# Multifunctional Platelet-Like Particles to treat Polytrauma-Traumatic Brain Injury

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Traumatic brain injury (TBI), referring to a mechanical insult to the brain, is the leading incidence for morbidity and mortality in all populations. Polytrauma-traumatic brain injury (PT-TBI) is when a patient sustains a TBI with significant secondary injuries and accounts for 78% of all TBI injuries. Regardless of PT-TBI or TBI alone, the complex pathophysiology that ensues comprises edema in the brain, hypocoagulation and platelet deficiencies that contribute to persistent neuroinflammation and blood-brain barrier (BBB) breakdown. In particular, platelets are crucial for post-traumatic hemostasis, angiogenesis, inflammatory regulation, and release of growth factors to promote tissue repair, including BBB restoration. Additionally, PT-TBI patients often present with concomitant respiratory infections due to systemic immune suppression. Current treatments inadequately address interconnected issues such as inflammation, infection, and recovery. We addressed this critical therapeutic gap by designing an “all-in-one” therapy for PT-TBI to stop hemorrhaging, prevent infection, and reduce inflammation using a nanosilver platelet-like particle (Ag-PLP). The PLP is a novel synthetic platelet system based on an ultra-low crosslinked microgel displaying fibrin-binding antibody fragment, Sd-FV, that improves clot strength and structure. Here, the anti-microbial nanosilver particles embedded within the PLP serve to mitigate infection associated with PT-TBI.

A PT-TBI was produced using the well-established controlled cortical impact TBI model alongside a femoral artery injury during the same anesthetic event in Sprague Dawley rats (ASU IACUC approved). Male cohorts (n=5, 8-10 wks old) were used to assess the therapeutic effects of saline or PLP alone at 3 days post injury. Naïve cohorts served as baseline controls. Bleeding after the femoral artery injury was compared amongst cohorts to evaluate differences in total volume and rate. We also observed differences in immune cell populations in the brain, blood, and spleen between uninjured and polytrauma cohorts. In concordance with previous reports, we observed elevated microglia population in the ipsilateral injured brain compared to contralateral, indicating immune cell activation post-injury. Additionally, we assessed lung infection by culturing lung tissue homogenates on blood agar, where we observed saline treated injured cohorts exhibit α and  hemolytic bacteria growth, which was not observed in PLP treated cohorts. Future studies will focus on the Ag-PLP formulation. Ultimately, the base PLP formulation demonstrates promise in reducing pathological polytrauma outcomes.

Type 1 diabetes (T1D) results from autoimmune-mediated destruction of pancreatic beta cells, necessitating lifelong exogenous insulin therapy. Allogeneic stem cell-derived beta cell (sBCs) transplantation can restore endogenous insulin production but requires chronic systemic immunosuppression to prevent graft rejection. These drugs increase the risk of infections, malignancies, and teratoma formation from residual pluripotent cells. To address these safety concerns, we have developed a scalable hydrogel injection molding-based macroencapsulation method; macroencapsulation may reduce or eliminate the need for immunosuppression by preventing direct antigen recognition by host immune cells. This high-throughput method produces spiral-shaped alginate constructs with high surface area-to-volume ratios, optimized for nutrient and oxygen exchange.

Macroencapsulated human sBCs derived from the Mel1 INSGFP cell line expressing a GFP insulin reporter and constitutively expressing luciferase demonstrated comparable function and viability to unencapsulated controls via metabolic activity, live/dead confocal microscopy, and dynamic glucose-stimulated insulin secretion assay. sBC-specific CAR-T cells were used to evaluate macroencapsulation protection against direct antigen recognition, where encapsulation significantly reduced CAR-T proliferation and activation in vitro.

Control and encapsulated sBCs, transplanted into the epididymal fat pads of NSG mice, demonstrated comparable survival via longitudinal bioluminescent imaging and human C-peptide levels to at least 54 days. sBC-specific CAR T cells were adoptively transferred 54 days post-transplant, and a significant decline was observed in C-peptide and bioluminescence for sBC controls relative to macroencapsulated sBC. Overall, in vitro and in vivo assessment demonstrate that sBC macroencapsulation preserves cell function and viability relative to unmodified control cells, and prevents T cell-mediated sBC rejection.

