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**3. Title:**

Bioengineered niches that recreate physiological extracellular matrix organisation to support long-term haematopoietic stem cells.

**2. Authors:**

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**Abstract**

Long-term reconstituting haematopoietic stem cells (LT-HSCs) are used to treat blood disorders via allogeneic stem cell transplantation (alloSCT), to engraft and repopulate the blood system. The very low abundance of LT-HSCs and their rapid differentiation during *in vitro* culture hinders their clinical utility. Previous developments using stromal feeder layers, defined media cocktails, and bioengineering have enabled HSC expansion in culture, but of mostly short-term HSCs (ST-HSC) and progenitor populations at the expense of naïve LT-HSCs. Here, we report the creation of a bioengineered LT-HSC maintenance niche that recreates physiological extracellular matrix organisation, using soft collagen type-I hydrogels to drive nestin expression in perivascular stromal cells (PerSCs or pericytes). We demonstrate that nestin, which is expressed by HSC-supportive bone marrow stromal cells, is cytoprotective and, via regulation of metabolism, is important for HIF-1α expression in PerSCs. When CD34+ve HSCs were added to the bioengineered niches comprising nestin/HIF-1α expressing PerSCs, LT-HSC numbers were maintained with normal clonal and *in vivo* reconstitution potential, without media supplementation. We provide proof-of-concept that our bioengineered niches can support the survival of CRISPR edited HSCs. Successful editing of LT-HSCs ex vivo can have potential impact on the treatment of blood disorders.

**5. Funder information:**

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**6. Date data set can be made public:** immediately.

**7. Restrictions:**  No restrictions.

**8. Ethical approval:** not required.

**9. preferred licence:** No preferred.

**Data folders**

**Figure 2**

AFM > contains AFM images for figure 2b and sup fig1a

BCA > contains BCA raw values from plate reader for figure 2c .xlsx

BMP-2 ELISA > raw plate reader data for figure 2g .xlsx

IN CELL WESTERN HFN7.1 > Licor scan raw values and images for figure 2e .xlsx and .tif

IN CELL WESTERN P5F3 AND TOTAL FN > Licor scan raw values and images for figure 2d & f .xlsx and .tif

**Figure 3**

Brefeldin > raw .tif microscope images for Figure 3e and sup Figure 4e, c & d.

Nestin > raw .tif microscope images for Figure 3c, sup Figure 4a.

PERICYTE PHENOTYPE FLOW > flow cytometry .FCS files from PerSCs for Figure 3f and sup Figure 5.

Rheology > .xlsxcontaining raw rheology measurements

VIMENTIN MICROSCOPY > raw .tif microscope images for figure 3d.

**Figure 4**

The RNAseq data generated from PerSCs in this study have been deposited in NCBI’s Gene Expression Omnibus and are accessible through GEO Series accession number GSE265789 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE265789).

**Figure 5**

HIF > contains raw .tif microscope images for Figure 5, each condition from each donor in a file. Also contains CellProfiler script for measuring nuclear HIF as .cpproj

HYPROXYPROBE AUG 18 > raw .tif microscope images for figure 5c. .xlxs file containing work sheets for analysis.

TARGETTED METABOLOMICS > contains LC-MS data processed by Glasgow Polyomics facility as .xls and .PDF.

**Figure 6**

6b siRNA > raw .tif microscope images for figure 6b

PI > raw .tif files for Figure 6d and e.

PNES> raw .tif files for Figure 6a.

siRNA HIF > raw .tif files for Figure 6c.

supplementary > collagen I > .xls and .tif files containing raw data from Licor scan for supplementary Figure 10c.

**Figure 7**

CRISPR > .ab1 sequencing files for Figure 7h.

HSC FLOW > raw .fcs files for HSC flow cytometry for Figure 7b, c.

IN VIVO > .ppt containg analysed FACS plots, then cull lineage/Cull progenitor/Stem containing .fcs raw flow cytometry files and flowjo .wsp files.

LTC-IC > .xlsx containing CFU counts and analysis, .wsp flowjo workspace containing FACS sort,

 LTC-IC > evos images > contains .tif raw images for CFU replicates imaged.

**Supplementary figure 3**

HOESCHT CELL NO 280521 > .TIF evos microscope coverslip scans used to calculate cell number in SF3.

LiverDead stain distribution > .pfzx file containing analysed Z stack intensity used to generate graph in SF3a. .PDF containing raw images. .tif containing stacks and colour bar.

**Supplementary Figure 12**

.xlsx containing file containing analysed Z stack intensity used to generate graph in SF12b.

**Supplementary Figure 14**

.TIF raw microscopy images for SF14.

**Supplementary figure 2**

.jpg raw light miscropy images used for figure SF2.

**Supplementary Figure 4**

Raw .TIF microscopy images for SF4a and b.

**Supplementary Figure 7**

HIF 7D 14D > Raw .TIF files for SF7 b and c.

HIF co-loc to N microscopy pericyte 0317 SUP FIG 7A > contains individual files for each conditions raw .TIF microscope conditions. And cellprofiler script as .cpproj file.

.xlsx and .tif files from Licor scan for SF7d

.xlsx raw scan from using PHERAstar FSX microplate reader for SF7e

**METABOLOMICS**

Contains LC-MS data processed by Glasgow Polyomics facility, contains .xlsx, .PDF, and .mzML raw files.