Megakaryocytes from human pluripotent stem cells

new perspectives for biological models and clinical applications

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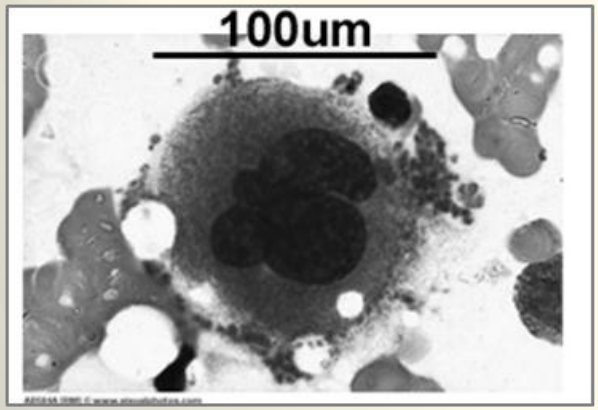
NHS Blood and Transplant

UNIVERSITY OF CAMBRIDGE

MEGAKARYOCYTE PROFILE

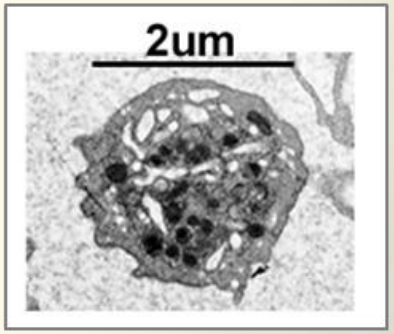


<1% blood cells

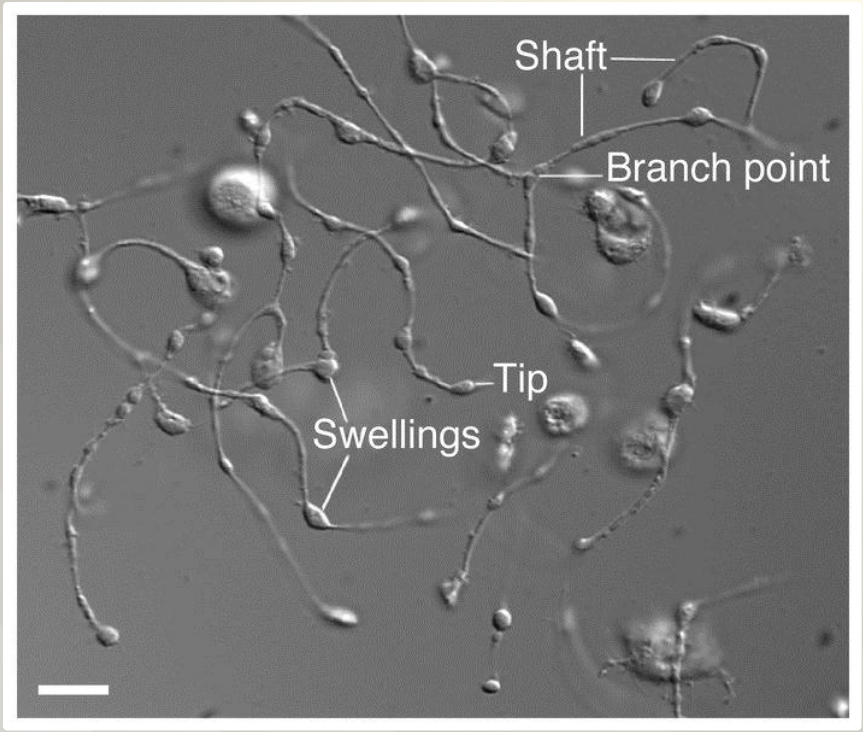
Bone marrow

endothelium

Circulation



> 200,000 /uL



IN VITRO PATHWAYS TO MEGAKARYOCYTES

Forward programming

ES/iPSC

Mesoderm

Haemogenic endothelium

LT-HSC

ST-HSC

Directed differentiation

LMPP

CLP

T-cell P

B-cell P

B-cell

Plasma-cell

NKP

NK

DCP

DC

MDP

DCP

GMP

Monocyte

Macrophage

CMP

Myeloblast

Eosino

Baso

Neutro

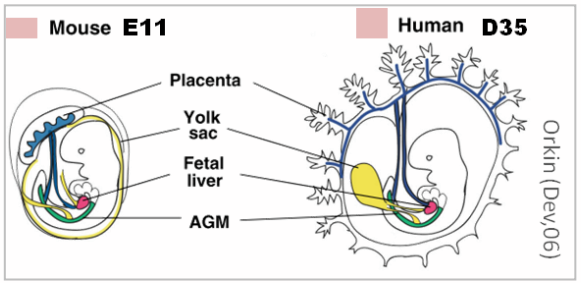
Platelets

MKEP

MK

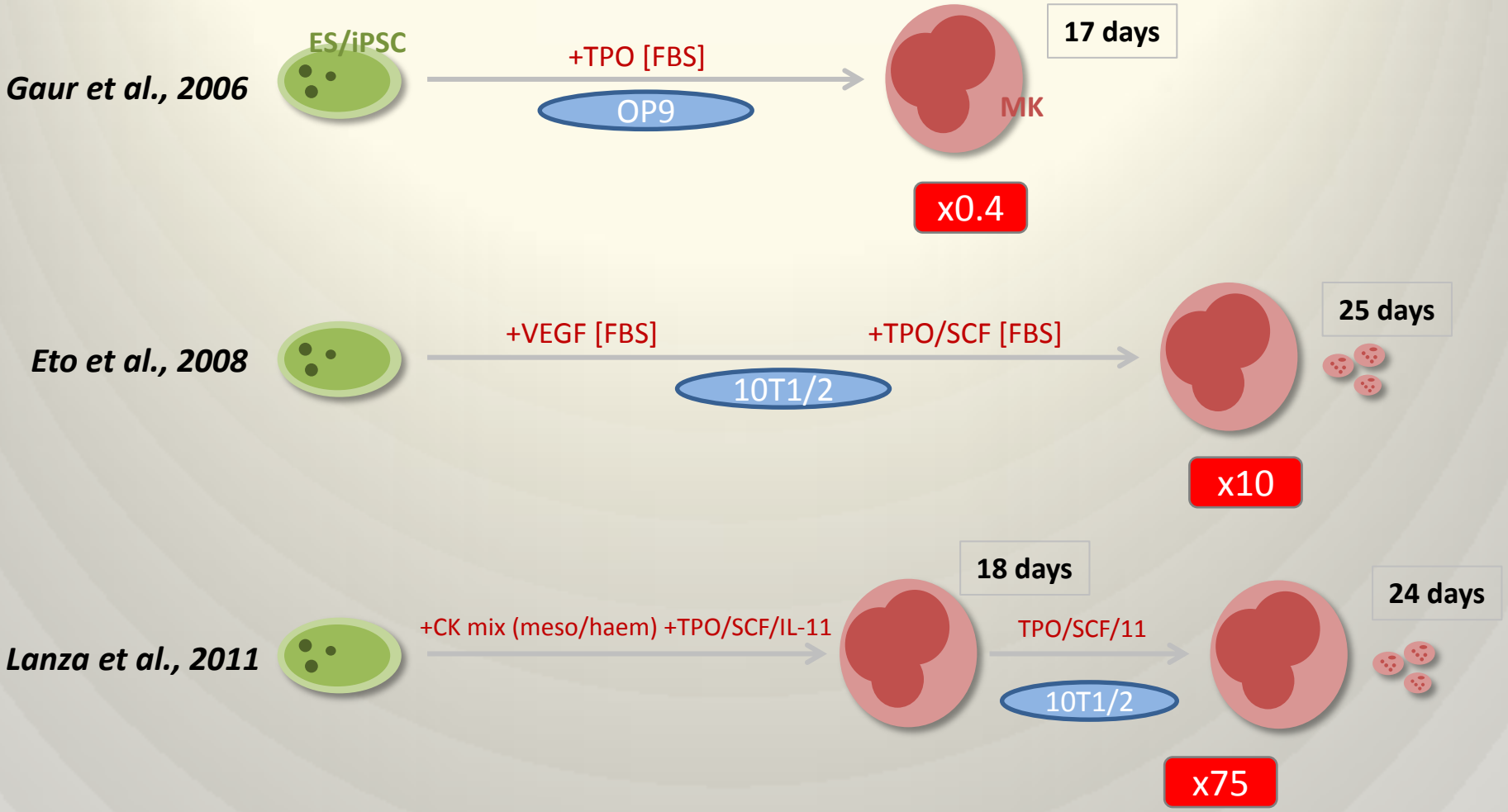
EB

RBC



FROM hiPSC TO MEGAKARYOCYTES (and platelets?)

