

DESIGN BRIEF

Engineering hematopoietic stem cell niches *in vitro* via mRNA-LNP-induced paracrine signaling in a microfluidic model

Sajani Brown, Kirsten Choi, and Tanishka Singh ■ Weston High School, Weston, Massachusetts, United States

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Hematopoietic stem cells (HSCs) comprise less than 0.01 percent of bone marrow, yet they are essential for bone marrow transplantation and lifelong blood production. Currently, HSCs cannot be significantly expanded *in vitro*, meaning clinicians can only transplant what a donor provides. As a result, many patients, especially those from underrepresented ethnic backgrounds with reduced access to compatible donor matches, die waiting for a compatible match. The central barrier is our inability to recreate the functional bone marrow stem cell niche outside the body to keep HSCs in an undifferentiated state. *In vivo*, endothelial cells regulate HSC quiescence through localized paracrine signaling. However, existing culture systems rely on externally added growth factors that degrade rapidly and fail to replicate sustained niche signaling. We propose a programmable microfluidic niche model in which a basal layer composed of endothelial cells is transfected with lipid nanoparticle-encapsulated mRNAs (mRNA LNP) encoding necessary proteins and factors in order to mimic paracrine signaling. We hypothesize this approach will preserve CD34-positive stemness in the HSC cells and enable controlled, sustained expansion of HSCs while maintaining their undifferentiated state. By enabling scalable HSC expansion in an *in vitro* system, this platform could help improve the accessibility of bone marrow transplantation for patients with limited donor availability.

Keywords: Stem cell niches, microfluidic device, hematopoietic stem cells, mRNA-LNPs, induced paracrine signaling

The authors were mentored by *Neurath, Rachel (Weston High School, Weston, Massachusetts, United States); Horanyi, Peter (UCB Biosciences, Cambridge, Massachusetts, United States). Please direct correspondence to: Rachel Neurath, neurathr@weston.org. This is an Open Access article, which was copyrighted by the authors and published by BioTreks in 2026. It is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

HSCs are the foundation of bone marrow transplantation, a life-saving treatment for leukemia and other blood disorders. These cells reside in a specialized stem cell niche in the bone marrow, where endothelial cells regulate their self-renewal through localized paracrine signaling. While transplantation is highly effective, it is severely limited by a shortage of human leukocyte antigen (HLA) matched donors. HLAs are specialized proteins found on the surface of nearly all cells in the human body, allowing the immune system to identify whether a cell is foreign or not. If there are any unknown proteins displayed, the immune system reacts to remove any potential threats. However, HLAs are encoded by the most polymorphic genes in the human body, meaning there is a great number of variations from person to person. In the case of transplantations, when the HLAs do not match, the immune system can get triggered and cause rejection within the body, leading to illnesses such as graft-versus-host disease (GVHD) (Park, 2012). An-

other problem that limits bone marrow HSC transplantations is that it depends greatly on the quantity of HSCs being transplanted, which is inherently insufficient, as HSCs make up only 0.01 percent of bone marrow (Mayle et al., 2012). Traditional approaches attempt to alleviate these hurdles by seeking HLA-matched donors or using immunosuppressive strategies to manage immune complications. However, these approaches still fail to replicate the natural bone marrow niche signals required for sustained, large-scale HSC expansion *in vitro* (Khaddour et al., 2023).

Furthermore, existing *in vitro* culture systems rely on externally added growth factors that degrade rapidly and fail to replicate sustained niche signaling (Sharma et al., 2012). As a result, HSCs often lose stemness and differentiate prematurely, preventing their therapeutic use. Patients with blood disorders like leukemia must rely on donor bone marrow, which carries risks of painful procedures, immune complications, and limited HSC counts.

To address this, we propose a synthetic biology-based microfluidic system that models the stem cell niche *in vitro* by mimicking paracrine signaling, enabling HSC culture and expansion for transplantation. By utilizing mRNA-LNPs, we would instruct a set of feeder cells to constantly produce the essential proteins rather than adding proteins through perfusion. This would provide the sustained signaling required to keep the stem cells from losing their potency. Developing a reliable method to transform somatic cells into functional, transplant-ready HSCs could enable autologous transplantation, which utilizes the patient's own cells to eliminate the risk of immune rejections. Engineering a niche that maintains HSC multipotency is a critical step toward safer, scalable transplantation therapies.