# Cell-to-cell communication analysis to interrogate trophoblast tolerance induction in placental mimicry cell therapy

David Ruiz-Pardo, Chishiba Chilimba, Shivani C. Hiremath, Jessica D. Weaver

Chronic systemic immunosuppression is required to prevent rejection of transplanted allogeneic cells, tissues, and organs. However, immunosuppression varies in efficacy and significantly increases the risk of life-threatening infections and malignancies. By contrast, placental pregnancy is a model of allogeneic graft acceptance, where immunological tolerance is induced toward allogeneic tissue. We have shown that alginate encapsulated human placental trophoblast cells (JAR cell line) can evade immune destruction in a xenogeneic transplant model (mouse), and prevent or delay rejection of encapsulated xenogeneic bystander cells. To elucidate the mechanism by which trophoblast soluble factors modulate immune rejection through communication with immune cells, we used a JAR proteomics dataset and immune cell scRNA-seq dataset to perform receptor-ligand analysis of JAR secreted factor interactions with immune cell surface receptors. First, JAR secretome was mapped onto the human interaction database at IntAct to uncover all potential interactions between the JAR cell secretome and immune membrane proteins. Over-representation analysis of the resulting interactome showed that JAR secretome interacts predominantly with innate immune processes, such as neutrophil degranulation. Receptor-ligand (R-L) pair analysis demonstrates pairs between JAR ligands and immune receptors across 18 innate and adaptive immune cell subtypes, with the top 50 R-L pairs demonstrating a greater number of significant pairings in innate immune cells. Overrepresentation analysis demonstrates these pairs significantly overlay with pathways associated with innate and adaptive immune system, cytokine signaling, and complement cascade; notably, there is a high overlap ratio with IL-4 and IL-13 signaling, which is associated with type 2 immunity. Overall, cell-to-cell communication analysis between trophoblasts and immune cells provides insights into how placental mimicry cell therapy may prevent bystander cell rejection in a xenogeneic model. We are currently validating these findings using high dimensional myeloid (25 markers) and lymphoid (32 markers) flow cytometry analysis.

**Sustained Delivery of Alpha Ketoglutarate and BMP2 in Hyaluronic Acid Hydrogels for Enhanced Bone Repair**

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**Introduction:** Bone cell metabolism is an important research area that remains largely understudied in orthopedics despite 2.2 million bone grafts implanted annually worldwide (1). Bone morphogenetic proteins (BMPs) are soluble growth factors clinically approved to promote bone repair; however, supraphysiological concentrations required for osteogenesis cause adverse clinical events and high expenses (2), which have reduced the usage of existing BMP2-based therapies. We have designed novel polyester of alpha ketoglutarate (paKG) microparticles (MP). Monomeric aKG has been shown to promote osteoblast differentiation and decrease osteoclast activity (3), indicating the potential of aKG to both enhance bone formation and inhibit bone loss. By encapsulating paKG and BMP2 in a proteolytically-degradable hyaluronic acid hydrogel, we enabled the co-delivery of paKG with a low concentration of BMP2. Additionally, paKG MP are highly tunable, and hydrolytically degradable, so, as the hydrogel degrades there will be a controlled, sustained release of aKG and low concentrations of BMP2 to promote bone repair in a synergistic fashion for the full cycle of bone healing.

**Methods:** PaKG was synthesized through the reaction of alpha ketoglutaric acid with 1,10-decandiol. Maleimide-modified hyaluronic acid (MaHA) was generated by reaction of 2-aminoethylmaleimide trifluoroacetate salt with the tetrabutylammonium salt of hyaluronic acid using a BOP coupling reagent. 2-5 wt% hydrogels +/- paKG MP were crosslinked using a bifunctional thiol crosslinker at a 1:1 maleimide:thiol molar ratio. PaKG *in vitro* bioactivity was examined using human mesenchymal stem cells (hMSCs) and RAW264.7 macrophages. *In vivo* studies were performed in Sprague Dawley rats with 8 mm critical-sized calvarial defects.

**Results:** PaKG MP were synthesized through double emulsion (Fig 1A). MaHA was synthesized and paKG MP were successfully encapsulated within MaHA hydrogels which did not interfere with hydrogel crosslinking (Fig 1B). Monomeric aKG release from paKG microparticles was confirmed through high performance liquid chromatography (Fig 1C) with sustained release for a minimum of 14 days.*In vitro* studies were performed to confirm paKG bioactivity (without BMP2) and select concentrations for *in vivo* investigation with BMP2.

