# Engineering a 3D Human Ex Vivo Model of Insulitis Using Donor-Derived Islets and Autologous Immune Cells

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**Introduction:** Type 1 diabetes (T1D) is a chronic autoimmune disease, most commonly diagnosed in children, in which the immune system selectively destroys pancreatic insulin-producing beta cells, leading to insulin deficiency and chronic hyperglycemia. Affecting an estimated 9–10 million people worldwide, its incidence continues to rise, particularly among adolescents and young adults. A central pathological hallmark of T1D is insulitis, an inflammatory lesion marked by immune cell infiltration around and within pancreatic islets that progresses through distinct stages. This process is driven by a dynamic and self-amplifying dialogue between infiltrating immune cells and beta cells: $CD4^+$ and $CD8^+$ T cells and macrophages release pro-inflammatory cytokines such as IFN-α, IFN-γ, TNF-α, and IL-1β, as well as chemokines, which recruit additional immune cells, sustain local inflammation, and activate beta-cell apoptotic pathways and stress, leading to dysfunction and loss. Importantly, beta cells also actively contribute by mounting stress responses and secreting inflammatory mediators that further amplify immune activation. While animal models such as the non-obese diabetic mouse have been invaluable, their rapid disease kinetics differ substantially from the slower and more heterogeneous progression in humans. Therefore, developing a dynamic human-relevant in vitro model of insulitis that integrates pancreatic islets with key immune cell populations and reproduces the pro-inflammatory microenvironment would provide a physiologically meaningful platform to dissect disease mechanisms, and support preclinical evaluation of therapies aimed at preventing beta-cell loss.
**Materials and methods:** Human islets and autologous peripheral blood mononuclear cells (PBMCs) were isolated from brain-dead organ donors. To recreate the immune–islet interactions of insulitis, islets were embedded in Matrigel domes and co-cultured in 3D with CFSE-labeled PBMCs under a pro-inflammatory cytokine cocktail (IL-1β+TNFα+IFNγ), enabling real-time fluorescent tracking of immune cell infiltration and islet interactions for up to 6 days. Insulitis (infiltration, inflammation, stress, death and dysfunction) was evaluated by life cell imaging, real time PCR, dynamic glucose-stimulated insulin secretion (GSIS) using perifusion system and ELISA.
**Results:** The 3D co-culture model successfully reproduced both the initiation and progression of insulitis. The cytokine cocktail drove robust immune infiltration, increased inflammatory mediator and endoplasmic reticulum stress, impaired insulin secretion and induced beta cell death.
**Conclusions:** This human-relevant in vitro model captures the dynamic crosstalk between islets and immune cells across early and progressive stages of insulitis, providing a valuable platform for mechanistic studies and therapeutic testing.