Systems level

Our proposed solution relies on repurposing Organ-on-a-Chip (OoC) models, microfluidics devices typically used for disease modeling, to recreate the HSC niche *in vitro*. The OoC platform investigates the use of mRNA lipid nanoparticles to create a more accurate representation of an *in vivo* bone marrow (BM) HSC niche. OoCs are a form of microtechnology that ultimately mimic the natural environment of cells *in vitro* through the use of microfluidics and cell culturing (Emulate, 2023). By utilizing the microtechnology of the Emulate BM Chip, which is shown in Figure 1, we aim to transform mature somatic cells into healthy, stable HSCs that would be viable for BM transplants in leukemia patients (Emulate, 2018). The main goal of the experiment is to compare the efficacy of two distinct approaches to delivering niche signals to HSCs. Specifically, we are evaluating the impact of directly delivering exogenous differentiation factors via perfusion against a simulated paracrine approach, which uses mRNA LNPs to prompt endothelial cells to produce those same factors.

Cellular Source and Microtechnology Assembling

Primary Umbilical Vein Endothelial Cells (HUVEC; ATCC PCS-100-013™) will be utilized as the foundational cell source. HUVECs are selected for their strong response to inflammatory signals and their established role as a substitute for the bone marrow vascular niche. Following the standardized protocols for the Emulate BM-OoC, cells will be recovered from cryopreservation and seeded into a dual-channel microfluidic device (Chou et al., 2020). There are two channels within the Emulate BM-OoC: the vascular channel (bottom) and the marrow channel (top) (use Figure 1. as a refer-

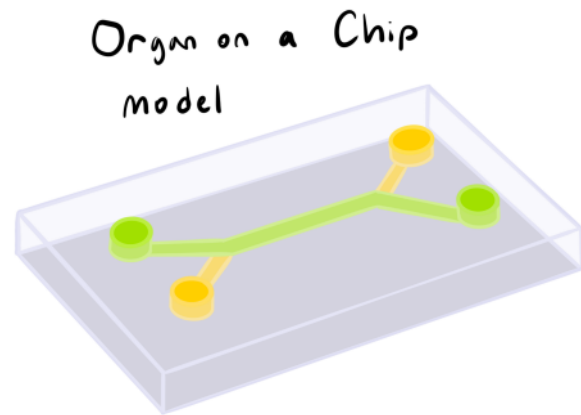


Figure 1. Illustrates the general structure of the Emulate BM OoC which we will be using. The green area represents the marrow channel while the yellow area represents the vascular channel.

ence). The bottom channel will be lined with HUVECs to mimic the bone marrow endothelial cells, while the top channel will be constructed with a secondary layer of HUVECs adjacent to a 3D fibrin gel scaffold, mimicking the extravascular hematopoietic space (see Figure 2 for a better understanding of the structure). Using the perfusion strategy from Chou et al., the system is fed via the microfluidic channels to establish nutrient gradients and physiological shear stress (Chou et al., 2020).

Turning the HUVEC into iPSC

To conduct the “undifferentiation” from HUVECs into iPSCs, Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc) are introduced to the system via the top perfusion layer. While iPSC reprogramming generally resets a cell’s epigenetic and phenotypic state, it doesn’t correct somatic genetic mutations. Therefore, to derive healthy HSCs, the backbone of BM transplants, the starting somatic cells must be sourced from a non-malignant lineage. iPSCs derived directly from acute myeloid leukemia cells retain any leukemia-based mutations and express the leukemic phenotype upon redifferentiation (Kotini et al., 2023). Thus the success of this method relies on the utilization of nonmutated endothelial cells. However, since finding unmodified cells is not the main focus of the experiment, the use of HUVECs is acceptable.

Hematopoietic differentiation

Once the HUVECs are undifferentiated, the iPSCs in the top layer are re-differentiated into HSCs by delivering the specific differentiation factors mentioned in the Parts level section. This mimics the transition

from hemogenic endothelial cells to HSCs within a controlled environment. Live-cell imaging will be used to track structural changes as the plate iPSC-derived cells group into round, non-adherent HSCs.

Maintaining HSC multipotency

The experiment will have one experimental group and one control group. The experimental group (OoC 1) will introduce the necessary proteins and factors via mRNA LNPs into the bottom vascular channel (more details about which proteins/factors in the Parts level). Ideally, the bottom HUVECs will uptake the LNPs and will translate & secrete the stabilizing proteins through the gel layer. The control group will be exposed to the same factors; however, the delivery method will differ, with the proteins being delivered directly via perfusion into the top channel.

Evaluation and data collection

To evaluate whether the LNP-mediated paracrine approach sustains stemness better than exogenous protein perfusion, the chips will be analyzed using a combination of confocal imaging and flow cytometry.

First, the total hematopoietic output must be quantified without interference from the underlying HUVEC layer. Because both HUVECs and HSCs are CD34+, standard CD34 screening cannot accurately distinguish between the two cell types in this co-culture system. Instead, fluorescently labeled CD45 binding antibodies will be perfused into the marrow channel, as CD45 is exclusively expressed on cells of the hematopoietic lineage. Confocal Z-stacking microscopy will then be used to capture 3D images of the fibrin gel scaffold, allowing us to count the distinct CD45+ fluorescent rings and calculate total cell yields (Rix et al., 2022).

