

Heraeus Workshop, Hanau 24.02.2011:

Wechselwirkung Material/Zelle – Oberflächeneigenschaften
beeinflussen die Zelldifferenzierung

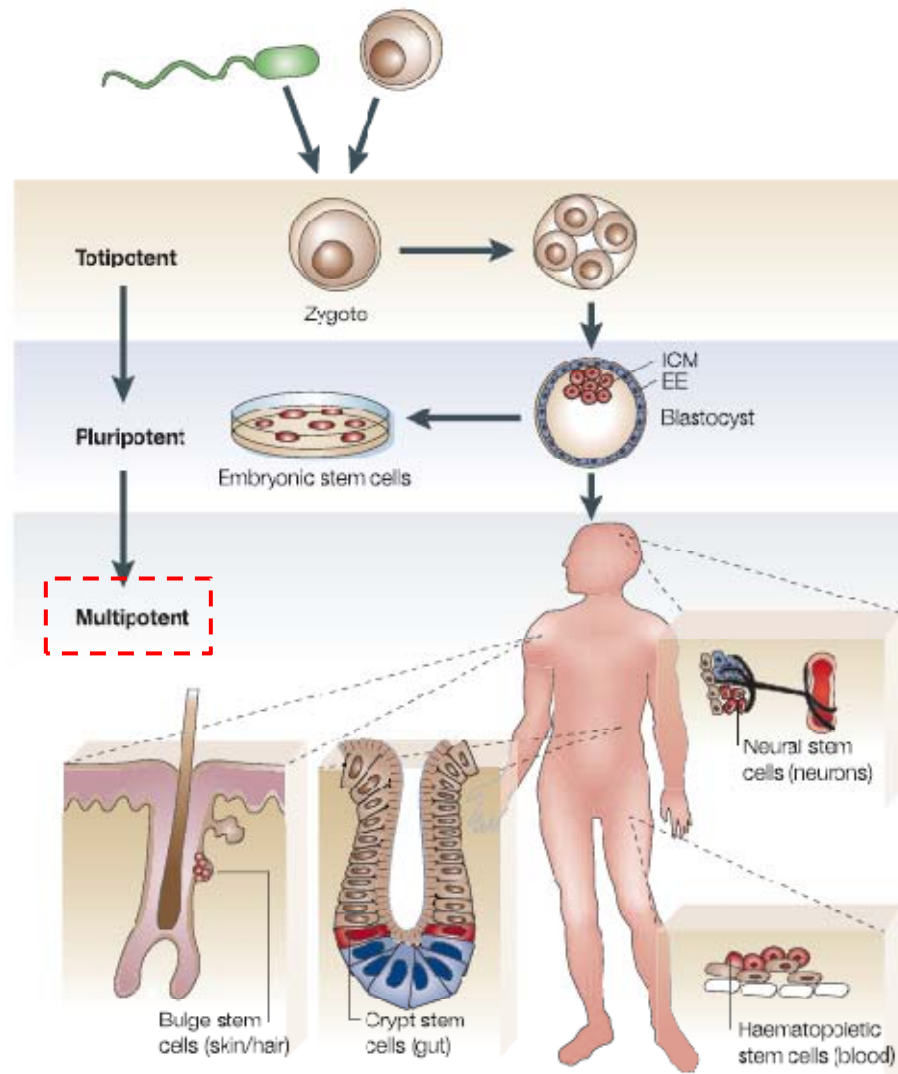


*Optimierung von
Zellkulturbedingungen für die
Kultivierung und Expansion von
Zellen für regenerative Therapien*

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Leibniz Institut für Polymerforschung
Max Bergmann Zentrum für Biomaterialien, Dresden