*In vivo* models tested combinations of paKG MP (0.25 or 1 mM) and a low concentration of BMP2 (0.1 ug) within MaHA hydrogels. MaHA hydrogels were placed in rat critical-sized cranial defects. After 6 weeks, X-ray radiography (Fig 2), microcomputed tomography (bone volume, bone volume/total volume), and histological staining were performed to evaluate the impact of a low concentration of BMP2 co-delivered with metabolites on bone healing. Preliminary findings indicate the combined delivery system of paKG MP in MaHA hydrogels with low dose BMP2 increases bone formation *in vivo* comparable to a high dose of BMP2 alone.

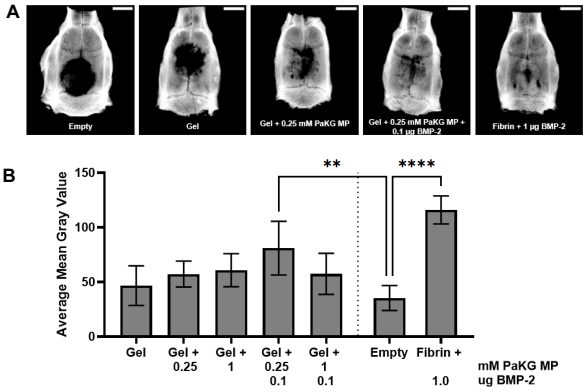


**Fig 2**. (A) Representative Faxitron images of cranial defect with PaKG MP in MaHA hydrogel with low dose BMP2 compared to empty defect (negative control) and fibrin with high dose of BMP2 (positive control). Scale bar is 5 mm. (B) Quantification of average mean gray value within the 8 mm defect from analysis of Faxitron images. Asterix indicates statistical significance ($p < 0.05$).

**Conclusion**: Together, this data highlights the potential of paKG MP to modulate osteoblast/osteoclast behavior and potentially reduce the BMP2 concentration needed for bone repair.

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# Modulating Discogenic Low Back Pain Development Using Chondroitin Sulfate Microparticles

Disc degeneration is strongly associated with low back pain and is characterized by reduced cellularity, inflammation, altered mechanics, loss of proteoglycans, and nerve ingrowth. Chondroitin sulfate (CS) is a key proteoglycan that maintains water content and inhibits nerve growth in the disc. Given the importance of CS in the disc, our lab has fabricated methacrylated CS microparticles (CS MPs) and evaluated whether intradiscal injection of CS MPs could prevent pain-like behavior and reduce nerve ingrowth in a rat model of low back pain.

CS MPs were fabricated using methacrylated CS-A via water-in-oil emulsion. The animal study included Sprague Dawley (60M, 60F) rats divided evenly into three groups: sham, injured+PBS, and injured+CS MPs. All animals underwent surgery to expose the L5-L6 disc. In the injured groups, the disc was punctured and scraped to induce degeneration, followed by immediate injection of PBS or CS MPs. Animals were assessed bi-weekly for 14 weeks for pain-like behavior using pressure algometry, and disc volume was measured in vivo using microcomputed tomography. Following euthanasia, L5-L6 discs were harvested for histological assessment of degeneration and immunohistochemical evaluation of nerve and blood vessel presence.

Over the 14 weeks, disc volume in injured+CS MP animals was not significantly different from sham animals, whereas injured+PBS animals exhibited a significant decrease in disc volume compared to sham animals. These findings suggest that CS MPs help maintain disc volume. Pressure algometry showed a significant reduction in normalized values in injured+PBS animals compared to sham animals, beginning at 6 weeks in males and at 10 weeks in females. Injured+CS MP animals showed significantly improved pressure algometry values compared to injured+PBS animals at week 6 in males and week 10 in females. However, values did not differ significantly from sham or injured+PBS animals at later time points. These results suggest that CS MP did not fully alleviate pain-like behavior.

Histological analysis showed that both injured+PBS and injured+CS MP discs were significantly more degenerated than sham discs in both sexes. Immunohistochemistry results demonstrated significantly greater nerve and blood vessel presence in injured+PBS animals (4.34% ± 3.27% and 2.03 ± 2.22%) compared to sham animals (0.04% ± 0.07% and 0.09 ± 0.13%). Injured+CS MP animals exhibited lower nerve and blood vessel presence (2.02% ± 2.01% and 1.12% ± 1.33%), but these differences were not statistically significant compared to injured+PBS animals. There may be a potential reduction in nerve ingrowth in the injured+MP CS animals, but there was high variation suggesting a more sensitive assay may be needed.