Published methods



The Long-Term Goal and Drive for This Research

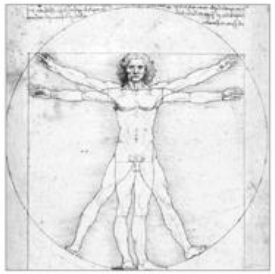
Therapeutic application

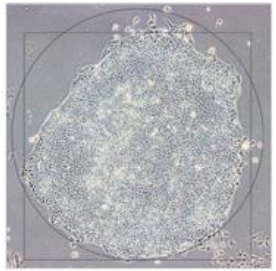
In vitro production of platelet for transfusion

Platelet needs

- **240K platelet concentrate units** per year in the UK.
- **Annual cost of £55M** for the NHS.
- **Short shelf life** (5 days).
- **Voluntary donor** dependency.

The iPS option


2E+8 PLT/ml
1E+11 PLT/day


1E+7 PLT/million
2E+9 iPS/unit

iPS platelets

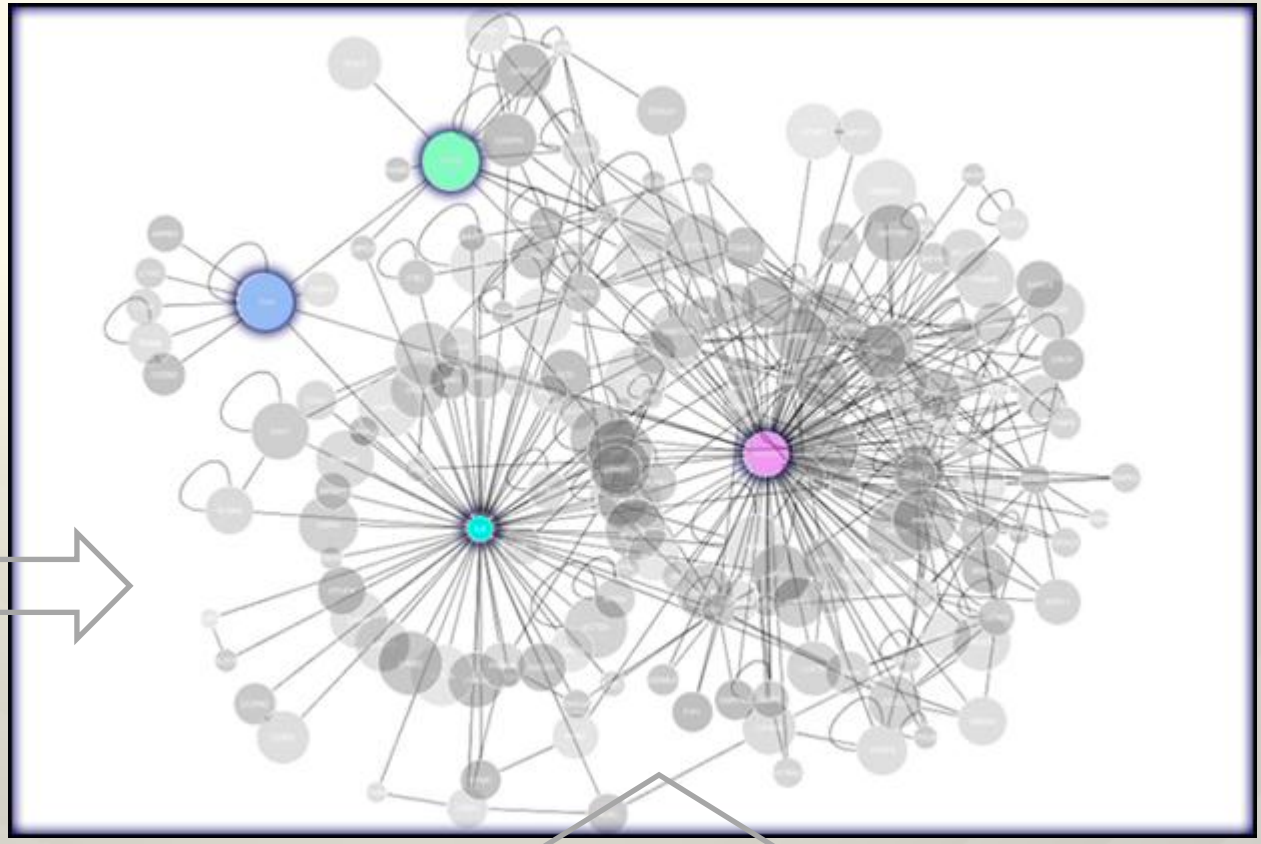
- **On-demand/controllable** production.
- **Biocompatibility** (HLA, HPA, ABO).
- Anucleate end product **safety** (irradiation).

26 days culture
Mouse feeder cells and FBS
High inter cell line variability

State of the art

MK PROGRAMMING APPROACH HYPOTHESIS

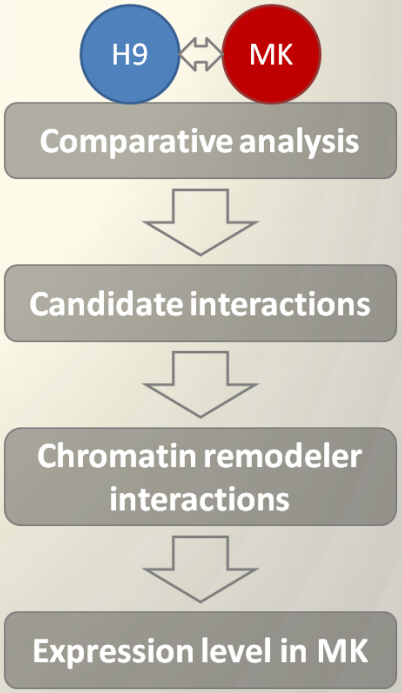
Ectopic expression of master transcription factors to impose a MK phenotype to unrelated cell types