Following imaging, cells will be harvested from the marrow channel to confirm true long-term HSC identity and rule out premature differentiation. To maintain cost-effectiveness while ensuring high accuracy, the harvested cells will be screened using a streamlined 6-marker human panel. Successful expansion of undifferentiated HSCs will be verified via positive selection for CD34+ (the clinical baseline), EPCR+ (a marker for *in vitro* expansion), and GPRC5C+ (a marker confirming a dormant, quiescent state) (Rix et al., 2022). Concurrently, downstream multipotent progenitors and mature blood lineages will be excluded via negative selection for Lin-, CD38-, and CD45RA-.

Ultimately, we will compare the total yield of this precise Lin-, CD38-, CD45RA-, CD34+, EPCR+, GPRC5C+ population between the experimental LNP group (OoC 1) and the protein-perfused control group. A higher yield of these quiescent cells in the LNP group

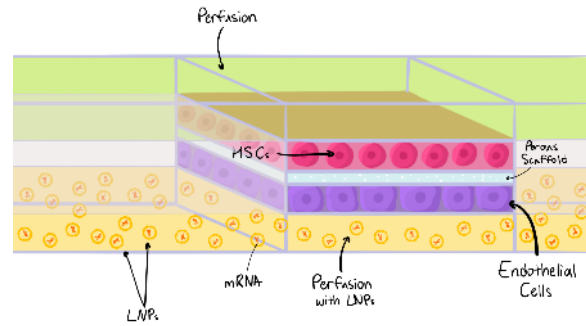


Figure 2. Breaks down the overall structure of the OoC model, specifically for the experimental group. The created HSCs are on the top channel with a green colored perfusion while the endothelial feeder cells are on the bottom channel with a yellow perfusion containing mRNA LNPs.

will demonstrate that continuous paracrine signaling accurately mimics the native bone marrow niche mechanics required to preserve stem cell potency.

Device level

To model the hematopoietic stem cell (HSC) niche *in vitro*, we propose a microfluidic organ-on-chip system based on the Emulate Bone Marrow (BM) Chip. This platform will be adapted to recreate key structural and biochemical features of the native bone marrow microenvironment to support HSC maintenance, expansion, and controlled differentiation.

The proposed device consists of two parallel microchannels separated by a semi-permeable porous membrane. The upper channel will be seeded with HSCs derived from patient-specific iPSCs, while the lower channel will contain feeder cells. This compartmentalized architecture is intended to mimic the spatial organization of the bone marrow niche, allowing distinct cell populations to interact through soluble signaling while remaining physically separated.

Continuous microfluidic perfusion will establish physiologically relevant flow conditions within both channels. This dynamic flow is expected to support nutrient delivery, waste removal, and the formation of stable signaling gradients. The porous membrane between channels will enable paracrine exchange of factors, allowing feeder cells to regulate HSC behavior through the secretion of cytokines, growth factors, and other niche-specific signals.

To engineer sustained and tunable paracrine signal-

ing within the device, we propose the use of mRNA-loaded lipid nanoparticles (mRNA-LNPs) to feeder cells. These nanoparticles will deliver synthetic mRNA encoding key niche factors, enabling feeder cells to act as *in situ* producers of essential proteins. mRNA will be generated using plasmid construct #222990 as a template for *in vitro* transcription (Chen et al., 2023).

By integrating mRNA-LNP-mediated protein expression with a microfluidic co-culture system, the proposed device aims to more accurately simulate the dynamic signaling environment of the bone marrow. The device is designed to guide iPSC-derived cells toward functional HSC states, while preserving their multipotency. This proposed microfluidic device represents a modular and scalable approach for engineering synthetic stem cell niches, with potential applications in regenerative medicine, disease modeling, and cell therapy development.

