Biodegradable Stealth Polymer Coatings to Enable Universal Hematopoietic Stem-Cell Grafts for Curative β-Thalassemia Transplantation

Huanxuan (Shawn) Li

Stanford International Genetically Engineered Machine (iGEM) Team, Stanford University

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Abstract

β-Thalassemia produces chronic hemolytic anemia that demands lifelong transfusions and iron-chelation therapy for more than 60 000 newborns every year. Allogeneic hematopoietic stem-cell transplantation (HSCT) can be curative, yet <30 % of patients identify a fully HLA-matched sibling donor and unrelated donor registries serve ethnically diverse populations poorly. Partially matched grafts trigger graft-versus-host disease (GvHD) with five-year survival <25 % in severe cases.

This study asks whether a transient, biodegradable "stealth" corona—formed with NHS-activated poly(ethylene glycol) (PEG, 5 kDa) or degradable zwitterionic poly(carboxybetaine methacrylate, PCB, 6 kDa)—can mask donor hematopoietic stem-cell (HSC) epitopes long enough to blunt early alloresponses and then hydrolyze to restore native signaling.

Aim 1: Optimize coating density while preserving ≥90 % viability and critical chemokine-receptor function.

Aim 2: Quantify in-vitro immune evasion (≥70 % reduction in T-cell proliferation/IFN-γ) and multilineage potency (≥85 % colony-forming units).

Aim 3: Demonstrate engraftment, reduced GvHD, and polymer clearance in a BALB/c → C57BL/6 murine HSCT model powered for 30 % effect size. Successful completion will provide pre-clinical proof-of-concept for off-the-shelf HSC therapies that could democratize curative treatment for β-thalassemia and related hemoglobinopathies.

Introduction

β-Thalassemia arises from inherited β-globin mutations that impair hemoglobin assembly, driving ineffective erythropoiesis, transfusion dependence, and life-threatening iron overload. Lifetime transfusion and chelation expenses average $5 000–10 000 per year in India and exceed $40 000 in the United States (World Health Organization, 2024), imposing crushing financial burdens on regions where allele frequency is highest.

HSCT remains the sole curative option but is bottlenecked by (i) the scarcity of HLA-matched donors and (ii) lethal GvHD when mismatched grafts are used. Current immunosuppressive regimens dampen allo-reactivity at the cost of opportunistic infections, organ toxicity, and malignant relapse.

Polymer-mediated immunocloaking offers an orthogonal strategy: physically hide donor antigens long enough for host adaptation, then leave no residue. PEGylation prolongs nanoparticle circulation and has shielded pancreatic islets and CAR-T cells; however, permanent PEG shells increasingly provoke anti-PEG antibodies. Degradable zwitterions such as PCB resist protein fouling and hydrolyze into benign osmolytes, but have never been systematically applied to fragile HSCs whose CXCR4-mediated homing and self-renewal must remain intact. Bridging this gap could unlock universal HSCT.

Literature Review

- **PEGylated biomaterials.** Abuchowski et al. (1977) first masked bovine serum albumin with PEG. Krishnamurthy et al. (2022) showed transient immune evasion of PEGylated CAR-T cells but highlighted anti-PEG antibodies.

- **Anti-PEG immunity.** Yang et al. (2024) reported anti-PEG IgM/IgG seroprevalence >40 % in healthy donors, underscoring the need for degradable alternatives.
- **Zwitterionic polymers.** Zhang & Jiang (2023) and Liu et al. (2019) demonstrate PCB surfaces resist fouling and hydrolyze within weeks. Wu et al. (2021) used PCB shells to prolong mesenchymal stromal-cell survival across xenogeneic barriers.
- **HSC transplantation biology.** Engraftment requires intact CXCR4–SDF-1α signaling (Orban et al., 2020); acute/chronic GvHD remain major mortality drivers (Sakac et al., 2018).

Research Questions & Hypotheses

	Question	Hypothesis
RQ1	Can PEG- or PCB-based nanoscale coatings achieve ≥80 % surface coverage on donor HSCs while preserving ≥90 % viability?	**H1:** NHS-ester conjugation at 4 °C for 30 min forms a ~20 nm corona meeting these criteria.
RQ2	Do coated HSCs reduce allogeneic T-cell activation without impairing homing and multilineage potential in vitro?	**H2:** Coated grafts will cut T-cell proliferation and IFN-γ/IL-2 secretion by ≥70 %, yet retain ≥85 % colony-forming efficiency and CXCR4 migration**.**
RQ3	Will transiently cloaked grafts engraft across MHC barriers, cut GvHD severity, and clear polymer debris in vivo?	**H3:** In BALB/c → C57BL/6 transplants, coated HSCs will achieve ≥60 % donor chimerism by week 4, halve GvHD scores, and show complete polymer clearance and normal serum chemistry by day 42.

Methodology

1. **Polymer Synthesis and Characterisation**
    - **Reagents**. PEG‑NHS (5 kDa) and PCB‑NHS (6 kDa) dissolved in HEPES, pH 7.4.
    - **Coating protocol.** $CD34^+$ HSCs (mouse or cord‑blood human) incubated with 0.5–10 mg mL^{-1} polymer for 30 min at 4 °C, gentle agitation.
    - **Analytics**. Trypan‑blue viability, dynamic‑light scattering for hydrodynamic diameter, FT‑IR for amide‑bond confirmation. Fluorescein‑labeled polymer enables flow‑cytometric coverage quantification. Degradation tracked in 50 % human plasma at 37 °C over 28 days.
2. **In-Vitro Immune Evasion and Stem-Cell Potency**
    - **Mixed‑lymphocyte reaction**. BALB/c HSC stimulators vs CFSE‑labeled C57BL/6 T‑cells (E:T = 1:10–1:1); readout at 72 hours.
    - **Cytokines**. IFN‑γ and IL‑2 via ELISA.
    - **Potency**. 500 cells per dish in MethoCult; CFU‑GEMM/GM/E enumerated day 14.
    - **Chemotaxis**. Trans‑well migration toward 100 ng mL^{-1} SDF‑1α, 4 hours.
3. **Murine Transplant Model**
    - **Conditioning**. C57BL/6 recipients (8 weeks) receive 9 Gy total‑body irradiation.
    - **Graft**. 1×10^6 BALB/c HSCs (coated or uncoated) IV; n = 8/group (power = 0.8 for 30 % difference).