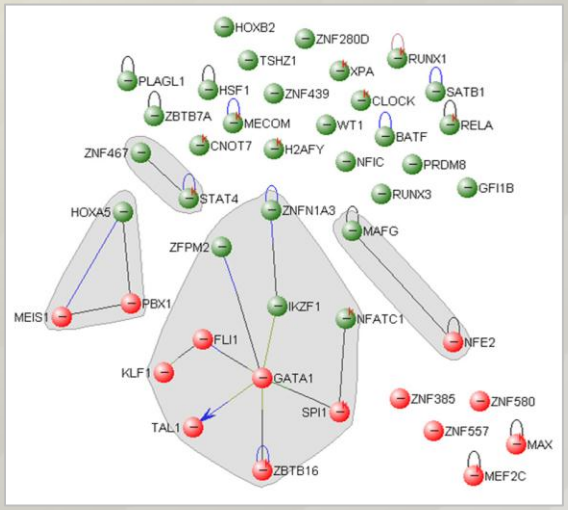
Ectopic expression

Environmental stimuli

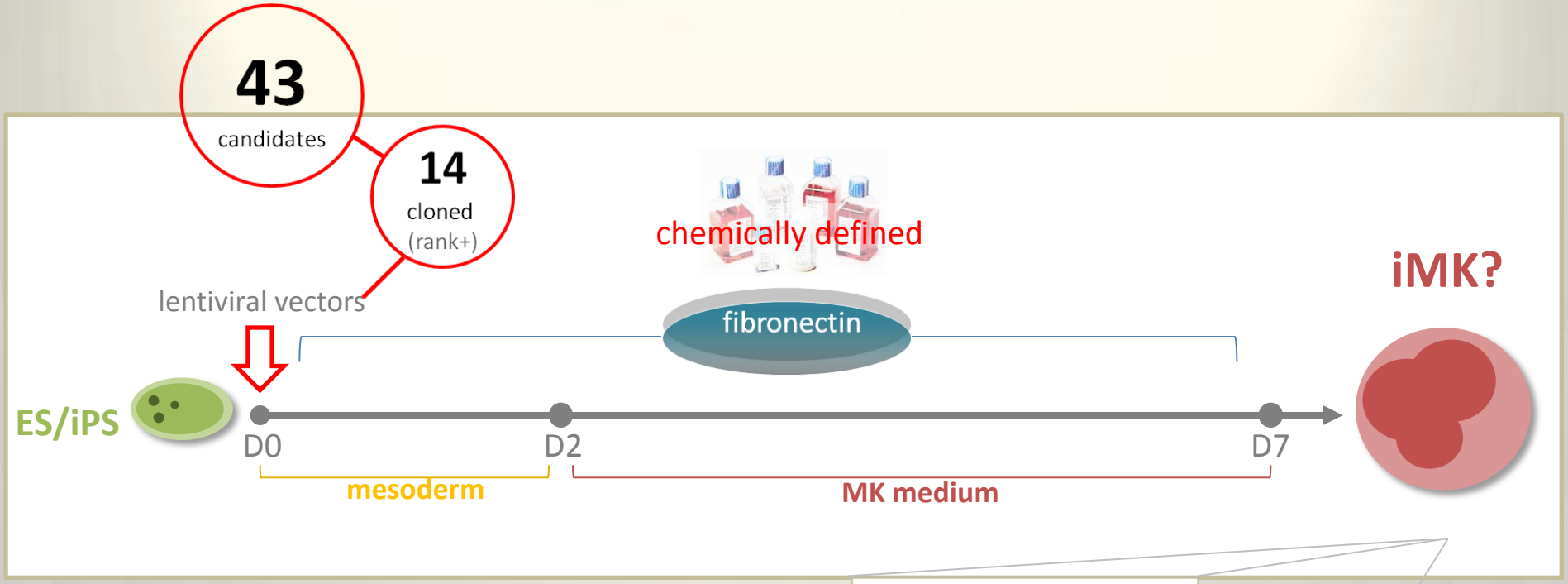
MK Forward Programming – 1st STEPS

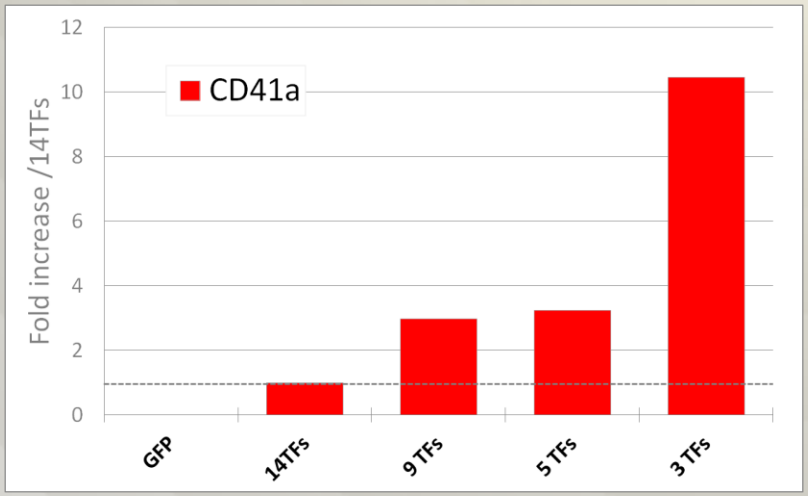


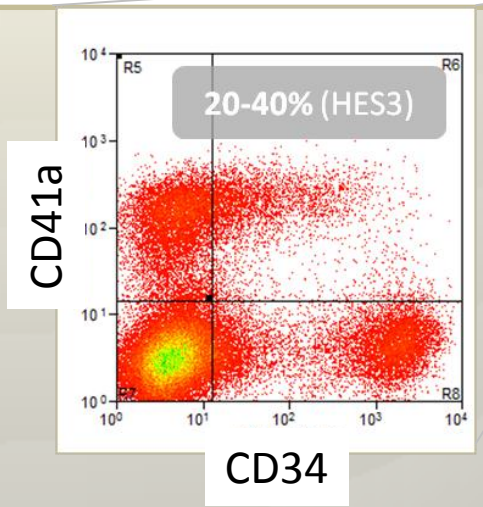
Candidate RANK	Symbol
1	GATA1
2	IKZF1
3	SPI1
4	HOXA5
5	MEIS1
6	FLI1
7	PBX1
8	ZBTB16
9	ZNFN1A3
10	TAL1
11	NFATC1
12	KLF1
13	ZFPM2
14	STAT4
15	NFE2
16	ZNF467
17	RELA
18	MEF2C
19	RUNX3
20	RUNX1
21	ZBTB7A
22	HSF1
23	MECOM
24	SATB1
25	PLAGL1
26	MAX
27	HOXB2
28	CLOCK
29	ZNF385
30	XPA
31	NFIC
32	MAFG
33	WT1
34	GFI1B
35	CNOT7
36	BATF
37	ZNF280D
38	TBXA2R
39	PRDM8
40	ZNF557
41	TSHZ1
42	ZNF580
43	ZNF439