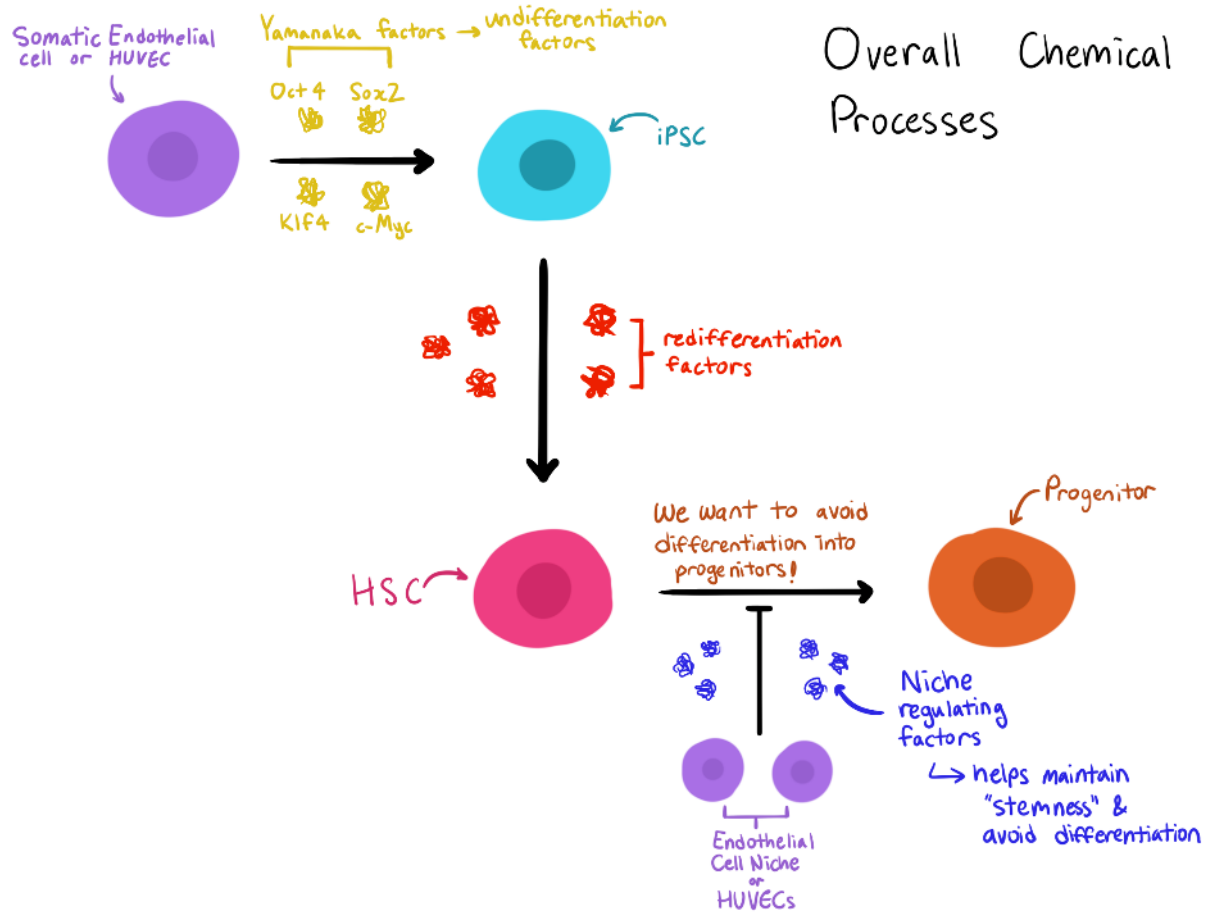


Figure 3. This diagram shows the overall chemical processes to give a full visual of the main biological checkpoints of the experiment.

Parts level

In our method, there is first the re-programming of patient-derived somatic cells (HUVECs) into iPSCs, the differentiation of those iPSCs into HSCs, and finally, signaling to maintain those HSCs in a quiescence state (refer to Figure 3 diagram). Our proposed idea utilizes mRNA LNPs and feeder cells to mimic *in vivo* paracrine signaling. All of these biological processes and the creation of mentioned factors are addressed in this section in detail.

mRNA LNP production

A visual of proper transfection is depicted by Figure 4. We utilized Addgene-sourced plasmids shown in Table 1 as templates for independent *in vitro* transcription of each mRNA needed. Producing each mRNA species in isolation minimized the risk of cross-contamination and unwanted molecular interference prior to LNP formulation.

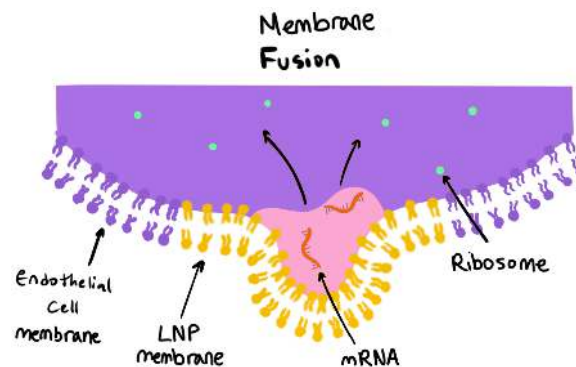


Figure 4. This diagram magnifies membrane fusion or the process in which the mRNA LNP is taken up by the endothelial cell.