Regenerative medicine: stem cells for therapeutic purposes



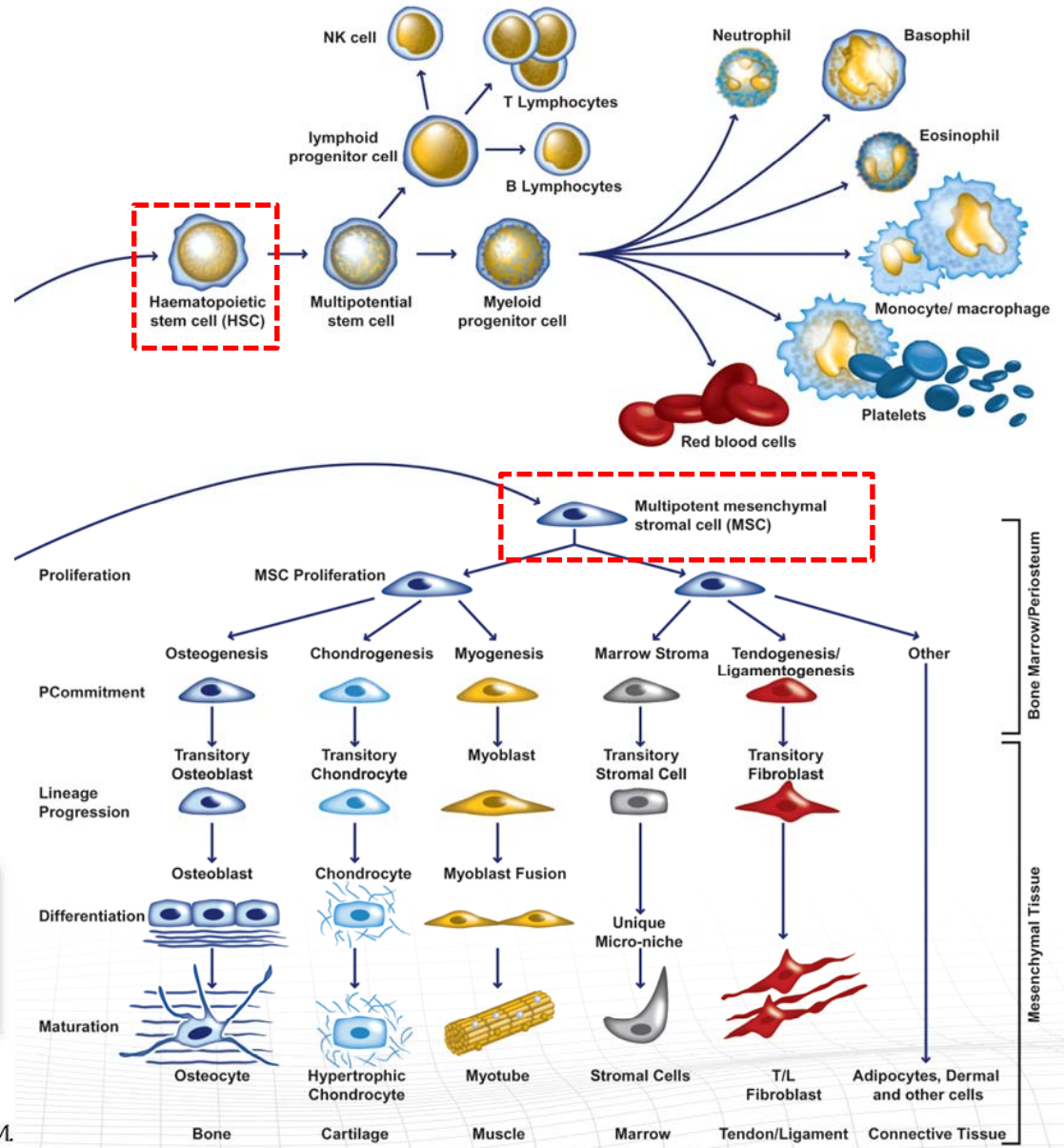
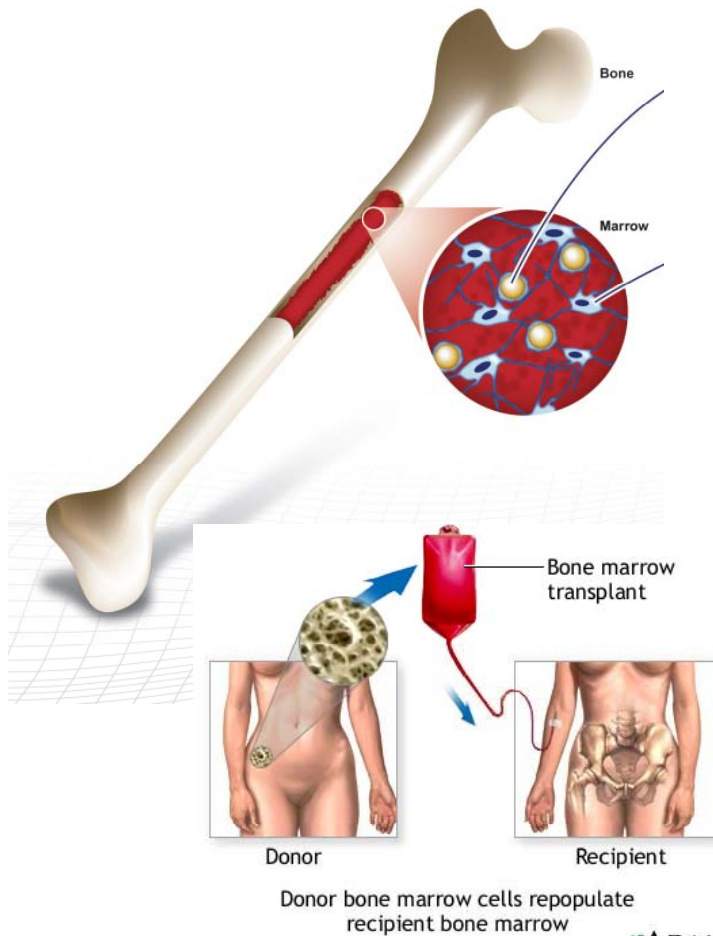
Adult multipotent stem cells