MK Forward Programming – 1st STEPS

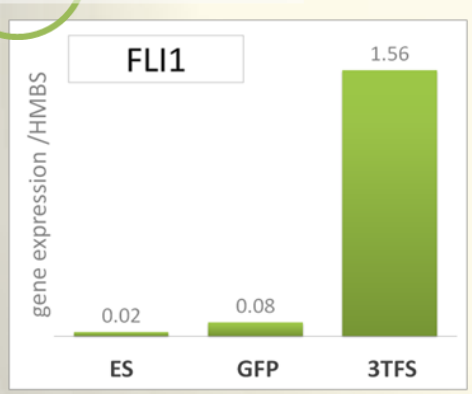


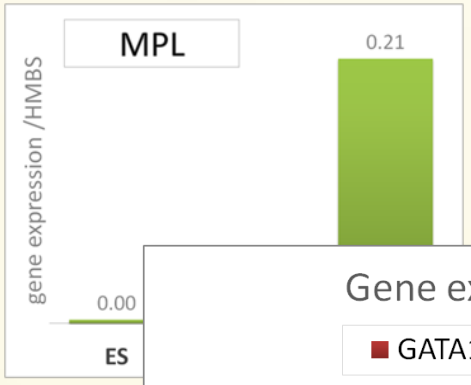


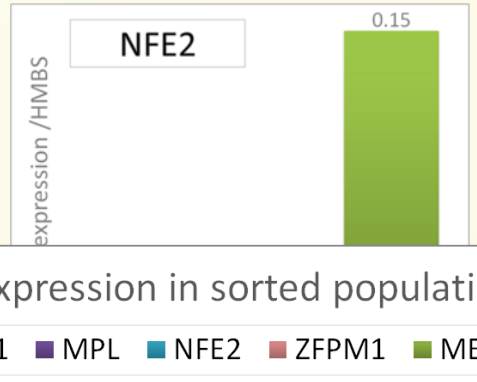


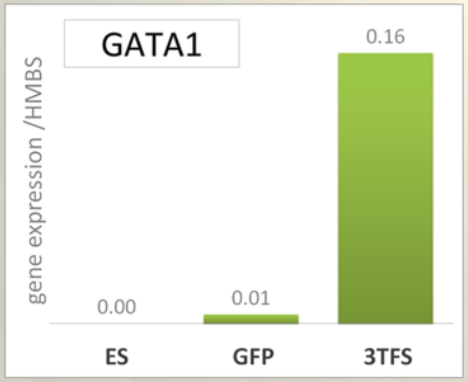
MK Forward Programming – 1st STEPS

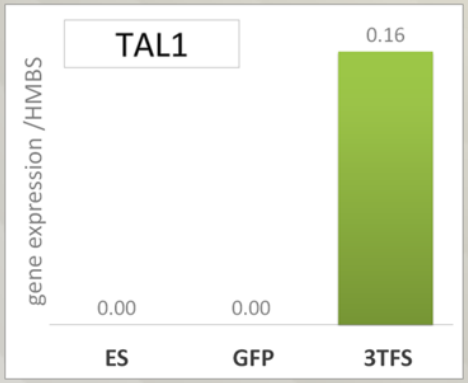
whole population D7

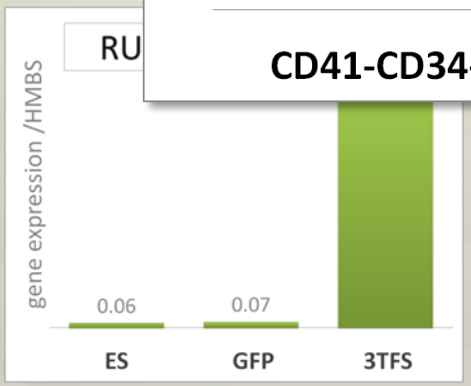


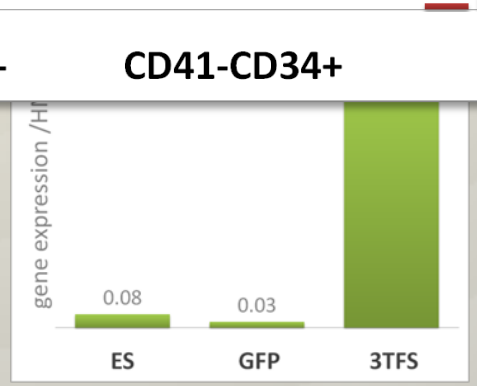








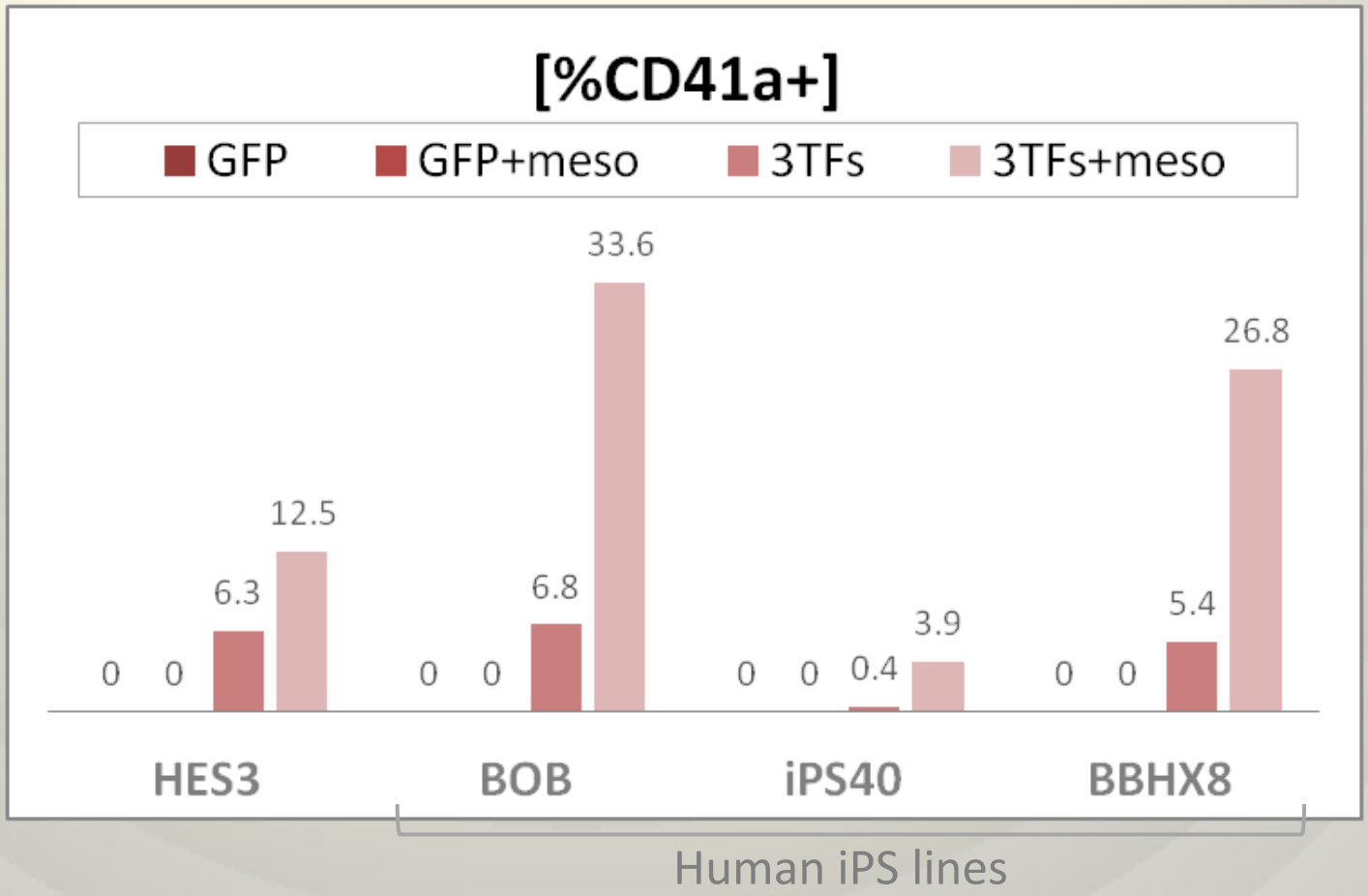




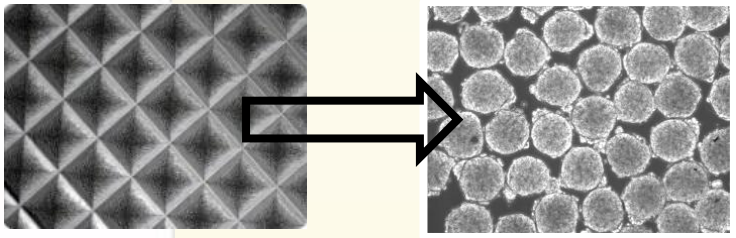
early

late

DEVELOPMENT – MESODERM INDUCTION



DEVELOPMENT – GOING 3D



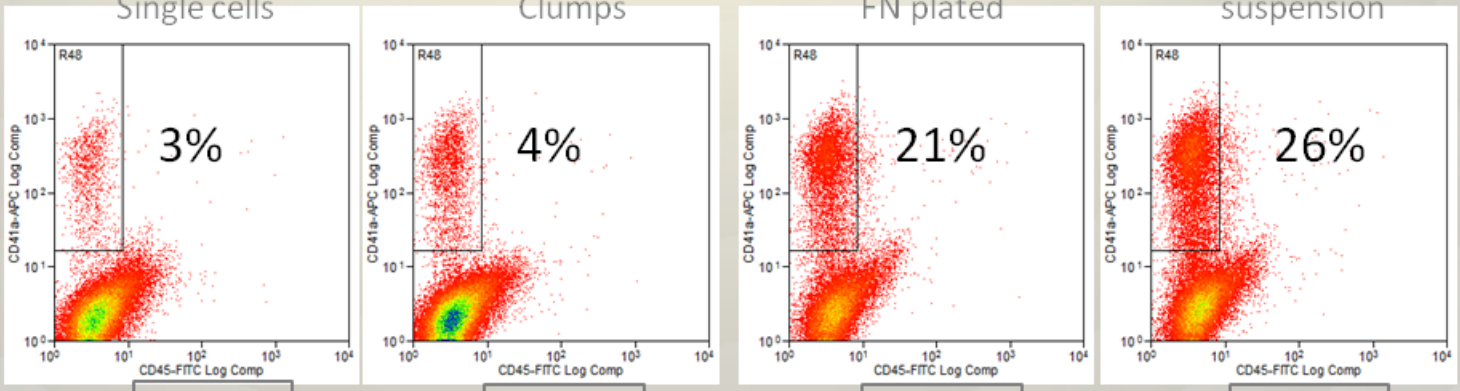
Standardisation
(cell number, EB size control)

Transduction efficiency+
(single cells in suspension)

Improved cell viability

2-D on fibronectin

3-D (spin EB_{400})

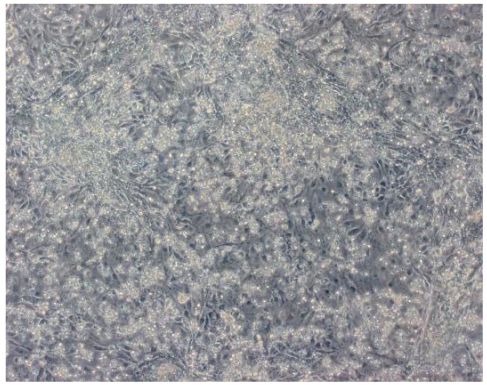


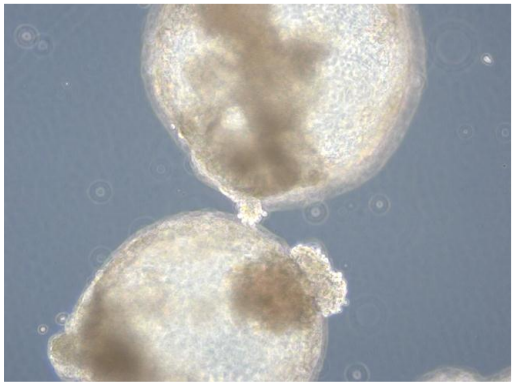
Cell count
Day7 (CD41a)

