure 1. Cell-laden spheroids generated by the novel 3D bioprinter technique. Schematic depiction of INS-1E-laden spheroids fabrication crosslinked with TA solution.

RESULTS

1. Successful Production of ColTA Spheroids Demonstrating Long-Term In Vitro Viability and Sustained β-Cell Functionality

Spheroid viability and metabolic activity were assessed using the AlamarBlue® assay (Invitrogen™, A50100). Each sample was placed in a well of a 48-well plate containing culture medium with a 1:10 dilution of AlamarBlue and incubated at 37 °C. For ColTA spheroids, incubation ranged from 2–8 hours for absorbance measurements or 2 hours for fluorescence analysis. Subsequently, 100 µL from each condition was transferred to a 96-well plate and analyzed using a SYNERGY H1M fluorescence plate reader. Fluorescence was recorded with excitation at 560 nm and emission at 590 nm. Fluorescence measurements were used to quantify spheroid viability across three independent batches. As shown in Figure 2, variability was observed between batches; however, spheroids remained viable even after two months of encapsulation. These results indicate that ColTA spheroids can maintain long-term viability.

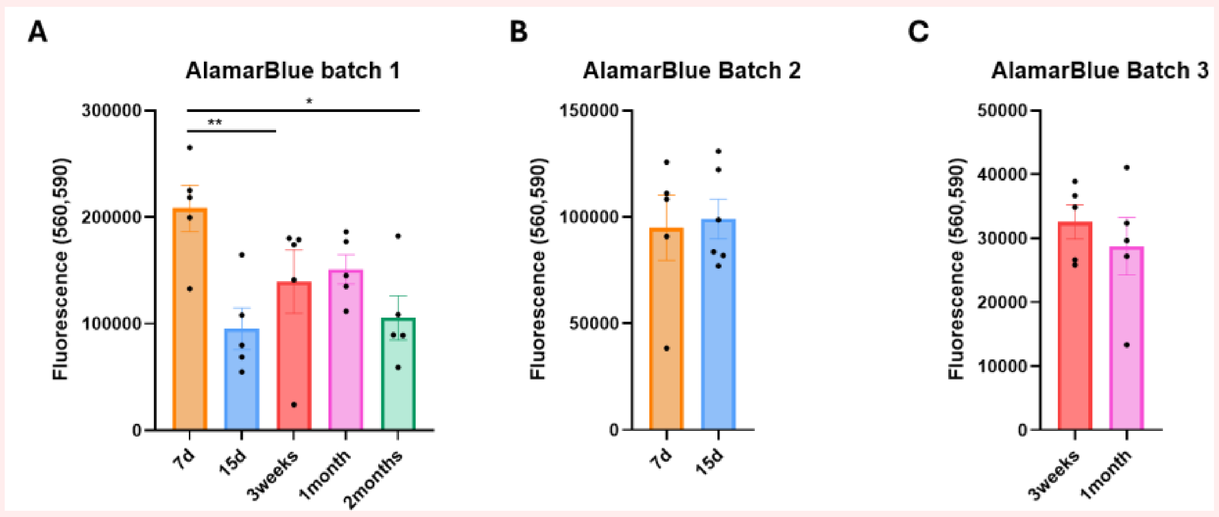


Figure 2. ColTA-INS1E spheroids maintain their viability during time, despite their variability. A) Batch 1. Alamar Blue test of ColTA INS1E at 7d, 15d, 3weeks, 1month and 2 months after encapsulation and maintained in culture. **B)** Batch 2. Alamar Blue test of ColTA INS1E at 7days and 15 days after encapsulation. **C)** Batch 3. Alamar Blue test of ColTA INS1E at 3weeks and 1 month after encapsulation. N = 5-6 spheroids per condition. Data were analysed using a one-way ANOVA with Dunnett's post hoc test to compare cryopreserved conditions to the fresh control group. * $p < 0.05$, ** $p<0.01$.

After assessing the viability of our spheroids, to evaluate the functionality of the spheroids, we performed glucose-stimulated insulin secretion (GSIS) assays. We performed the assay with different batches of 7d and one for 15d, 1month and 2months. Spheroids were first incubated under low-glucose conditions (2.8 mM) to establish basal insulin release, followed by exposure to high-glucose medium (16.7 mM) to trigger insulin secretion. Supernatants were collected at each stage and insulin levels were quantified by ELISA (CrystalChem). An increase in insulin secretion in response to glucose at different time points after encapsulation confirmed the functional activity of the spheroids, demonstrating their ability to sense glucose and release insulin accordingly (figure 3). However, the insulin secretion following glucose stimulation was modest, indicating the need for further refinement of the procedure.

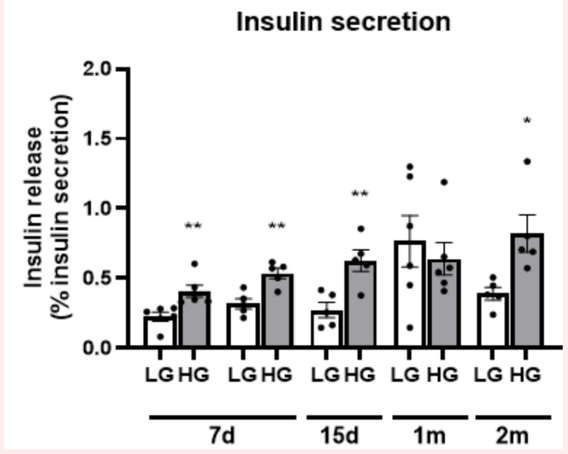


Figure 3. Insulin secretion increased in ColTA-INS1E spheroids after a glucose stimulus. Functionality of spheroids from 7d, 15d, 1month and 2 months were assessed, and insulin release was determined by ELISA. N= 5-6 per condition. Statistical analysis between LG condition and HG condition for each batch was performed by t-test. *$p < 0.05$, ** $p < 0.01$.