- **Read-outs**. Peripheral blood chimerism (CD45.1/2) weekly; GvHD score daily (0–10); IVIS imaging of near-IR-labeled polymer; serum ALT/AST/BUN/Cr; day 42 histopathology.
- **Statistics**. Repeated-measures ANOVA for chimerism/GvHD; Kaplan-Meier survival; $\alpha = 0.05$.
- **Risk & alternatives**. If coating impairs CXCR4, reversible click chemistry or lipid-anchored stealth lipids will be trialed; if anti-PEG IgM emerges, PEG arm will be dropped.

4. Data Analysis

All quantitative data will be represented with its standard deviation, Normality will be assessed by Shapiro-Wilk testing. For multi-group comparison, one-way ANOVA followed by Tukey's post-hoc test will be applied. Power analysis performed G*Power indicated the triplicate biological replicate in vitro and eight mice per group in vivo yield 80% power (alpha = 0.05) to detect 30% differences.

Ethical Considerations

All procedures follow Institutional Animal Care and Use Committee (IACUC) guidelines emphasizing refinement, reduction, and replacement. Humane endpoints: >20 % weight loss, severe hunching, or GvHD ≥8. Human cord-blood cells will be de-identified under IRB oversight. Personnel complete blood-borne-pathogen training. PEG and PCB are FDA-listed excipients, yet systemic toxicity will be monitored via complement activation and cytokine panels in addition to serum chemistry.

Budget and Timeline

Quarter	Milestone	Cost (cumulative)
Q1 (M1 - M3)	Go 1: ≥ 80 % coverage & ≥ 90 % viability confirmed	$3 200
Q2 (M4 - M6)	Go 2: ≥ 70 % T-cell suppression & ≥ 85 % CFU potency	$6 800
Q3 (M7 - M9)	Optimize polymer dose (0.5–5 mg mL^{-1}) and HSC/recipient ratio (0.5–2 × 10^6)	$9 700
Q4 (M10 - M12)	Go 3: ≥ 60 % donor chimerism & ≤ 50 % GvHD score vs control; manuscript drafted	$12 000

Major line items: polymers $1 200; culture media/cytokines/assays $4 600; DLS & FT-IR core fees $2 000; animal purchase/housing $2 880; IVIS imaging $1 500; consumables $1 000. Institutional flow cytometers, biosafety cabinets, and −80 °C storage provided in-kind. A 20 % contingency is reserved.

Closing Remarks

Growing up with transfusion-dependent β-thalassemia, I spent a decent amount of my childhood time running from one hospital to another, trying to see if a donor's HLA matches myself or not. Each transfusion and medicine had strained my family's finances, myself, and my family. In regions where transfusion-dependent β-thalassemia are populated, an HLA-matched stem-cell graft was almost out of the equation for most children who share my condition. After moving to the United States and having a successful transplant, I now live a normal life, but I have never forgotten the friends I met on the way who did not get that chance. Their stories drive this project. By engineering a biodegradable immunocloak that turns any donor stem cell into a universally compatible graft, I aim to make "luck" an unnecessary and curative therapy routine.

References

Abuchowski, A., Van Es, T., Palczuk, N. C., & Davis, F. F. (1977). Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. *Journal of Biological Chemistry*, 252(11), 3578-3581.

Zhang, L., & Jiang, S. (2023). Zwitterionic materials for biomedical applications: from design to translation. *Advanced Materials*, 35(17), 2208605.

Krishnamurthy, S., Ross, K. H., & Nair, S. K. (2022). Polymer cloaking strategies for cellular immuno-engineering. *Blood*, 139(11), 1629-1639.

Wu, C., Li, J., & Chen, Y. (2021). Transient immune camouflage of mesenchymal stromal cells enhances xenogeneic survival. *Biomaterials*, 275, 120939.

Orban, P. C., Chapman, P. L., & Peled, T. (2020). Homing and engraftment of hematopoietic stem cells: an emerging target for therapeutic intervention. *Cell Stem Cell*, 27(3), 402-423.

Liu, Y., Liang, Q., & Zhang, D. (2019). Carboxybetaine methacrylate coatings resist protein fouling and promote cell compatibility. *Acta Biomaterialia*, 84, 280-290.

Sakac, D., Smith, C. N., & Brezar, V. (2018). Graft-versus-host disease: current pathophysiology and novel therapies. *Clinical Immunology*, 195, 1-8.

Cho, J. H., Lee, H. J., & Ko, Y. J. (2018). Near-infrared fluorescent polymers for in-vivo tracking of biomaterials. *ACS Applied Bio Materials*, 1(3), 773-783.

Salas, D., & Porada, C. D. (2017). Advances in β-thalassemia curative therapies: gene editing and beyond. *Molecular Therapy*, 25(8), 2011-2026.

Thomas, E. D., Storb, R., & Clift, R. (2015). Hematopoietic stem-cell transplantation: past and future. *The New England Journal of Medicine*, 372(19), 1811-1820.