X0.1 | X0.2 | X0.8 | X0.8

DEVELOPMENT – GOING 3D

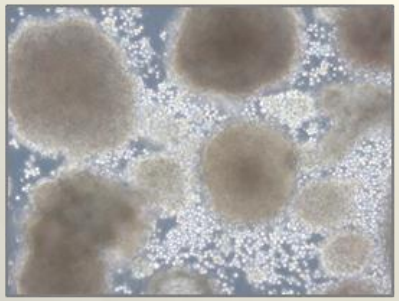
day7

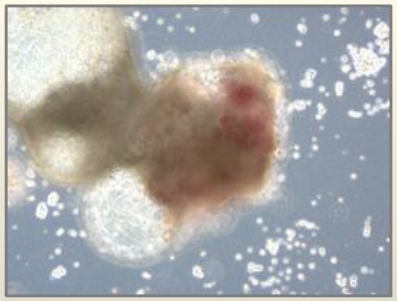


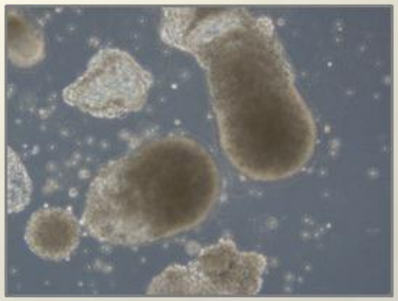


day12

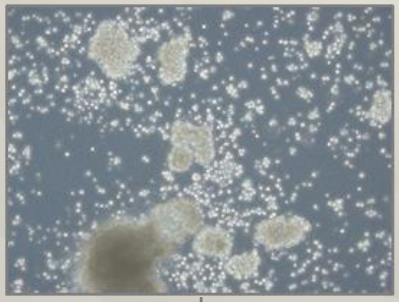
BBHX

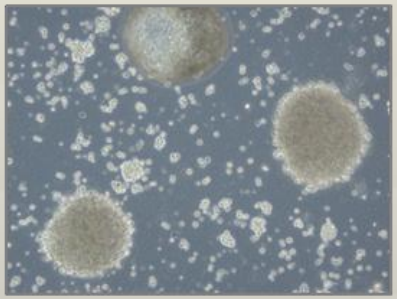


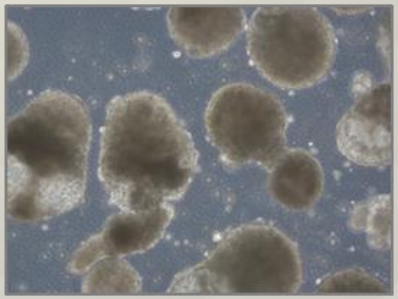




BOB



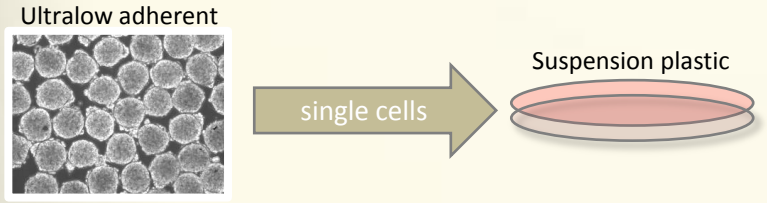


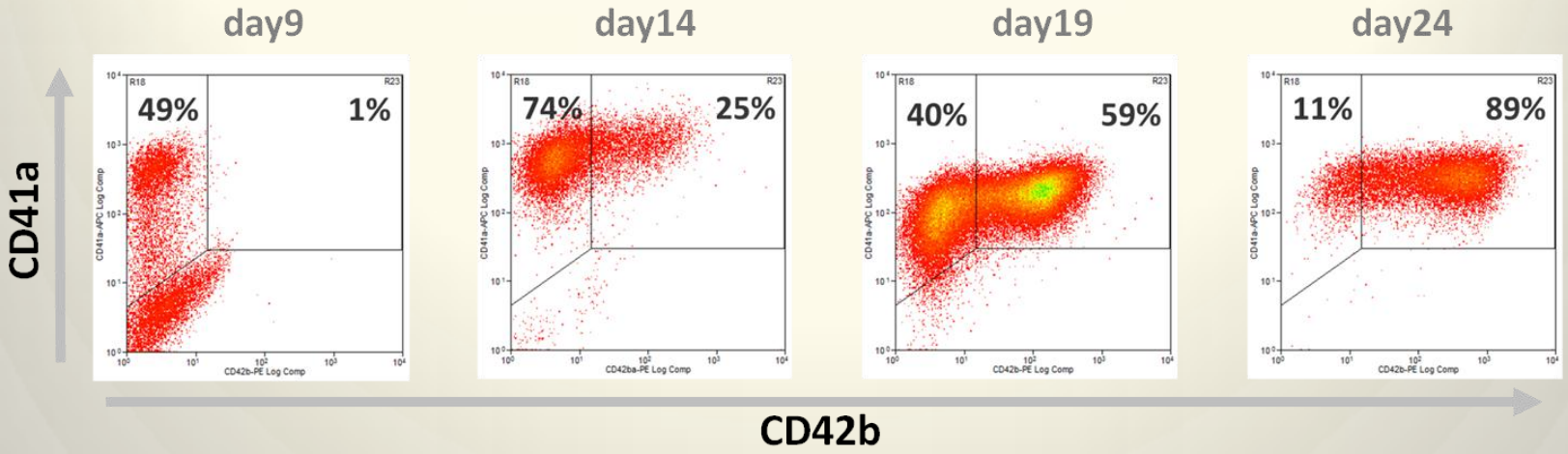


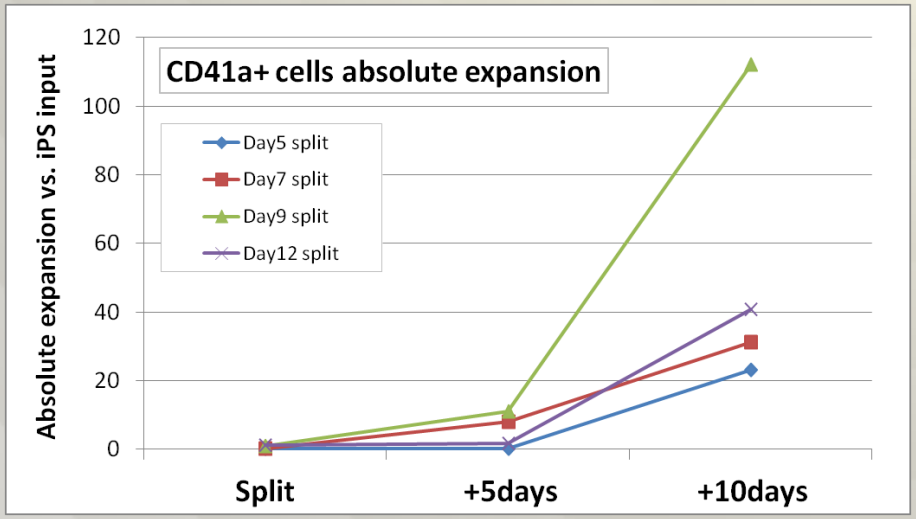
3TFs

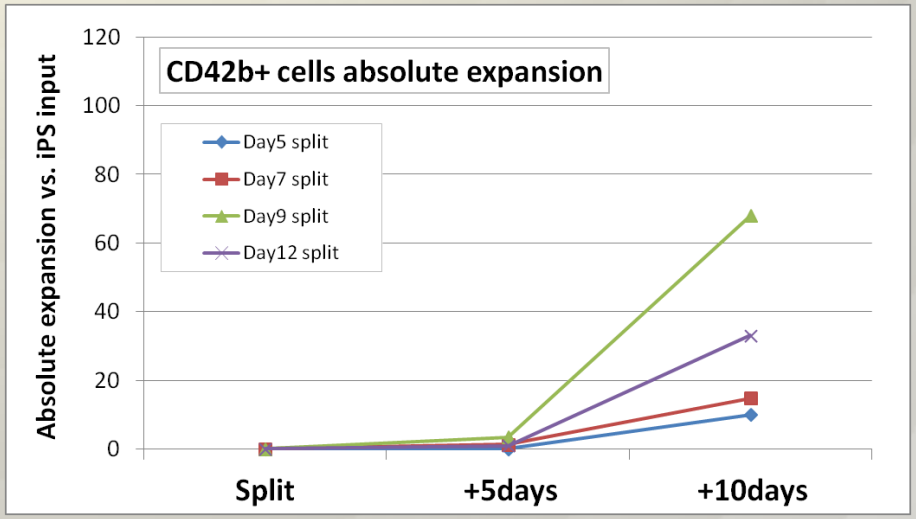
Non transduced

FoP MK MATURATION – 2nd CULTURE STEP



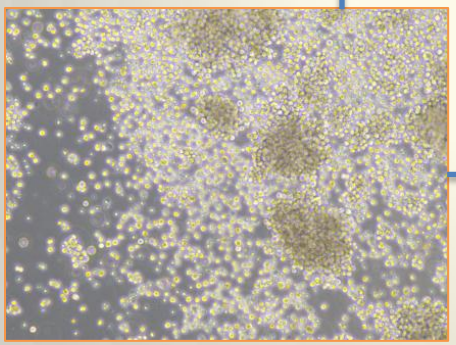


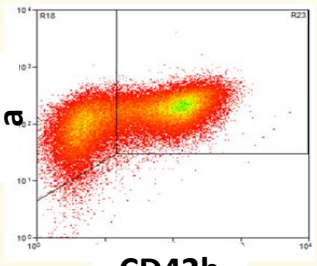


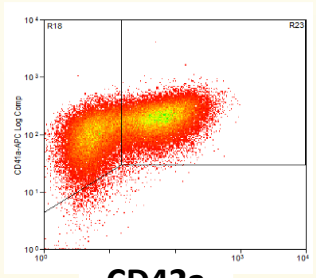