Together these findings demonstrate CS MPs can improve some features of the degenerated disc, but more work is needed to characterize the results from this study. This includes using immunohistochemistry to stain for CS in the disc to determine if particles are maintained and PCR to evaluate changes in the dorsal root ganglia which contains the neurites that innervate the disc.

**Abstract**

Bacterial vaginosis (BV) is a common and burdensome condition affecting up to 30% of women of reproductive age, with recurrence rates as high as 80% within one year following standard antibiotic treatment. BV is driven by a dysbiosis of the vaginal microbiome, characterized by overgrowth of Gardnerella vaginalis and depletion of protective Lactobacillus species. A key contributor to treatment failure and recurrence is the formation of a resilient G. vaginalis biofilm, which limits antibiotic penetration and promotes bacterial persistence. Current adjunct therapies, such as boric acid, provide limited long-term efficacy, underscoring the need for preventative, biofilm-targeted solutions.

To address this unmet need, Olora Health has developed BVBalance, a bioresorbable intravaginal scaffold designed to be used as an adjunct to oral metronidazole therapy for the prevention of recurrent BV. BVBalance is fabricated from polylactic-co-glycolic acid (PLGA) and incorporates an auxetic honeycomb geometry that expands upon insertion to conform to the vaginal canal while maintaining structural stability and fluid flow. The scaffold contains interconnected void spaces that act as reservoirs for a chitosan-based hydrogel delivering a dual-combination therapy of Lauramide Arginine Ethyl Ester (LAE), a naturally derived antimicrobial that disrupts bacterial biofilms, and encapsulated Lactobacillus to support microbiome restoration following antibiotic treatment. A hyaluronic acid surface coating further enhances comfort during insertion, resulting in a triple-combination therapeutic approach.

The device is inserted via a single-use barrel-plunger applicator concurrent with the first oral dose of metronidazole. Following insertion, the scaffold expands to achieve intimate contact with the vaginal walls and undergoes controlled bioresorption, eliminating the need for device removal. Current proof-of-concept efforts focus on three critical performance domains: in-vitro evaluation of LAE-mediated biofilm disruption, assessment of PLGA degradation under simulated vaginal conditions, and mechanical testing to define design-relevant structural parameters. Results from these studies will inform final device specifications and guide future prototyping.

BVBalance represents a novel biomaterials-based approach that integrates bioresorption, localized biofilm disruption, and microbiome restoration to improve antibiotic efficacy and reduce BV recurrence. This platform has the potential to significantly improve long-term outcomes and quality of life for women affected by recurrent BV.

**Engineering an Electrically Conductive Cardiac Patch Incorporating Genetically Engineered Cardiomyocytes**

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***Abstract :***

Myocardial infarction (MI), a leading cause of mortality worldwide, occurs due to the coronary artery obstruction, resulting in ischemic injury and subsequent loss of cardiomyocytes (CMs), the specialized contractile cells of the myocardium. Due to the limited regenerative capacity of these cells, the damaged myocardium undergoes fibrotic remodeling, leading to scar tissue formation, resulting in decreased cardiac function and subsequent heart failure. Over the past few years, a central focus has been on the development of cell-based therapies that directly deliver human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to the injured myocardium to promote cardiac repair. The most widely explored strategy for this is the establishment of cardiac patches encapsulating the hiPSC-CMs within hydrogel scaffolds mimicking the properties of the native myocardium. Although extensive research has focused on developing cardiac patches, their therapeutic potential has remained limited due to poor cellular viability and engraftment of the cardiac patch, potentially leading to the risk of arrhythmias at the interface of the implanted patch and the host myocardium. To address these challenges, this study focuses on developing an electrically conductive cardiac patch by encapsulating genetically modified hiPSC-CMs that overexpress the cyclin D2 gene (CCND2-CMs) and retain proliferative capacity, together with isogenic hiPSC-derived cardiac fibroblasts (hiPSC-CFs), within gelatin methacrylate (GelMA)-fibrin hydrogel composites. We performed a series of optimizations by varying hydrogel composition, crosslinking, and culture conditions (static vs dynamic culture) to identify the optimal formulation for a conductive cardiac patch. To further assess the viability, proliferative capacity, and contractile properties of CCND2-CMs within these hydrogels, we compared them against control wild-type iPSC-CMs (CNTRL-CMs). Hydrogel scaffolds with varying stiffness were fabricated by modulating crosslinking parameters like UV light intensity, crosslinking time, and hydrogel concentration, and the mechanical properties were characterized using compression testing. Subsequently, Live/Dead assays were performed to assess cell viability post-encapsulation under different conditions, and the contractile behavior of the patches was analyzed using brightfield imaging. Our results demonstrated that the optimized GelMA-Fibrin hydrogel composite supports the co-culture of encapsulated CCND2-CMs or CNTRL-CMs and hiPSC-CFs, leading to improved cell viability and contractility. This optimized hydrogel matrix is currently being integrated with conductive nanoparticles (i.e., gold nanorods) to develop fully functional and conductive cardiac patches with enhanced cell viability and electrophysiological properties.

