In Vivo High-throughput Screening of Immunomodulatory Biomaterials using Cellular Barcoding for Human Islets Transplantation in Immunocompetent Type 1 Diabetic Mice Models

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Statement of Purpose: Encapsulation of pancreatic cells to treat type 1 diabetes (T1D) has emerged as a promising approach to overcome donor shortages and eliminate the need for immunosuppression. However, implanted biomaterials often trigger foreign body responses that lead to fibrosis and subsequent implant failure[1,2]. Our objective is to develop a high-throughput screening method to find anti-fibrotic encapsulation materials for therapeutic cell delivery. Based on the prior library of small molecule conjugated-alginate analogs[1], we synthesized hundreds of novel alginates with triazole modification, identified their anti-fibrotic properties *in vivo*, and found a leading biomaterial that can not only enable long-term protection of encapsulated human islets but also preserve their functions in T1D mice model.

Method: A library of triazole containing alginate analogs was devised, and a total of new 210 alginate analogs was synthesized via click chemistry. After material characterization, 150 alginate analogs were chosen as a suitable material to fabricate capsules and screened in profibrotic C57BL/6J mice. A unique single-nucleotide polymorphism (SNP) profile from 20 different human umbilical vein endothelial cells (HUVECs) donors was designed, and the genotype patterns were used to barcode material's identity by encapsulating each donor within new biomaterials (**Fig. 1**).

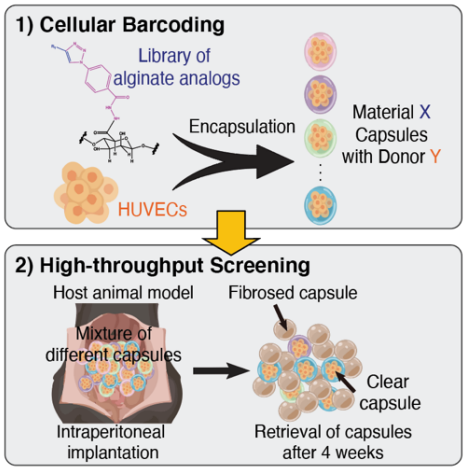

Figure 1. *Schematic of in vivo high-throughput material screening.*

This method can screen up to 20 distinct material formulations in the same implant site with next-generation sequencing (NGS) analysis. In addition, we were able to increase the material barcoding capacity up to 400 and finally screened 100 materials in the NHP model with a dual donor barcoding strategy. The lead found from mice screening was used to encapsulate human islets and implanted into STZ-induced T1D mice, and long-term islets viability and functions were investigated.

Results and Conclusions: After four weeks post-implant, the low fibrosed capsules were selected, and their identity was revealed using NGS by demultiplexing each material's SNP profile. This *in vivo* material screening method enables the evaluation of the biocompatibility and anti-fibrotic properties of various alginate analogs in unbiased conditions with a high-throughput manner. The lead material (Z4-A10), showing the best anti-fibrotic response during screening, was utilized to deliver human islets in diabetic immunocompetent mice. Islets within Z4-A10 capsules in IP space were successfully engrafted and allowed long-term diabetic correction compared with SLG20 capsules without immunosuppressive treatment (**Fig. 2**). The explanted capsules showed minimal fibrosis depositions on their surface with highly viable islets after 80 days post-implant. We also found that the lead material improved potency preventing the medical-grade catheter from fibrosis upon implant. Taken together, our newly discovered lead has a substantial potential for clinical translation in improving the performance of medical devices and encapsulated cell-based therapeutics.

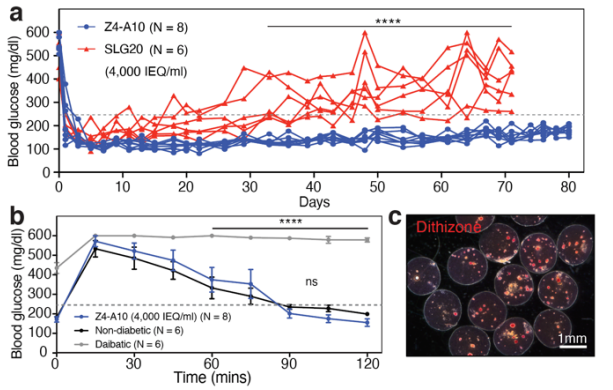

Figure 2. *Lead biomaterial (Z4-A10) mitigates host immune response and protects islets in long-term: (a) BG monitoring after human islets transplantation (2,000IEQ per mouse) in STZ-induced C57BL/6J mice, (b) IVGTT test, (c) DTZ staining image of explanted Z4-A10 capsules at 80 days post-transplantation.*

References: Vegas AJ. *Nat Biotechnol*. 2016; 34:345-352. Veiseh O. *Nat Mater*. 2015; 14:643-651.

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