2. In Vivo Evaluation of UNIINK Spheroids in Diabetic Mouse Models

To test the efficacy in vivo of UNIINK, we selected two different diabetic mice models: a spontaneous one (NOD mice) and an induced one by injections of streptozotocin (STZ mice). After the apparition of hyperglycaemia in our mice models, we inserted spheroids with INS1E cells (β-cells) embedded within them. We measured the glycaemia with a glucometer (AVIVA Accu-Check, Roche) by obtaining a drop of blood from the tail vein. For the STZ model (induced model) the chronic concentration of **50 mg/kg** was defined and used for the whole project. We fabricated spheroids with ColTA and INS1E (in a cell density of $7 \cdot 10^6$cells/mL) and we maintained them for one week in culture. After that, we implanted the equivalent of 2000 islets in 12-week-old mice in this case (n=5 NOD and n=5 STZ). We implanted the spheroids intraperitoneally and measured the glucose until day 30. The spheroids of ColTA with INS1E embedded within it were able to decrease the blood glucose over time. However, this implantation could not restore the normoglycaemia in the mice for a long period of time (Figure 4)

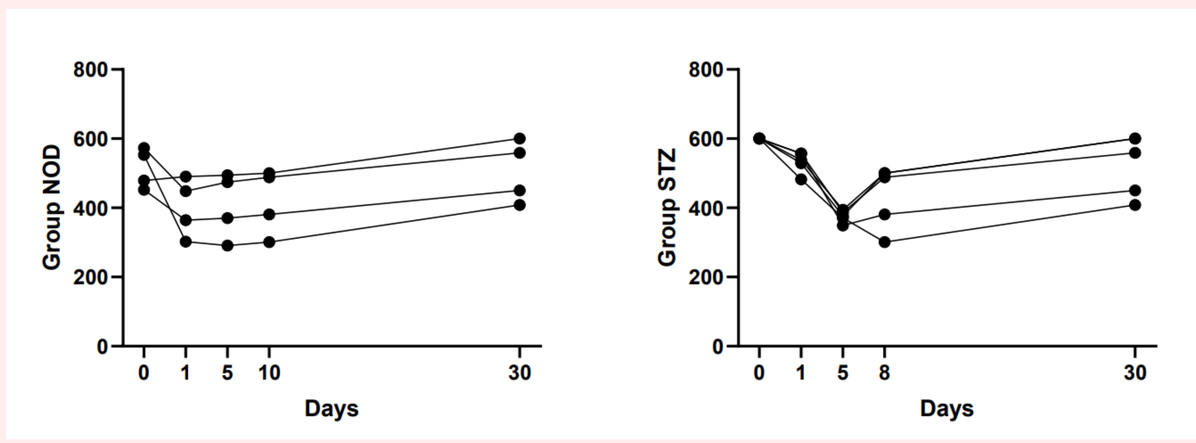
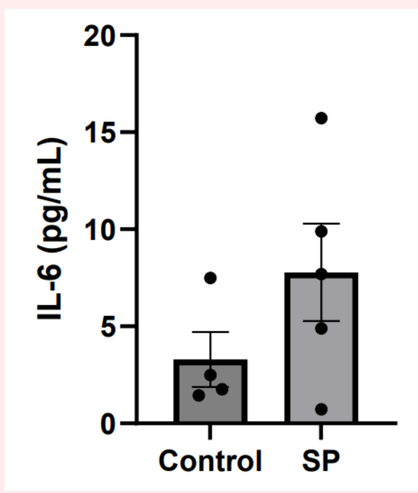


Figure 4. The implantation of spheroids decreased the levels of glucose in diabetic mice models. A) NOD mice (n=5), B) STZ-mice (n=5).

3. Assessment of the inflammatory response against UNIINK



Five 12-week-old C57BL/6 mice were transplanted with 10 cell-free spheroids. After 7 days, plasma samples were collected and compared with those from five sham-operated control mice (surgery without transplantation). Plasma IL-6 levels were quantified by ELISA (R&D Biosystems). No significant increase in IL-6 concentration was observed in transplanted mice compared with controls. Nevertheless, a trend toward higher IL-6 levels was detected, suggesting that the procedure may induce a mild inflammatory response.

Figure 5. Plasma IL-6 levels in transplanted mice showed a non-significant increase compared with sham-operated controls. Statistical analysis was performed using Student's t-test.

CONCLUSIONS

The **UNIINK platform** enables the rapid and scalable fabrication of **3D ColTA micro-spheroids** containing functional β-cells for potential **T1DM** therapy. These spheroids maintain **long-term β-cell viability and insulin secretion in vitro** and demonstrate **biocompatibility and immune tolerance in vivo**. Implantation of **INS1-E–laden spheroids** in hyperglycaemic mice significantly reduced blood glucose levels, approaching near-physiological values. Overall, these results highlight the **therapeutic potential of the UNIINK platform** as a promising cell-based strategy for **glycaemic control in T1DM**, supporting its **continued development and future clinical translation**.

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