The “gold standard” for constructing an LNP that is taken in by endothelial cells involves blending four primary ingredients: an ionizable lipid, a helper lipid,

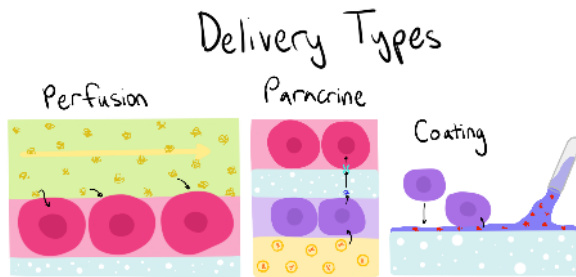


Figure 5. This diagram demonstrates the three different methods of introducing each protein or factor to the cells.

cholesterol, and PEG-functionalized lipids at carefully optimized ratios. The LNPs would be made out of SM-102, phosphatidylcholine, cholesterol, and polyethylene glycol lipid which would be assembled with a NanoAssemblr Ignite (McKenzie et al., 2023). To ensure the LNP's transfection into the HUVECs is sufficient, the mRNA LNP would be conjoined to anti-CD31 using maleimide-thiol chemistry and subsequently purified (Leo et al., 2026). All methods of creating the mRNA LNPs (except for the mRNA contents and the mentioned plasmid) will follow the process used by Leo et al. (2026).

Stage 1 (Reprogramming into iPSCs)

The reprogramming of somatic cells is achieved by the introduction of four transcription factors, known as the Yamanaka factors, into the patient-derived somatic cells (Figure 5). These factors, Oct4, Sox2, Klf4, and c-Myc, override the cell's existing purpose and induce a pluripotent state (Aguirre et al., 2023). Oct4 is the most critical factor, as it activates the genes responsible for pluripotency and suppresses genes that define a specialized cell type (Radzishchanskaya & Silva, 2014). Sox2 works along with Oct4, helping to open regions of DNA that are typically in mature cells, which allows pluripotency to be activated. Klf4 stabilizes the process by preventing the cell from undergoing apoptosis during reprogramming, while also reinforcing the activity of Oct4 and Sox2 (Narayan et al., 2017). c-Myc acts as an accelerant, increasing overall gene expression and making chromatin more accessible, which speeds up reprogramming (Li et al., 2013). The mentioned Yamanaka factors will be delivered to cells via perfusion through the upper channel layer.

Stage 2 (Differentiation into HSCs)

Once the iPSCs are established, they must go through a series of stages to become HSCs. As shown in Figure 5, there would be three forms of delivery: perfusion, paracrine, and coating. Perfusion is when the protein

factors are delivered via the solution in the OoC channel layers. Paracrine delivery refers to the usage of mRNA LNPs to create the necessary factors in feeder cells and deliver them to the targeting cells. Coating occurs before the cells are adhered to the scaffold and often carries factors that would help in current or later processes. It's important to note that while some deliveries must occur simultaneously, others must be stopped before others start.

The first step is to maintain the pluripotency of the iPSCs after establishment. To prevent the differentiation of iPSCs, it's important to add bFGF through the top perfusion layer (Asbrock et al.). Once ready to initiate differentiation, the perfusion should no longer consist of bFGF.

The next step is mesoderm induction and early specification where BMP4 should be delivered through the perfusion. This is an important step because it is the first major "commitment" where the cells it can now differentiate into are narrowed down into tissue like blood, bone, muscle, or heart rather than brain or gut tissue. Mesodermal cells can be characterized by the gene Brachyury (Lim et al., 2013).

Afterward, the prior perfusion should be replaced by a "hematopoietic cocktail" medium which would include the soluble factors SCF, Flt-3 Ligand, TPO, IL-3, and IL-6. This specifies the mesodermal cells into the hematopoietic precursors hemangioblast then into hematopoietic progenitor cells, forcing the cells to "commit" to a blood-forming pathway. At this stage, the cells begin expressing surface markers like CD34 (Lim et al., 2013).

Lastly, the factors HoxB4 and AGM would be delivered through paracrine signaling to ensure the progenitors turn into true HSCs. This is the most critical step for bone marrow transplants because only HSCs have the ability to live in an adult host and continuously create all types of blood cells for the rest of the patient's life (Lim et al., 2013). Look over Table 2 for more detailed information about the chosen factors.

Maintaining HSCs

To maintain the HSCs, both paracrine signaling and perfusion must be used for the most accurate representation of *in vivo* settings. The proteins and signals needed are listed and described in Table 3. For the experimental group, all proteins and factors that are delivered through paracrine signaling *in vivo* would be sent into the bottom channel of the OoC via mRNA LNPs and under paracrine signaling according to Figure 5 and Figure 6 while TPO would be delivered through the top perfusion channel. The control group would not use paracrine delivery and only use perfusion through the top channel using a solution of all of