**Engineering a 3D Printed Microfluidic Early Gestation Placenta-on-chip**

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The human placenta serves crucial roles including preventing infections and fetal rejection during pregnancy, but it is poorly understood. Ethical concerns of early human pregnancy research and poor homology of animal models constrain the study of the human placenta. Further, simple 2D in vitro models and many placenta-on-a-chip designs poorly recapitulate the native placenta microenvironment and early placental development (Turco and Moffett 2019). Additionally, the gold standard material for organ-on-a-chip device fabrication, PDMS, is not favorable for protein and drug assays because of its tendency to absorb small and hydrophobic molecules. Treatment to make PDMS surfaces hydrophilic are not permanent as surfaces revert. To close this critical gap, we have designed a microfluidic platform to replicate the placenta at early gestation stage. We use stereolithography -3D printing for rapid fabrication of complex geometric chip design (Hart et al. 2020) modeling fetal and maternal vasculature structures and use 3D hydrogel culture of placental cells to mimic early placental development. Further, we evaluate the biocompatibility of commercially available resins with a trophoblast cell line, evaluate hydrogel systems to generate placental organoid-like structures within the placenta-on-a-chip, and characterize the transport kinetics of molecules between the fetal and maternal compartments. We show that Parylene-C deposition on 3D printed cell culture devices prevents cytotoxicity, enabling microfluidic production for placental barrier replication.

# Sex Differences in Adaptive Immune Responses after Traumatic Brain Injury

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**Statement of Purpose:** Traumatic brain injury (TBI) results from a mechanical insult to the head leading to acute injury sequela, including cortical tissue loss, edema, and blood brain barrier dysfunction. Following the acute phase, TBI incites chronic neuroinflammation, long term disabilities, and a diminished quality of life. Recent studies have highlighted sex differences in TBI, including how females exhibit worse post injury outcomes [1]. Adaptive immune responses play a critical role in neurotoxicity after TBI [2]. Despite our understanding of how sex and adaptive immune responses can influence TBI outcomes, current treatments do not account for these variables. We highlight differential responses in age-matched male and female cohorts (28, 60, and 90 days) after TBI. This work will inform how adaptive immunological responses can be modulated using interventional therapies.

**Methods:** A controlled cortical impact (CCI) TBI model was used in C57BL/6 mice (mixed sex; n = 5-6). At 28, 60, and 90 days post injury, animals were sacrificed to collect the brain, cervical lymph nodes, peripheral blood, and spleen. Single cell suspensions were stained for T helper 1 (Th1) (CD4+Tbet+), T helper 2 (Th2) (CD4+GATA3+), T helper 17 (Th17) (CD4+RORγT+), regulatory T (Treg) (CD25+FoxP3+), for flow cytometric analysis. Data shown are percentages of the positively stained populations after gating for viable single cells.

**Results:** This data shows time dependent differences in the adaptive immune response in the ipsilateral hemisphere (Figure 1). At 28 days post injury, there was a significant increase in Th1 cells compared to naïve (i.e. uninjured) in both male and female cohorts ($p = 0.0089$, $p < 0.0001$, respectively). Immune cell percentages decreased significantly from 28 to 60 days for Th1 and Th2 cells in the males ($p < 0.0001$, $p = 0.0313$, and $p = 0.0062$, respectively) and females ($p < 0.0001$, $p = 0.0495$, $p = 0.0143$, respectively). From 28 to 90 days post injury, Th1 decreased significantly in both males ($p < 0.0001$, $p = 0.0080$, respectively) and females ($p < 0.0001$, $p = 0.0246$, respectively).