- possible autologous stem cell donation
- Expansion / manipulation in vitro
- Transplantation / cell therapy

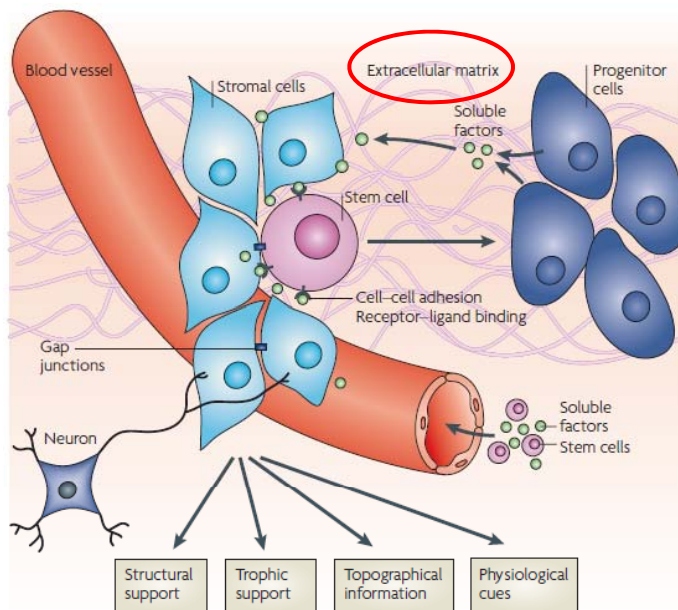
Problems:

- Cell yield / optimal cell numbers for transplantation
- Stem cell maintenance / specific differentiation