TABLE 1
THIS IS A LIST OF THE PROTEINS DELIVERED INTO THE OoC AND THEIR RESPECTIVE PLASMID AND/OR PROTEIN SOURCE

Protein or Signal	Addgene Plasmid	Protein Order
Oct4		R&D Systems (Recombinant Human OCT4/POU5F1, Cat# 9445-OD, Minneapolis, MN)
Sox2		Thermo Fisher Scientific (Gibco Human Sox2 Recombinant Protein, PeproTech, Cat# 100-03-25UG, Waltham, MA)
Klf4		R&D Systems (Recombinant Human KLF4, Cat# 10243-K4, Minneapolis, MN)
c-Myc		R&D Systems (Recombinant Human c-Myc, Cat# 10563-MC, Minneapolis, MN)
bFGF (Basic Fibroblast Growth Factor)		Thermo Fisher Scientific (Gibco Human FGF-basic 154 aa Protein, PeproTech, Cat# 100-18B-50UG, Waltham, MA)
Laminin		Sigma-Aldrich (Laminin from human fibroblasts, Cat# L4544, St. Louis, MO)
Collagen I		Thermo Fisher Scientific (Gibco Collagen I, Rat Tail, Cat# A1048301, Waltham, MA)
Entactin		R&D Systems (Recombinant Human Nidogen-1/Entactin, Cat# 2570-ND, Minneapolis, MN)
Heparan sulfate proteoglycan		Sigma-Aldrich (Heparan Sulfate Proteoglycan from basement membrane, Cat# H4777, St. Louis, MO)
BMP4 (Bone Morphogenetic Protein 4)		R&D Systems (Recombinant Human BMP-4, Cat# 314-BP, Minneapolis, MN)
Collagen IV		Sigma-Aldrich (Collagen from human placenta, Bornstein and Sage Type IV, Cat# C7521, St. Louis, MO).
Fibronectin		Sigma-Aldrich (Fibronectin from human plasma, Cat# F2006, St. Louis, MO)
SCF (Stem Cell Factor)	pCS2-zfKitlgb was a gift from Petr Bartunek (Addgene plasmid # 168200 ; http://n2t.net/addgene:168200 ; RRID:Addgene_168200)	R&D Systems (Recombinant Human SCF, Cat# 255-SC, Minneapolis, MN)
Flt-3 Ligand (Fms-like tyrosine kinase 3 receptor ligand)		R&D Systems (Recombinant Human Flt-3 Ligand, Cat# 308-FK, Minneapolis, MN)
TPO (Thrombopoietin)	pAc-His-zfTpo was a gift from Petr Bartunek (Addgene plasmid # 64309 ; http://n2t.net/addgene:64309 ; RRID:Addgene_64309)	R&D Systems (Recombinant Human Thrombopoietin/THPO, Cat# 288-TP, Minneapolis, MN)
Interleukins: IL-3 & IL-6		R&D Systems (Recombinant Human IL-3, Cat# 203-IL, Minneapolis, MN) R&D Systems (Recombinant Human IL-6, Cat# 206-IL, Minneapolis, MN)
HoxB4	HOXB4-WG-SP65 was a gift from Corey Largman (Addgene plasmid # 20986 ; http://n2t.net/addgene:20986 ; RRID:Addgene_20986)	
AGM (Aorta-gonad-mesonephros)	pscALPS-SIV-AGM-VERAGM3 vpr was a gift from Jeremy Luban (Addgene plasmid # 115826 ; http://n2t.net/addgene:115826 ; RRID:Addgene_115826)	
CXCL12	pcDNA3-CXCL12-sfGFP was a gift from Erik Procko (Addgene plasmid # 98961 ; http://n2t.net/addgene:98961 ; RRID:Addgene_98961)	
CXCL4	PF4-bio-His was a gift from Gavin Wright (Addgene plasmid # 53411 ; http://n2t.net/addgene:53411 ; RRID:Addgene_53411)	
TGF- β	pMT-TAK1-V5-His was a gift from Cyril Gueydan (Addgene plasmid # 63753 ; http://n2t.net/addgene:63753 ; RRID:Addgene_63753)	

TABLE 2
SEPARATES THE NECESSARY PROTEINS AND FACTORS INTO EACH STEP/PROCEDURE AND DELIVERY TYPE AND GIVES A DESCRIPTION OF WHAT EACH DOES FOR THE TRANSFORMATION OF CELL TYPES

Step/Procedure	Protein or Signal	Purpose	Delivery: Perfusion (Soluble)	Delivery: Paracrine (Cell-Secreted)	Delivery: Coating (Substrate)
Maintenance of Pluripotency Mesoderm Induction	bFGF (Basic Fibroblast Growth Factor) Laminin, Collagen I, Entactin, & Heparan sulfate proteoglycan	Maintains iPSCs in an undifferentiated, self-renewing state before induction Major matrix components required for initial differentiation of iPSCs into mesodermal cells	Yes		Yes
	BMP4 (Bone Morphogenetic Protein 4)	Recapitulates early embryonic signaling to help drive mesoderm emergence and early erythropoiesis	Yes		
Specification to Precursors (HPCs)	Collagen IV	Directs the generation of Flk1+ mesoderm and accelerates transition into hemangioblasts and progenitors			Yes
	Fibronectin	Provides physical support for the generation of CD34+ hematopoietic progenitors			Yes
	SCF (Stem Cell Factor)	Critical factor for hematopoietic development and the proliferation of immature hematopoietic cells	Yes		
	Flt-3 Ligand (Fms-like tyrosine kinase 3 receptor ligand) & TPO (Thrombopoietin) Interleukins: IL-3 & IL-6	Promotes the expansion and proliferation of immature hematopoietic progenitors Supports early hematopoietic development and the induction of myeloid/erythroid progenitors	Yes Yes		
Definitive HSC Maturation	HoxB4	Transcription factors that provide definitive engraftment potential and regulate the maturation of HSCs (upregulating CD34)		Yes	
	AGM (Aorta-gonad-mesonephros)	Provides the favorable niche microenvironment and secreted factors required to acquire long-term HSC activity		Yes	