**Conclusions:** This data suggests that after CCI, there are variable responses in adaptive immune cell populations infiltrating the brain. The data does not suggest sex differences between male and female cohorts for infiltrating adaptive immune cells in the ipsilateral hemisphere of the brain. Future analyses will focus on analyzing sex differences in the peripheral immune system, including blood, spleen, and cervical lymph nodes at the same points post injury. The long-term goal of this project is to use interventional therapies to modulate the adaptive immune response in the brain after TBI.

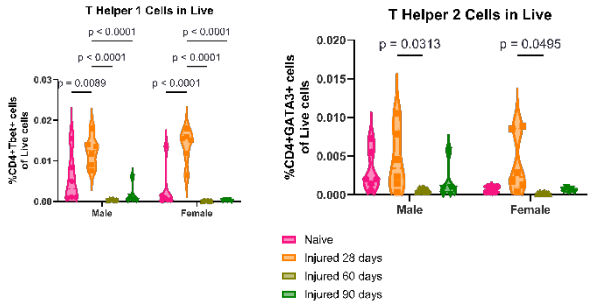


Figure 1. Percentages of Th1 cells (CD4+Tbet+), Th2 cells (CD4+GATA3+), in all live cells in the ipsilateral hemisphere for male and female cohorts.

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# Automated Coverslipping Design to Prevent Image Disturbances in Tissue Scans Used for Neurological Disorder Diagnosis

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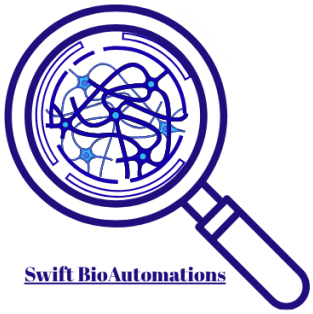


Our concept works to prevent image disturbances in pathology scans by making an automated design for coverslipping. The design will work to create reproducible quality slides through increased consistency via robotic activity. CND Life Sciences aids in the diagnosis of Parkinson's through skin biopsy in order to successfully provide a diagnosis before clinical onset. Current Laboratory processes are extremely manual with errors due to human variability. These errors cause delays and can result in the need for new biopsies, longer waits, and higher expenses for patients. These errors damage CND Life Science's profit margin, and the prevention of them would be a significant ROI, demonstrating a large need for automated devices in the pathology laboratory market.

Our design seeks to fill this market with an automated coverslipping process, ensuring slides are 95%+ readable with little to no need for technician input and preventing disruption of laboratory procedures outside of coverslipping. Our device is designed to be a discrete entity, ensuring it is easily inputted into any laboratory without having to adjust other procedures. The device moves the slides automatically between each step of coverslipping to avoid technician intervention. The device is consistent with a tight margin of errors such that the vast majority of slides have no clinically significant sample damage. Simple physics will be utilized to manipulate the slide. The dropper uses gravity to manipulate the fluid and the tilt table uses capillary forces to apply a uniform film. Suction cups that manipulate air pressure to control coverslip application. Pistons and conveyor belts use simple Newtonian mechanics.

Type 1 Diabetes Mellitus (T1D) is an autoimmune disease characterized by immune-mediated destruction of pancreatic β-cells, resulting in chronic dysregulation of blood glucose. Pancreatic islet transplantation is a promising therapeutic strategy; however, both direct and indirect antigen recognition can trigger graft rejection, requiring long-term systemic immunosuppressive therapy that carries significant toxicity. Therefore, there is a critical need for localized, biomaterial-enabled approaches that promote immune tolerance while preserving graft viability.

Trophoblast-derived extracellular vesicles (EVs) have emerged as a promising tolerogenic signaling platform due to their role in establishing fetal immune tolerance during pregnancy. These EVs contain immunomodulatory cargo that may regulate innate immune responses and reduce inflammatory cytokine signaling. In this work, we investigate the immunomodulatory capacity of trophoblast-derived EVs and engineer a synthetic hydrogel platform to enable controlled EV delivery for potential application in cell transplantation.