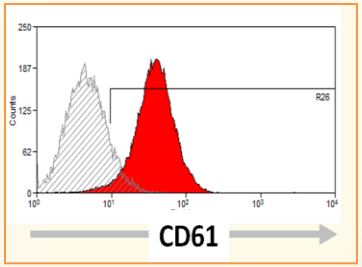
FoP MK MATURATION – 2nd CULTURE STEP

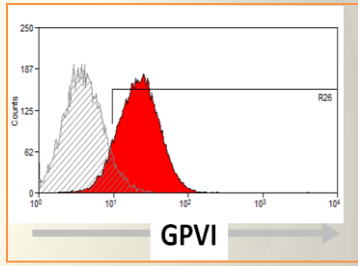
Suspension cells

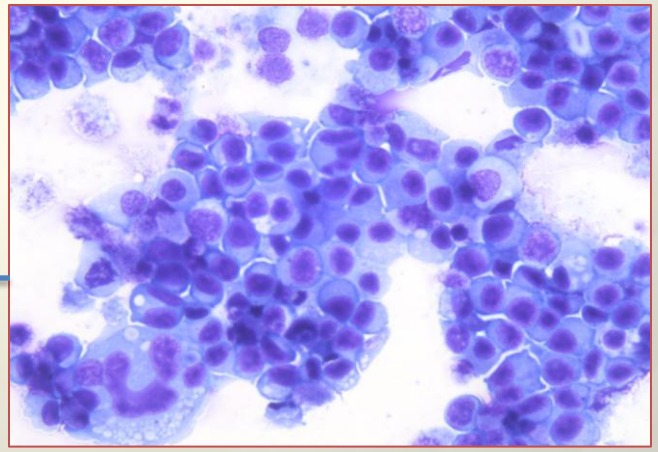


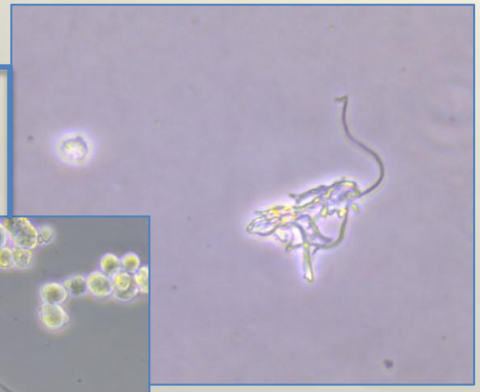


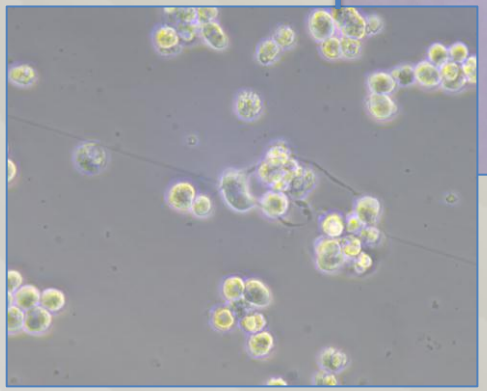




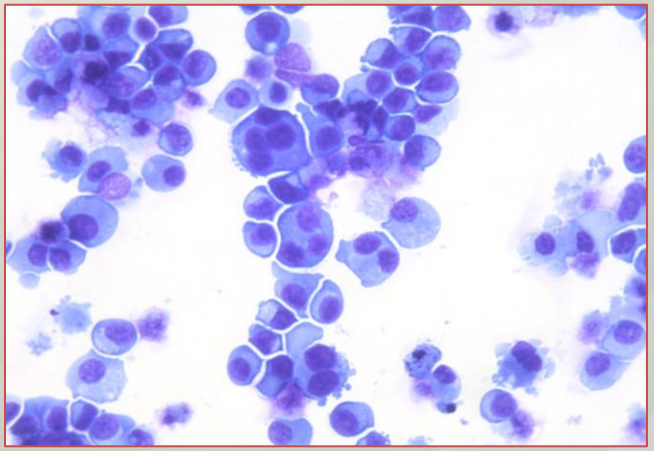




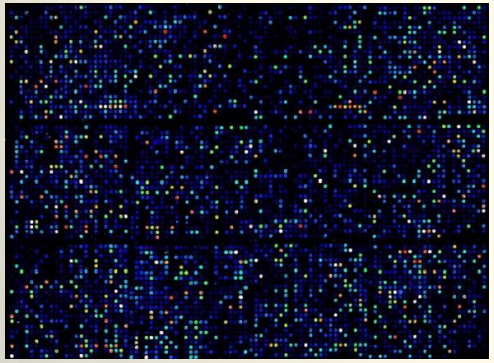




Proplatelet forming cells



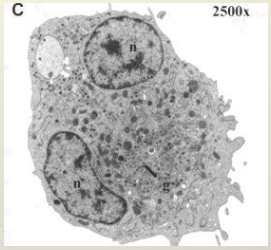
MK FORWARD PROGRAMMING – ONGOING…



iPS ⟺ CB/PB

- Whole genome gene expression
- Epigenetic marks (histone, 5mC)

- In vivo platelet production

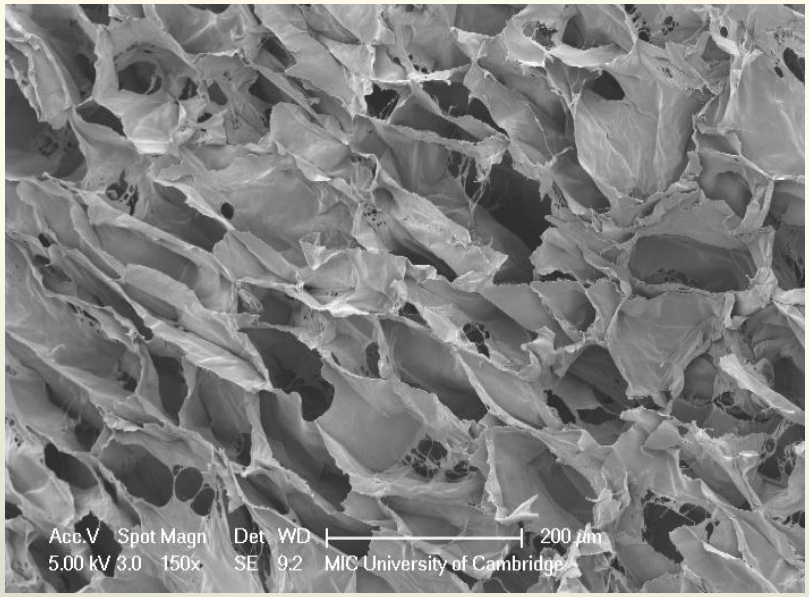


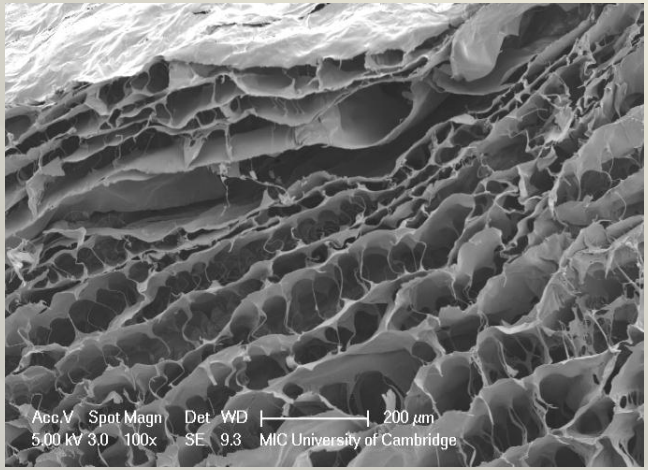
- Ultrastructure (e-micro)
- Functional tests

