Waffle-inspired micropatterned macrodevice for spatially controlled distribution and vascularization of encapsulated cellular therapeutics

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Statement of Purpose: Transplantation of islets in a macroencapsulation system is a promising treatment for type 1 diabetes[1]. However, the approach is limited by microtissue aggregation and inaccessibility of vasculature to the embedded cellular grafts, leading to insufficient mass transfer and eventually cell death[2]. Herein, we created a waffle-inspired hydrogel-based device enabling macro-encapsulation of microtissues in a homogeneous distribution using a micropatterned hydrogel pattern allowing the incorporation of vascular-inductive cells. Its potential in cell-based therapy for the treatment of type 1 diabetes was evaluated *in vitro* and *in vivo*.

Methods: We fabricated waffle-inspired micropatterned gelatin methacryloyl pattern (GelMA "lock) using photolithography. (**Fig. 1**). Human umbilical vein endothelial cells (HUVEC) were embedded in the GelMA precursors priors to UV exposure. Microtissues from INS-1E cell lines or primary rat islets mixed with alginate solution ("key") were deposited on the pattern then crosslink. The device design parameters were optimized to improve distribution of microtissues.

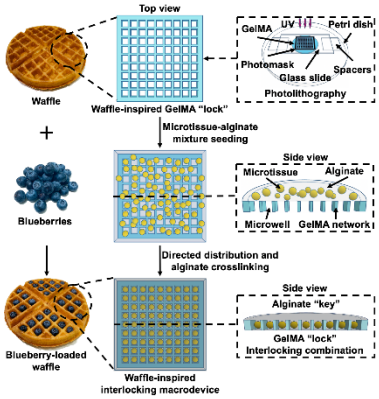

Figure 1. Schematic fabrication of waffle-inspired interlocking macrodevice ("WIM").

The viability of microtissues was characterized using live/dead staining and confocal microscopy. The distribution and function of HUVEC inside the GelMA grid were showed through immuno-staining of CD31. The glucose-stimulated insulin secretion (GSIS) was quantified by ELISA following microtissues incubation in 16.8mM and 2.8mM glucose solutions. Macrodevices encapsulating 500 islet equivalents (IEQ) of primary rat islets were implanted in STZ-induced diabetic mice to evaluate glucose control potential.

Results: We fabricated several designs of GelMA "lock" component by varying its micropattern to yield microwells with different shapes and sizes. We quantitatively investigate the relationship of microtissue distribution and various design parameters of the devices to maximize the distribution of one microtissue per one microwell. The S-300 design with square microwells of 300µm in length was proven as the optimal design to maximize homogeneous distribution of microtissues (**Fig. 2A**). Confocal microscopy confirmed that the microtissues remained well-distributed inside the microwells and exhibited high viability after alginate crosslinked (**Fig. 2B**).

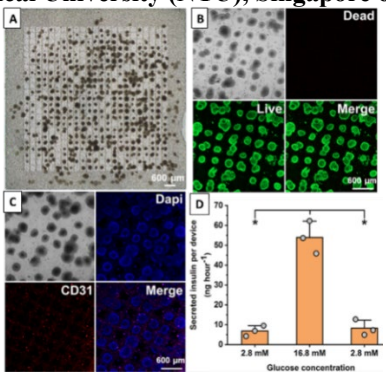

Figure 2. A) Bright field and B) live/dead images of HUVEC/ microtissues-encapsulating macrodevice. C) Immunofluorescent images of the macrodevice after staining of CD31. D) Static glucose-stimulated insulin secretion test by consecutively subjecting the device to different glucose solutions (n=3, N=2). (*) denotes statistical difference ($p < 0.05$).

We also performed immunofluorescent staining of CD31 – a marker for endothelial presence and function – to demonstrate the preserved functional potentials and viability of the laden HUVEC. The GelMA "lock" was stained red throughout, indicating the extensive CD31 expression of the laden HUVECs. Therefore, the system was confirmed to preserve the vascular-inductive potential of HUVECs while encapsulating them in proximity to INS-1E microtissues (**Fig. 2C**). During GSIS, a higher amount of insulin was secreted in response to the higher glucose level (**Fig. 2D**), confirming that microtissues in the device retained their glucose-responsive behavior. In preliminary *in vivo* evaluation, this device containing primary islets was able to lower the blood glucose level of diabetic mice after a simple subcutaneous implant using a marginal islet mass (**Fig. 3A, 3B**).

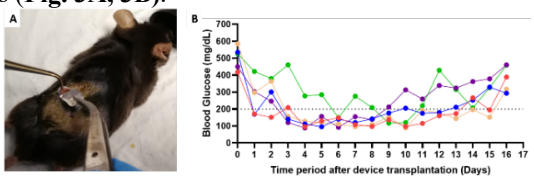

Figure 3. A) Subcutaneous implantation of macrodevice encapsulating primary rat islets into diabetic mice. B) Glucose monitoring of diabetic mice post-implant.

Conclusion: We demonstrated that the micropatterned hydrogel pattern homogenously distributed the encapsulated microtissues and provided spatial guidance for vascular cells. The macrodevice prototype has also demonstrated potential function in vivo. Thus, this macrodevice prototype might have various implications on cell encapsulation strategies for cell replacement therapy of type 1 diabetes and other hormone-deficient diseases.

References:

1. Wang L. Sci. Adv. 2021;7:eabd5835.

2. Colton C. Adv. Drug. Deliv. Rev. 2014;67:93-110.