First, the tolerogenic potential of trophoblast-derived EVs was evaluated in vitro by exposing M0 macrophages to EVs and quantifying time-dependent cytokine secretion using ELISA. This establishes baseline EV-driven immunomodulatory activity relevant to early inflammatory signaling following transplantation. Next, azide-functionalized polyethylene glycol (PEG) hydrogels were characterized as EV delivery vehicles through rheological testing to quantify mechanical properties and through in vitro degradation studies to assess hydrolytic stability over time. Finally, both hydrolytically and proteolytically degradable PEG hydrogel formulations were evaluated for their ability to sustain EV release, with EV release kinetics quantified over time in vitro.

Together, these studies integrate immunology and biomaterials design to develop a tunable hydrogel-based delivery system for trophoblast-derived EVs. This platform is intended to enable localized immune modulation as an alternative to systemic immunosuppression, supporting future strategies for improving graft survival in islet transplantation and other cell-based therapies.

**Trophoblast-mediated tolerance in Type 1 Diabetes autoimmunity**

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**STATEMENT OF PURPOSE-** Type 1 diabetes (T1D) is an autoimmune disorder in which the immune system attacks pancreatic beta cells, resulting in lifelong dependence on exogenous insulin. Despite improved insulin delivery and glucose monitoring, patients remain at risk for severe complications. Advancements have been made in the prevention of T1D onset, such as the recent approval of the T cell-targeting anti-CD3 therapy Teplizumab, which moderates the immune response by exhausting CD8+ T cells, but this therapy delays diagnosis by a median of only 2 years[1]. Therefore, there is a critical need for innovative approaches that provide enduring prevention of beta cell destruction. Pregnancy is associated with disease remission in numerous autoimmune diseases, including T1D[2], and this observation has been validated in the non-obese diabetic (NOD) mouse model[3]. We propose placental mimicry as a novel strategy to prevent beta cell autoimmunity. **We hypothesize that hydrogel-macroencapsulated trophoblast cell therapy will secrete soluble factors that will modulate the immune system comparably to pregnancy, resulting in a delay or prevention of T1D autoimmunity in NOD mice.**

**RESEARCH METHODOLOGY** Breeding: Female non-obese diabetic (NOD) mice were mated with MHC identical NOD males and fully MHC mismatched C57BL6/J male mice at age week 10 prior to the onset of diabetes. Blood glucose and body weights were measured weekly from age week 9 to 39. In vivo xenotransplants studies: Female and male NOD mice were transplanted with two macroencapsulated 1.5% alginate spirals with nano luciferase transduced JAR choriocarcinoma placental cells subcutaneous site along with a degradable vasculogenic hydrogel (10% 20 kDa PEG-maleimide, VEGF at 10 µg/mL concentration) or saline and viability of the JARs were quantified longitudinally with in vivo imaging system. Blood glucose and body weights were measured weekly from age week 9 to 39.

**RESULTS** Female NOD mice were mated with NOD and C57BL6/J males starting at age 9 weeks, after the onset of insulitis but before diabetes onset. By week 18, 80% of unmated female NOD mice spontaneously develop diabetes. In comparison to unmated female NOD mice, preliminary data indicate that female NOD mice mated with NOD male mice exhibit delayed diabetes and/or prevention of diabetes at the endpoint of 30 weeks (age 39 weeks) **(Fig. 1A).**

We hypothesized that macroencapsulated placental trophoblast cells could prevent or delay the onset of diabetes in NOD mice. We first evaluated macroencapsulated human trophoblast choriocarcinoma cell line JAR survival in NOD and B6 mice. JAR cells were transfected with luciferase, macroencapsulated and delivered to the subcutaneous site with a vasculogenic degradable synthetic hydrogel to enhance engraftment and survival, and monitored longitudinally with in vivo bioluminescent imaging. **(Fig. 1B).** We found that JAR xenografts survived long-term in B6 recipients, but exhibited a gradual rejection in NOD recipients over 100 days. By contrast, non-tolerogenic macroencapsulated HEK cells rejected in B6 mice within 2 weeks. Diabetes onset in the JAR recipient group was delayed comparably to the mated NOD females (**Fig. 1A**), coinciding with the rate of cell rejection (**Fig. 1B**), suggesting that placental mimicry cell therapy may prevent T1D autoimmunity.