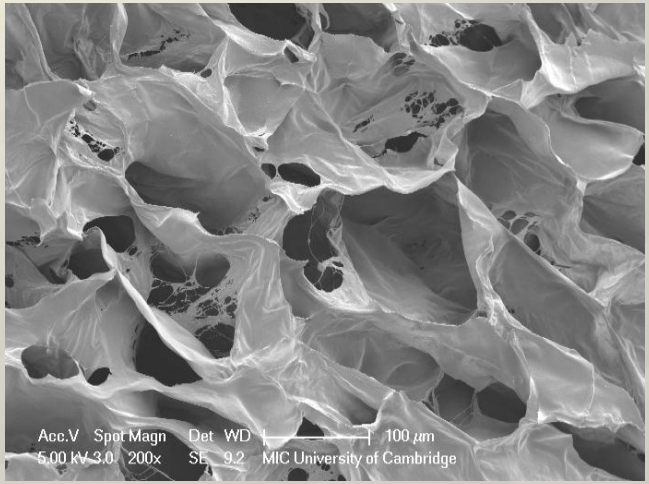
- Non integrative forward programming

Scaling up and promoting platelet production

COLLAGEN SCAFFOLDS



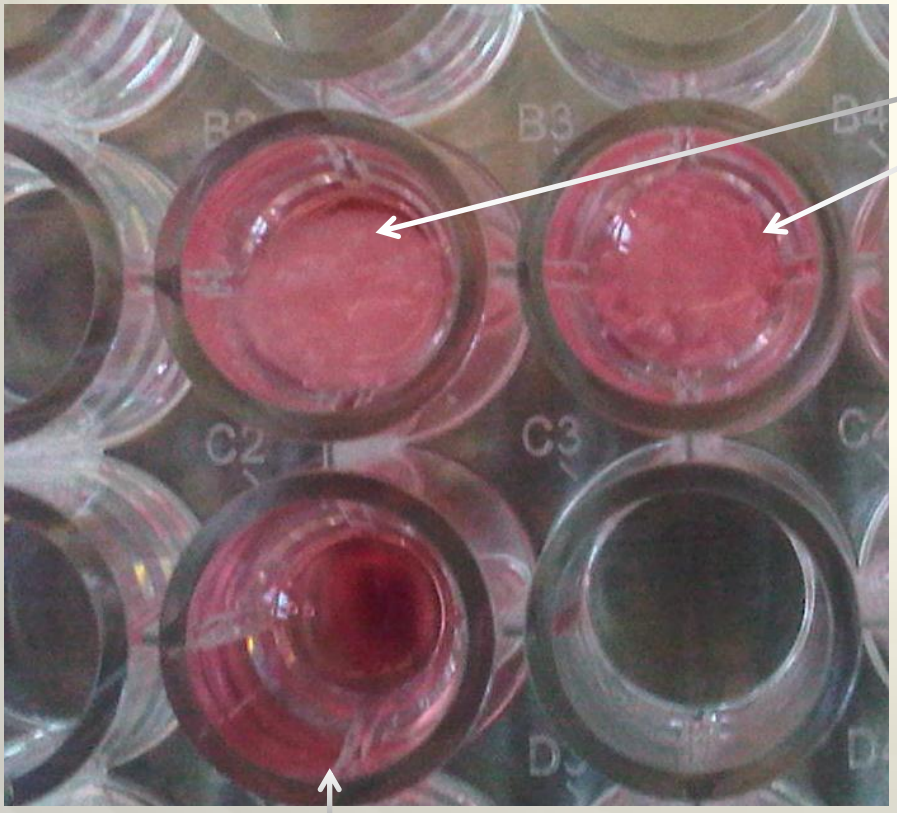


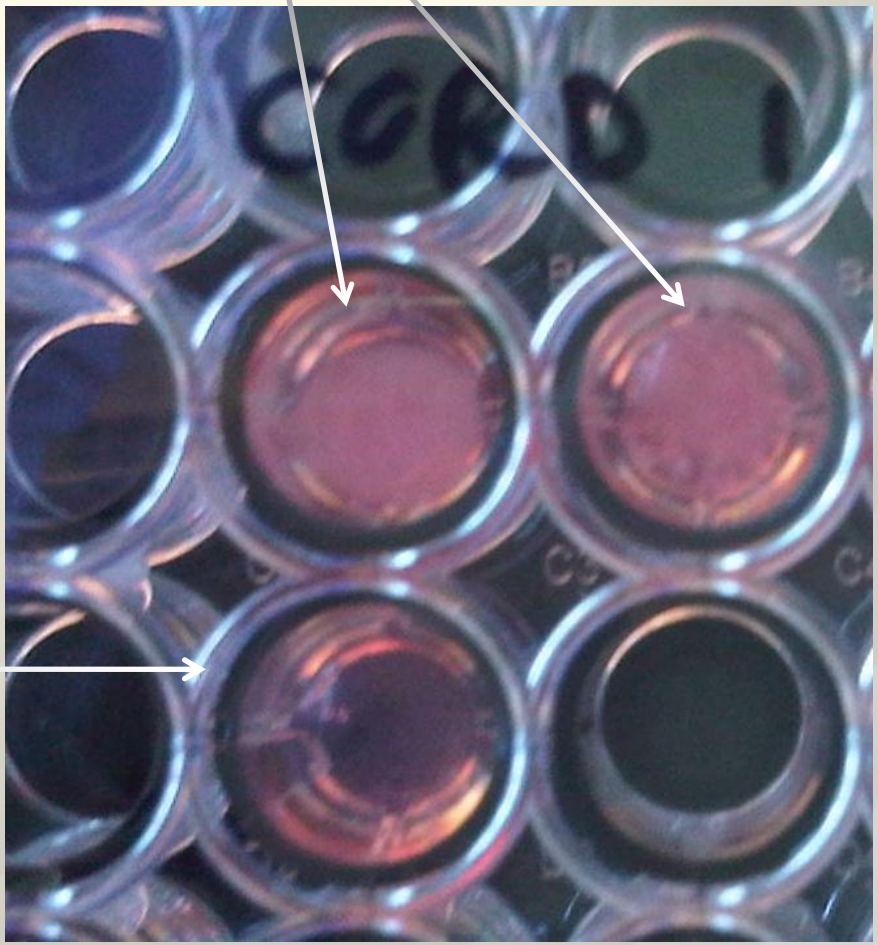


Variation of freeze dry conditions to provide different pore size and architecture

Scaling up and promoting platelet production

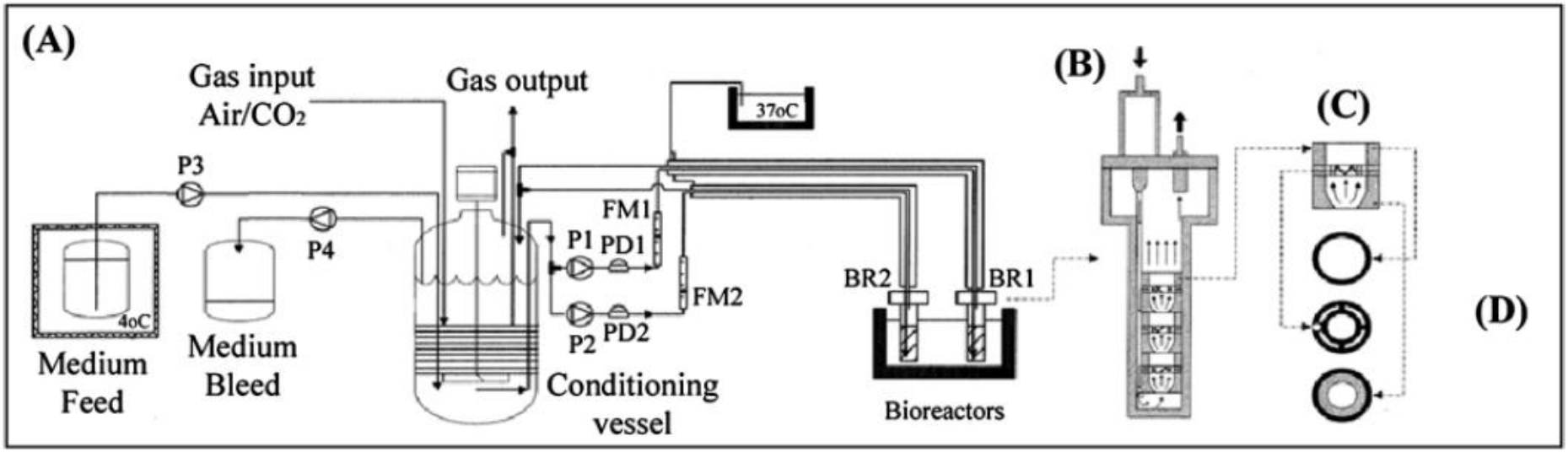
Collagen Scaffolds in
Culture with stem cells