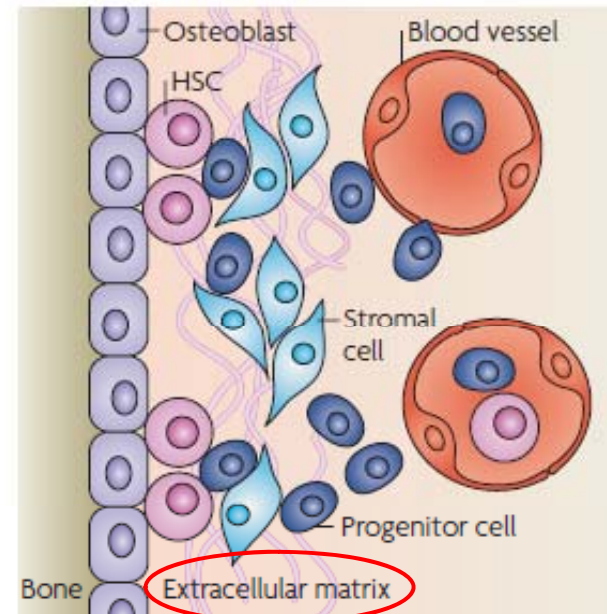
Bone marrow stem cells



Regulation of bone marrow stem cell fate



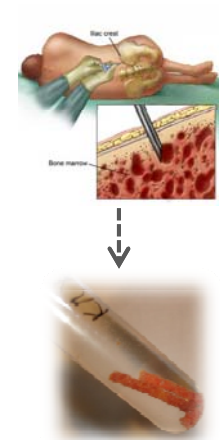
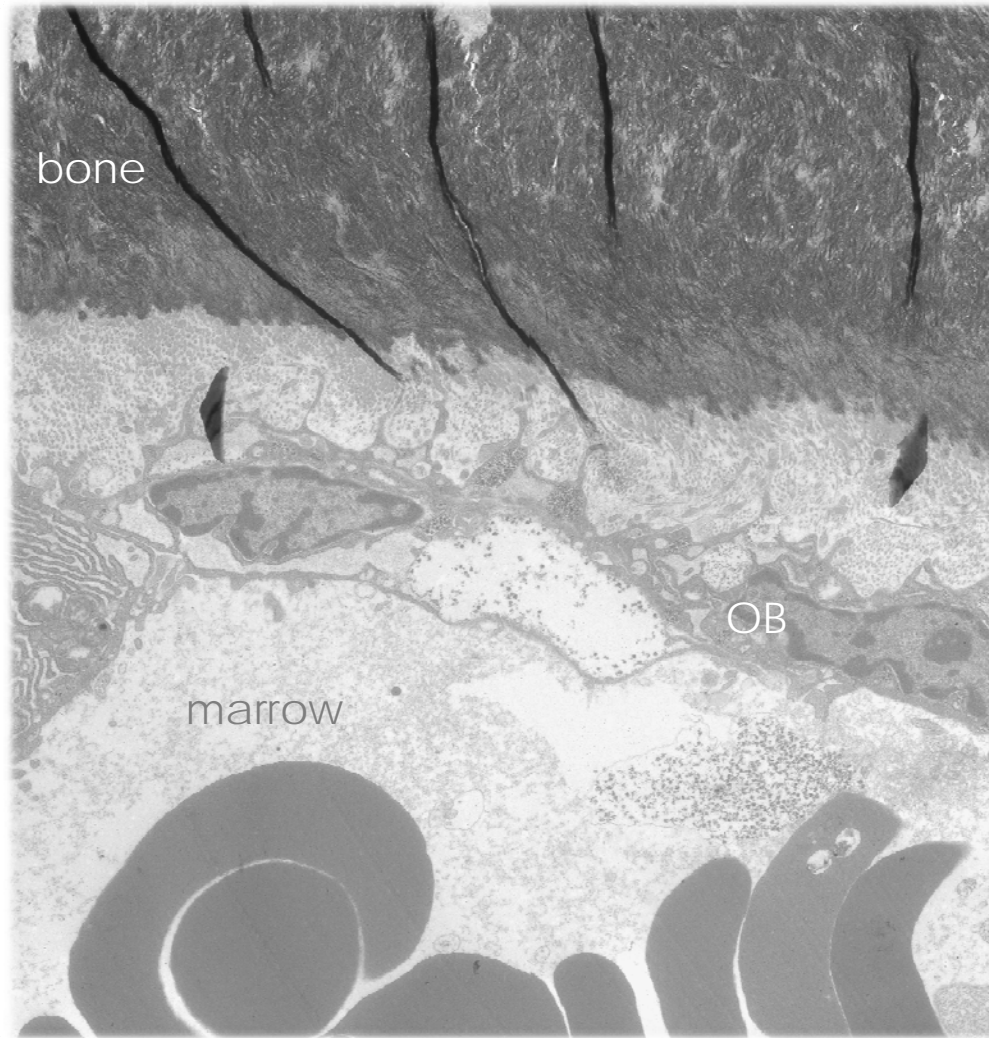
Nature Rev Mol Cell Biol (2008) 9 11



Nature Rev Mol Cell Biol (2008) 9 11

Extracellular matrix participates in cellular signalling, supports cell adhesion/migration and growth factor presentation and is part of the physical microenvironment.

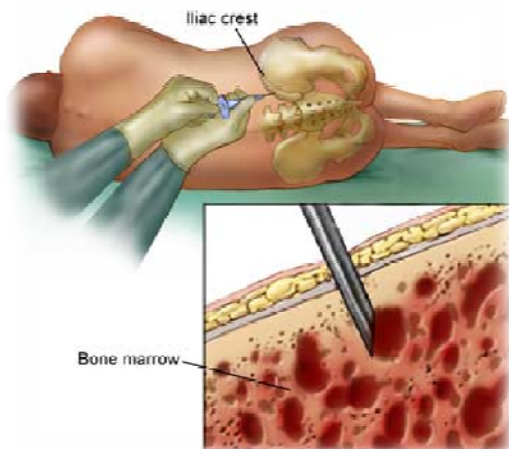
Human bone marrow microenvironment



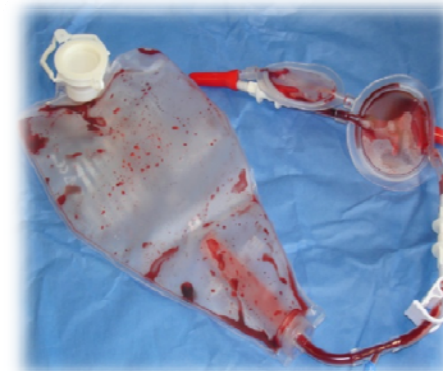
Bone marrow Mesenchymal Stromal Cells



Harvest of human bone marrow via bone marrow aspiration.