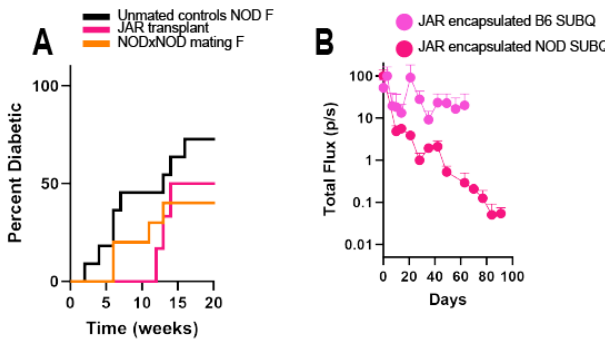


Figure 1: Diabetes incidence for MHC identical breeding, unmated control, and JAR transplants in NOD mice (A). JAR cell viability assessed through bioluminescence imaging in (B).

To further elucidate the impact of pregnancy and placental mimicry on T1D autoimmunity, we are using histological analysis of mated NOD female and JAR transplanted NOD female pancreata to analyze beta cell mass and degree of insulitis. Immune cell infiltration and beta cell positivity will be quantified to characterize the impact of treatment on the pathology of T1D.

**CONCLUSION** Preliminary data suggests that both pregnancy and placental mimicry cell therapy affects the immune progression of T1D. Future studies will evaluate the contribution of partial MHC mismatch to T1D onset in pregnancy, as well as the immunological mechanism by which trophoblast cells delay or prevent the onset of T1D.

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# *Engineered Glioblastoma-on-a-Chip for Assessing Biomimetic Nanomedicine-based Drug Delivery Approach*

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***Background:*** Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor, with a five-year survival rate of only 5–7%, largely due to therapeutic resistance and inevitable recurrence. A central driver of GBM aggressiveness is the glioma stem cell (GSC) population, which resides within the perivascular niche (PVN), a highly specialized, vascularized microenvironment that provides pro-survival cues promoting invasion, stemness maintenance, and resistance to standard therapies. Despite its critical role in disease progression, the PVN remains inadequately represented in conventional preclinical models. Moreover, effective therapeutic targeting of the PVN is hindered by the restrictive nature of the blood–brain barrier (BBB) and insufficient drug accumulation at the tumor site. Together, these limitations underscore the urgent need for advanced *in vitro* platforms that faithfully recapitulate the GBM–PVN microenvironment while enabling precise evaluation of targeted delivery strategies. To address these challenges, we developed and integrated two multi-disciplinary technologies: (i) an organotypic three-dimensional glioblastoma tumor-on-a-chip (TOC) model that recapitulates the structural and functional features of the PVN using patient-derived GSCs and brain-derived vascular cells, and (ii) biomimetic monocyte-membrane-coated nanoparticles (MoNP) engineered to enhance therapeutic delivery to the otherwise inaccessible PVN. Leveraging the innate homing and adhesion properties of monocytes toward inflamed and tumor-activated endothelium, we hypothesized that MoNP would selectively target the GBM-PVN and improve therapeutic efficacy.

***Methods:*** The TOC platform was fabricated using soft lithography and features a spatially organized architecture comprising distinct tumor, stromal, and perfusable microvascular network (μVN) compartments. Brain endothelial cells and pericytes were encapsulated in fibrin hydrogel to establish a functional μVN, followed by the injection of patient-derived GSCs into the tumor compartment on Day 7 and acellular hydrogel into the stromal compartment. On Day 8, MoNPs loaded with either fluorescent dye or verteporfin (VP) were administered, and nanoparticle uptake, vascular integrity, and tumor responses were monitored through Day 10.

***Results:*** Robust μVN formation was achieved, exhibiting physiologically relevant network morphology with high functionality. Upon co-culture with GSCs, the μVN displayed hallmark features of tumor-induced vascular dysfunction, including VE-cadherin disruption and upregulation of VCAM1, recapitulating key aspects of the GBM PVN. Fluorescently labeled MoNP preferentially accumulated within dysfunctional μVN and demonstrated enhanced penetration across the tumor–stromal interface compared to uncoated nanoparticles. Importantly, VP-loaded MoNP preserved microvascular integrity and significantly suppressed GSC proliferation and invasion relative to free VP treatment. These therapeutic effects were accompanied by a marked downregulation of pro-inflammatory cytokines associated with GBM progression.

***Conclusion:*** In conclusion, the integrated TOC–MoNP platform provides a robust and physiologically relevant preclinical system for interrogating GBM–PVN interactions and evaluating biomimetic drug delivery strategies. This approach offers a powerful framework for optimizing targeted therapeutics and advancing translational strategies for effective GBM treatment.