Note.—All of the information is from Lim et al. (2013).

the listed proteins and signals in Table 3.

Safety

Main idea

Safety is an important consideration at every stage of this proposed design and encompasses ethical standards, laboratory practice, and the responsible disposal of materials.

Ethical considerations

The reprogramming iPSC approach utilized in this design raises two ethical considerations. First, collecting somatic cells from a patient requires consent. All donors must be fully informed of the intended use of their cells, and samples must be handled in accordance with the IRB guidelines. Second, iPSC research does avoid the ethical concerns usually associated with embryonic stem cells, but the use of c-Myc as a reprogramming factor does introduce theoretical oncogenic risk, as c-Myc is a proto-oncogene. To mitigate this, it may be possible to use l-Myc as a substitute, and this should be evaluated. Additionally, the reach of this research is

limited to therapeutic applications and does not extend to germline modification.

Ethical clearance

To ensure that the experiment remains ethical with deriving and using iPSCs, researchers must undergo an NIH investigation and provide documents along with an additional IRB protocol number to then have IRB Approval. Not only that, but because this experiment utilizes iPSCs *in vitro*, all iPSCs must be reviewed and approved by the Institutional Biosafety Committee. There is also a form that must be completed along with restrictions on the use of iPSCs. One restriction states that human iPSC should not be introduced to non-human primate blastocyst (National Institutes of Health: Office of Intramural Research, n.d.). Overall, it is very important that researchers get the ethical clearance necessary to treat and use derived iPSCs with respect.

Laboratory safety

All mRNA synthesis and LNP formulation procedures must be conducted within a certified biosafety restriction to prevent the contamination of RNase and to

TABLE 3
LISTS ALL OF THE PROTEINS AND SIGNALS, ITS PURPOSE, AND DELIVERY TYPE NECESSARY FOR MAINTAINING THE HSCs IN A QUIESCENCE STATE

Protein or Signal	Purpose	Delivery Type
SCF (Stem Cell Factor)	Essential cytokine for the maintenance of HSCs	Paracrine (Produced locally by CAR/LepR+ cells and sinusoidal endothelial cells)
CXCL12	Essential for HSC maintenance and critical for maintaining stem cell quiescence	Paracrine (Produced locally by CAR/LepR+ cells and sinusoidal endothelial cells)
TPO (Thrombopoietin)	Essential for maintaining HSC numbers and their potential	Perfusion (Synthesized by hepatocytes in the liver and acts as a systemic hormone)
CXCL4	Specifically regulates homeostatic HSC quiescence	Paracrine (Secreted by megakaryocytes within the bone marrow)
TGF- β	Required for maintaining HSC quiescence	Paracrine (Produced by non-myelinating Schwann cells in the niche)

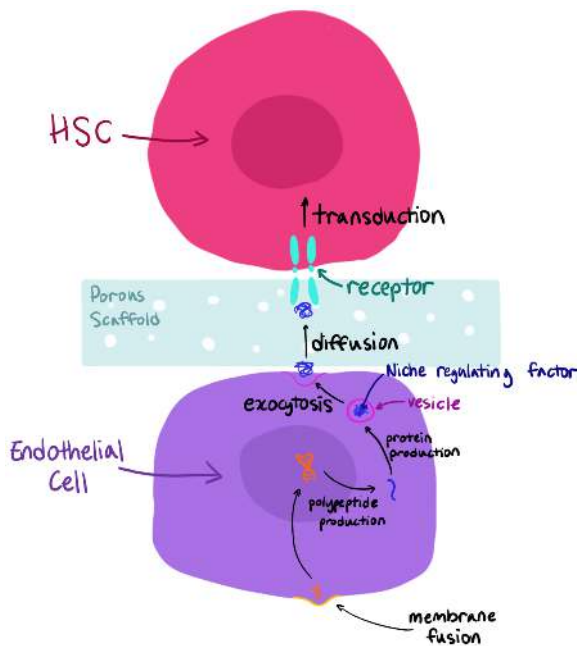


Figure 6. Shows the process of protein synthesis and delivery from the endothelial cell to the HSC. It helps to illustrate how the use of mRNA LNP would help model paracrine signaling.

protect researchers from exposure. Full PPE is required at all times during LNP preparation, as ionizable lipids can be cytotoxic at elevated concentrations. Because this design uses mRNA, a non-viral vector, there is no risk of generation of replicated particles, which reduces the biosafety burden overall (Hardee et al., 2017). All cell cultures must be performed under BSL-2 conditions, given the use of human-derived cells.