Aspirate leftovers for *in vitro* MSC culture.



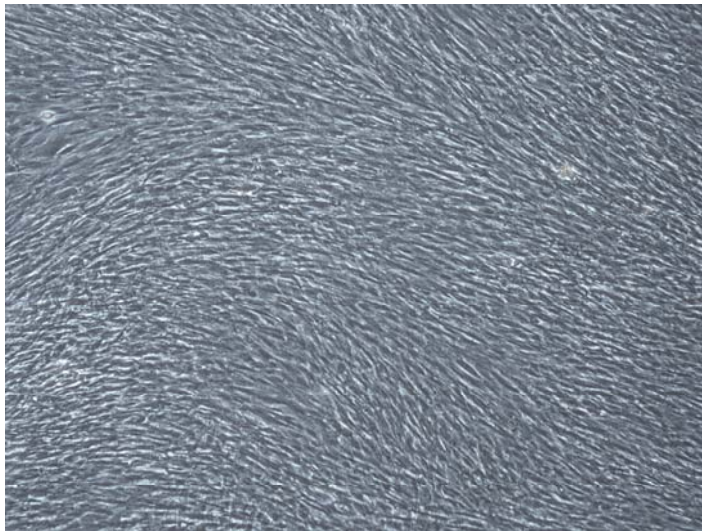
MSC isolation and *in vitro* culture

- Plastic adherence
- MSC marker expression

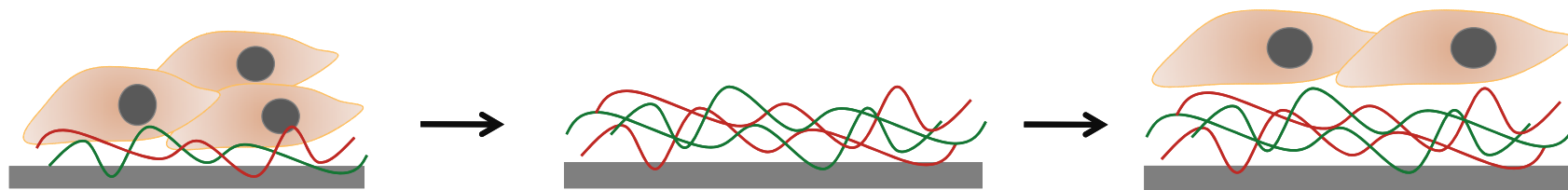
MSC-generated ECM as a culture substrate to simulate the bone marrow microenvironment



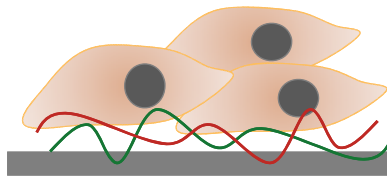
Standard MSC culture on tissue culture plastic (PTP)



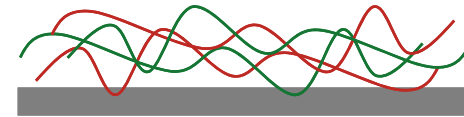
- MSCs senesce in culture over the period of several sub-passages
- Reduced proliferation and differentiation capacity after expansion and long-term culture



MSC-generated ECM as a culture substrate to simulate the bone marrow microenvironment