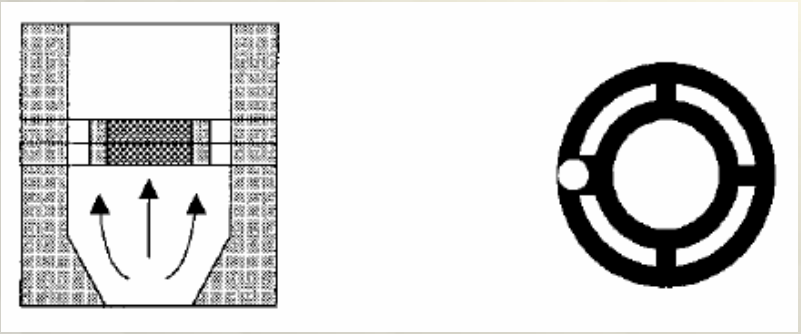


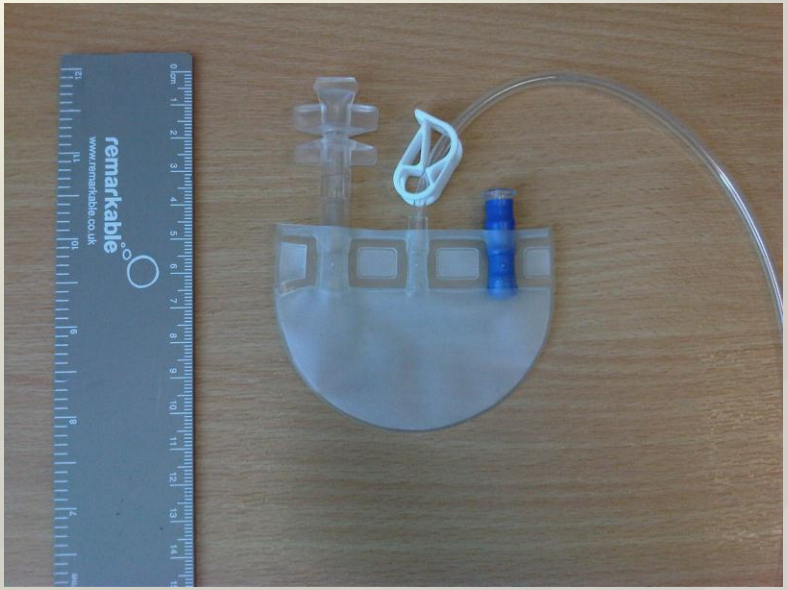


Standard Culture in 24 well plate

Scaling up and promoting platelet production

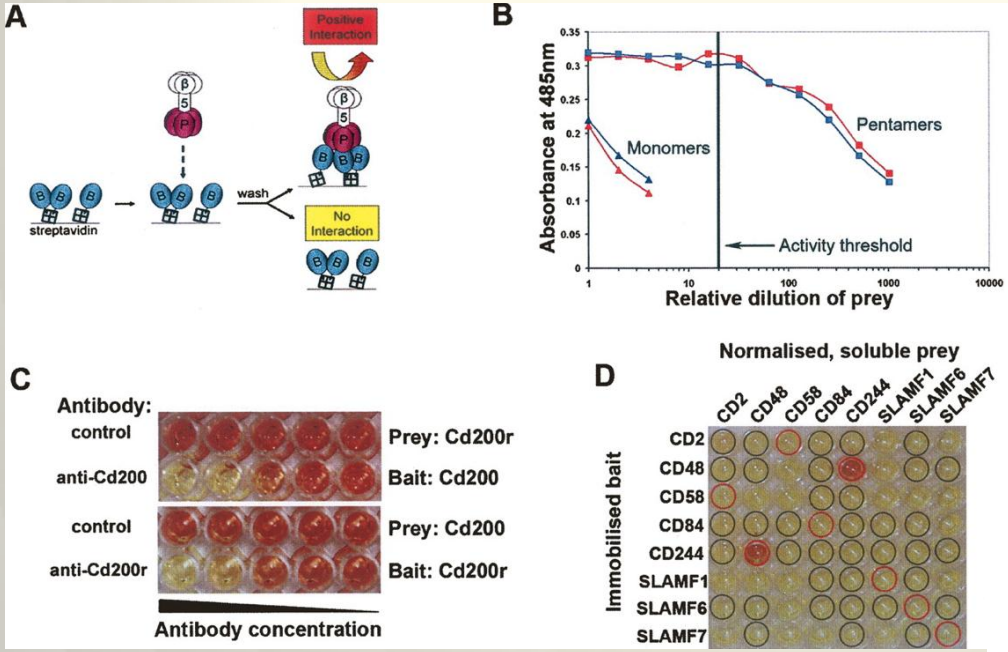






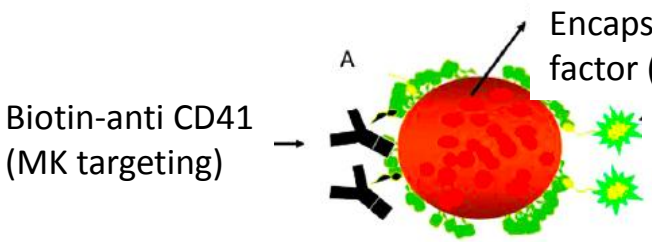
MK Forward Programming – FUNCTIONALISING SCAFFOLDS DELIVERING CYTOKINES

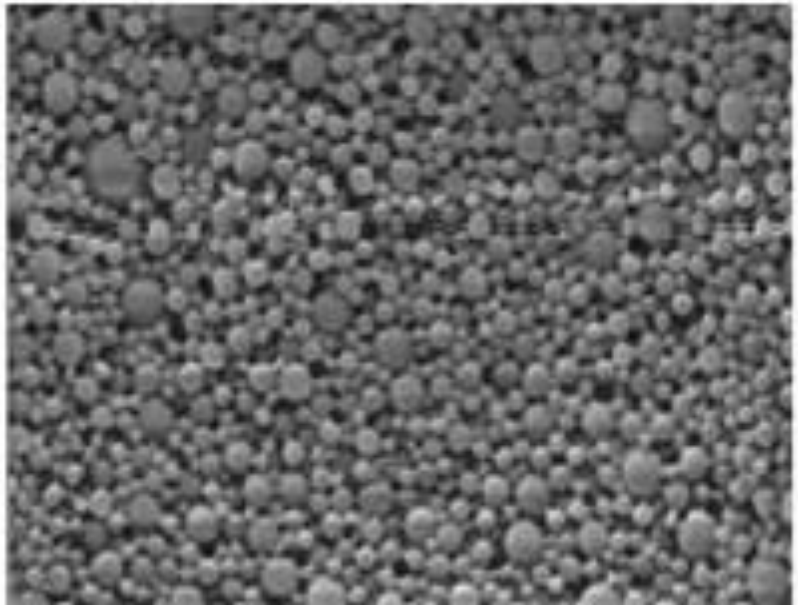
NICHE FROM PEPTIDE LIBRARIES



Bushell et al., Genome Res 2009

TARGETED NANOBEADS





Steenblock et al., Molecular Therapy 2008

CONCLUSIONS…

MK FORWARD PROGRAMMING OUTCOME

- **Cells expressing key features of mature megakaryocytes** have been obtained in chemically defined conditions in 19 days from several human ESC and iPSC lines
- **The numbers of cells obtained** is superior to any other protocol and compatible with the goal of in vitro production of platelets for human use.

SHORT TERM PERSPECTIVES

- **Transcriptome analysis** (+ epigenetic profiling) vs. cord blood derived MKs
- **Functional assays** (*in vitro* proplatelet formation, *in vivo* transplant studies in NSG mice)
- **Test of additional candidate TFs** to improve MK maturation step

MEDIUM TERM PERSPECTIVES

Biological models

Genetic engineering of iPSC lines for MK/PLT biology studies

- Tagging of novel MK genes for gene function studies
- MK targeted expression of maturation factors

Disease modelling

Derivation of iPSC lines from patients with inherited platelet disorders

- TAR and GPS follow up studies

Systems biology

Large scale genetic and epigenetic characterization of iPSC lines and derived MK

- Explore haematopoietic/MK potential inter line variability
- identify genetic and epigenetic factors controlling megakaryopoiesis from iPSC
- First quality assessment of a realistic iPSC derived cell therapy product, i.e. MK/PLT

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