Disposal

All biological waste must be autoclaved pre-disposal. Any materials that have come in contact with human-derived cells must be treated as biohazardous and dis-

posed of accordingly. Chemical waste generated during the LNP formulation must be collected and removed by a licensed hazardous materials contractor. (Environmental Protection Agency, n.d.) Finally, under no circumstances will biological or chemical waste enter the standard waste stream.

Discussions

Using OoC models to culture HSCs in order to proliferate them *in vitro* has many important applications in biology. It has the potential to change how we approach transplantation procedures for diseases such as leukemia, potentially allowing patients a less painful and far more successful recovery, as the use of their own somatic cells eliminates, or at least greatly diminishes, the chances of an acute immune response to the newly transplanted cells.

One matter that must be addressed is the use of HUVECs in the proposed experiment. HUVECs are cells found in the umbilical vein of an umbilical cord, which can only be derived from newborns, not adults. Therefore, they may not fully reflect the behavior of cells derived from patients as they are a differing cell type with differing properties. They were the chosen cell type due to their accessibility and because most research on endothelial cells utilized HUVECs as their main cell type. This means that we will be able to compare our results with other research to better understand the results. Such variations, however, can cause some fundamental changes to how the cells react in the experiment. HUVECs generally adhere to scaffolding due to their characteristic of responding well to lab coatings (Sgarioto et al., 2012). Other types of endothelial cells tend to have trouble adhering to surfaces unless such environments are encoded specifically to mimic *in vivo* settings due to the diversity between each type (Chi et al., 2003). Due to these differences, the procedure to adhere to the cells may change depending on the cell type. Additionally, because HUVECs are

macrovascular while other endothelial cells (such as those from bone marrows) are microvascular, they experience high-shear stress and thus require a significantly higher laminar flow (Garcia-Polite et al., 2016). While these differences may be significant, the main goal for this experiment is to see if mRNA LNPs can help HSCs maintain “stemness”, therefore, these differences may be temporarily undermined and looked into later into development.

Some other potential issues to address include unsupervised cell signaling from the feeder cells to the HSCs and the complexity of the system which may make it hard to identify the root of problems. In the event that these issues arise, they may be addressed by changing certain aspects of the experiment, such as the cell types, cell environment, protein factors, etc. in order to work towards achieving the desired results.

Next steps

This experiment will ideally allow researchers to culture HSCs *in vitro*, enabling controlled proliferation and retention of a stem-like state. The next steps will include further refining the model to more accurately replicate the niche, increase HSC proliferation, and ensure cost-effectiveness.

Refining the model will include making small changes to specific components of the device in order to test whether the changes resulted in the creation of a more accurate niche environment. One of the factors that will be changed is the laminar flow conditions. The microfluidic niche flow conditions will be modified to ensure the device can replicate the shear stress these cells are exposed to in their natural niche environment. The flow rate, direction, and pressure can be modified as needed to ensure maximum HSC proliferation. The composition of the solutions being used in the channels can also be experimented with in order to improve the fidelity of the niche and ensure cost-effectiveness.

Another avenue that might be explored to improve cost-efficacy is experimenting with the combination and concentration of the factors used to mimic paracrine signaling. The proposed model uses the “gold standard” which increases the true positive rate when determining the presence of HSCs. This is made possible by testing for multiple HSC markers (CD34+, EPCR+, CD49f+) and transfecting the feeder layer of cells with multiple proteins (SCFs, CXCL-12) encoded for by LNP encapsulated mRNA. This increases the chances of culturing and identifying true HSCs from other cell types that may be present, however, it also increases the overall cost, which can lead to challenges with accessibility of this technology.

Lastly, the most pressing issue to address following the experiment is determining how to obtain the cells from the OoC model and identifying the optimal transplantation method. It is imperative that the method for removal and use is precise and intentional since the goal is to have as many HSCs as possible to use for transplantation. Experimenting with varying protein factors, delivered through mRNA LNPs, to achieve higher success rates in iPSC proliferation and differentiation represents another promising avenue for future research.

Author contributions

TS worked on the Abstract, Next steps, and Device level sections. TS contributed to the Systems level and Parts level. KC worked on the Background, Parts, and Systems level. SB worked on the safety level and contributed to the Parts level. All images were drawn by KC, however, TS contributed to Figure 3.

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