Gelatin Maleimide Microdroplets for Hematopoietic Stem Cell Culture in Granular Hydrogels

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Statement of Purpose: Hematopoietic stem cells (HSCs) are the foundational units that give rise to the entire blood and lymph systems through a process termed hematopoiesis. They can be considered as residents of niches that comprise constellations of external physical and chemical stimuli which instruct HSC behavior. Treatments for blood and lymph disorders include bone marrow transplants in which donor HSCs are transplanted into a conditioned recipient with the goal of reconstituting healthy, lifelong hematopoiesis. Increased cell dosage has been associated with improved transplant outcome, but expansion of HSCs without sacrificing a critical subpopulation of long-term repopulating HSCs (LT-HSCs) remains a challenge in liquid and biomaterial-based culture. Previously, we have shown that a softer and more porous hydrogel improved LT-HSC maintenance, suggesting a need for highly diffusive biomaterials in effective HSC expansion.[1] In the same study, HSC-MSC co-culture was demonstrated to improve LT-HSC maintenance. In this study, we have cultured HSCs in a microdroplet suspension, and we will co-culture HSC-laden microdroplets with MSC-laden droplets in suspension to demonstrate the efficacy of highly diffusive, multicellular microdroplet culture. Further, we aim to develop a jammed granular hydrogel platform for coherent, multicellular culture to drive LT-HSC retention and enable construction of a more biomimetic bone marrow model.

Methods: HSCs and MSCs were isolated from the tibias and femurs of 4-8 week-old C57BL/6 female mice (The Jackson Laboratory). For HSCs, bones were crushed to free marrow cells, which were filtered and enriched via ACK lysis. Further enrichment was performed with EasySep™ Mouse Hematopoietic Progenitor Cell Enrichment Kit and via flow cytometry to isolate $Lin^-/Sca\text{-}1^+/c\text{-}Kit^+$ HSCs. MSCs were expanded from crushed bones for 10 days using MesenCult™ and the manufacturer's protocol. For HSC monocultures, $Lin^-/Sca\text{-}1^+/c\text{-}Kit^+$ cells were encapsulated in 3wt% maleimide-functionalized gelatin (GelMAL) macrogels or microdroplets at low (3.9×10^5 cells/mL) or high (1.56×10^6 cells/mL) densities and crosslinked with DTT. Cells were encapsulated in microdroplets using a flow-focusing microfluidic device, using an emulsion of DTT in light mineral oil/2% v/v SPAN80 to form droplets.[2] For co-culture experiments, HSCs and MSCs were encapsulated in discrete microdroplet cohorts (HSCs: 1.56×10^6 cells/mL, MSCs: 6.23×10^6 cells/mL) and combined in a 1:1 HSC:MSC ratio. Monocultures and co-cultures were performed in StemSpan™ SFEM supplemented with 100ng/mL SCF, and 0.1% Pen Strep for 4 days. Immunofluorescence was used to identify LT-HSCs. Granular hydrogel development will utilize vacuum filtration to jam microdroplets into hydrogel molds. We will functionalize GelMAL with guest and host molecules (e.g., ß-cyclodextrin and adamantane)[3] to enhance droplet-droplet interactions and stabilize the structure. Cohesiveness will be evaluated via microscopy by measuring the dispersion of droplets away from the granular hydrogel structure upon handling.

Results: Preliminary data I have helped collect suggest that encapsulating and culturing HSCs in suspended gelatin hydrogel microdroplets improves LT-HSC maintenance relative to a conventional bulk hydrogel (Figure 1). Current experiments will quantify the combined impact of microdroplet culture combined with HSC-MSC co-culture upon LT-HSC maintenance. Development of a granular hydrogel will evaluate structure stability as a function of guest and host molecule functionalization. These results will establish foundational principles for multicellular granular hydrogel culture and will enable assembly of independently tuned cell-laden microdroplets into granular hydrogels that support LT-HSC maintenance.

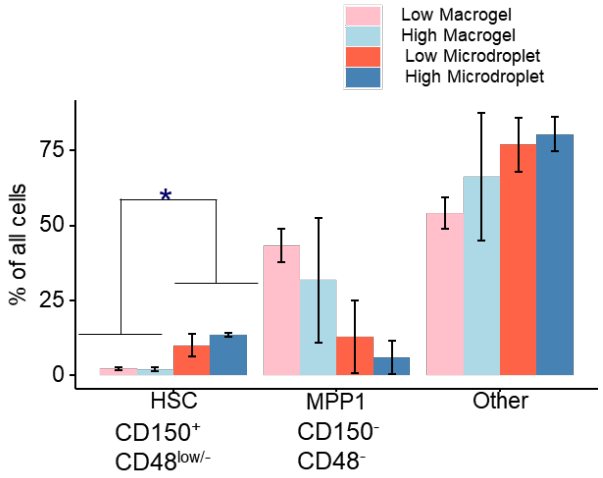

Figure 1. Hematopoietic stem and progenitor cells ($Lin^-/Sca\text{-}1^+c\text{-}Kit^+$) were encapsulated in either macrogels (bulk hydrogels) or microdroplets at low (3.9×10^5 cells/mL) or high (1.56×10^6 cells/mL) density. Immunofluorescence was used to determine the percentage of cells classified as LT-HSCs ($CD150^+/CD48^{low/-}$) or multipotent progenitor-1. ($CD150^-/CD48^-$). Microdroplet culture was associated with a significant increase in LT-HSC maintenance after 4 days in culture ($p < 0.005$, pairwise comparison, $n_{macrogel} = 2$, $n_{microdroplet} = 3$).

Conclusions: Microdroplet-based biomaterials improve diffusive transport and enable heterogeneous culture conditions. We have demonstrated that HSCs encapsulated in GelMAL hydrogel microdroplets better maintain a critical LT-HSC population, and we aim to demonstrate multicellular co-culture of HSCs with MSCs and advance toward a granular hydrogel system capable of replicating the heterogeneity of the HSC niche while enabling a higher and more biomimetic level of cell-cell interaction.

References:

1. Gilchrist et al., *Adv Healthc Mater.* 2019. 8(20) e1900751
2. Headen et al., *Adv. Mater.* 2014. 26(10): 3003-3008
3. Rodell et al. *Biomacromolecules*. 2013. 14: 4125-4134