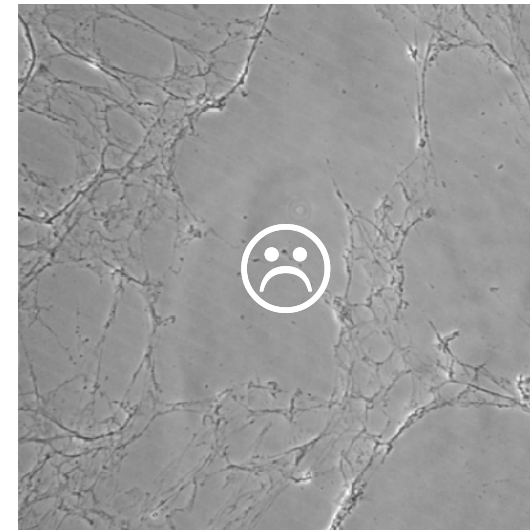
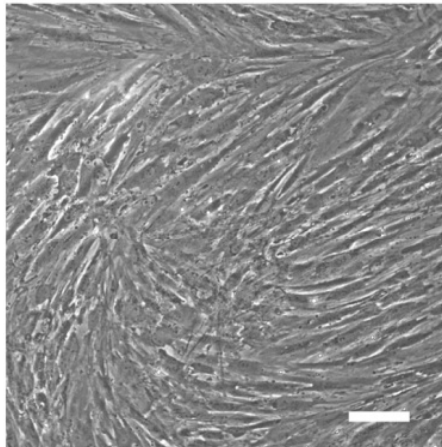


Extraction buffer
Phosphate-buffered saline
0,5% (v/v) Triton X-100
20mM NH₄OH

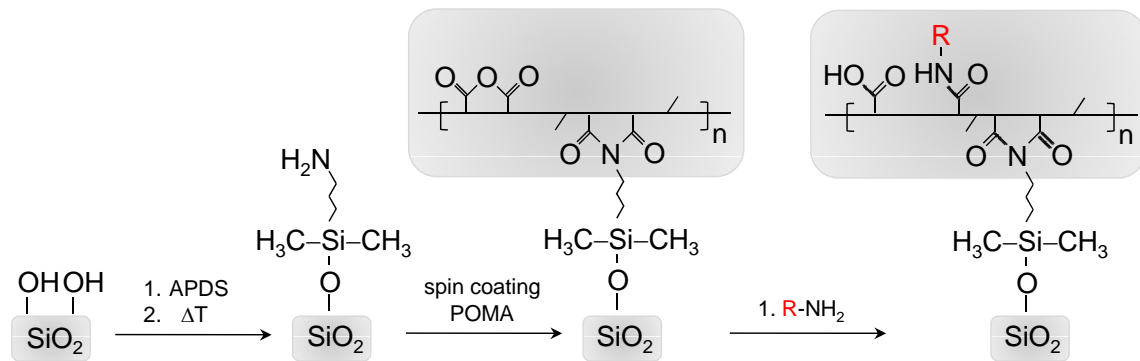
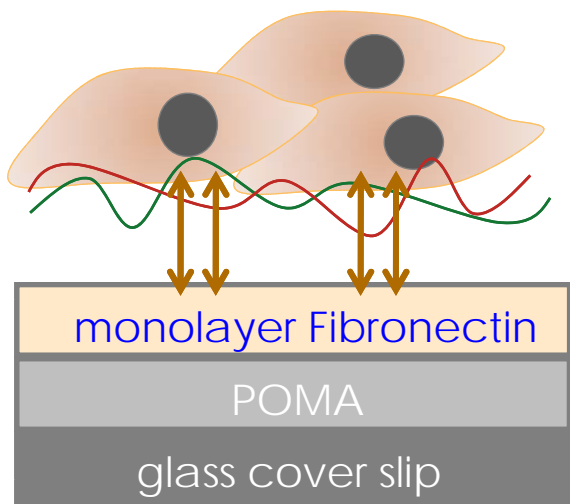


MSCs p1
ECM production

cell-free ECM



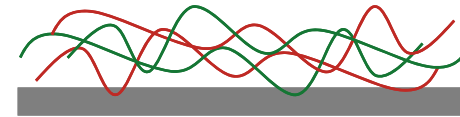
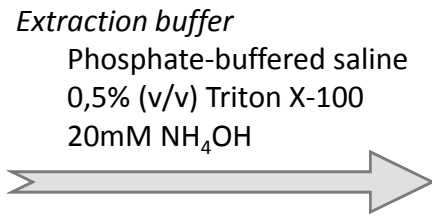
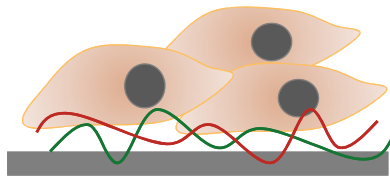
Coupling the cell-made ECM to the surface



APDS 3-Aminopropyl-dimethyl-ethyl silan
POMA Poly (octadecene *alt* maleic) anhydride

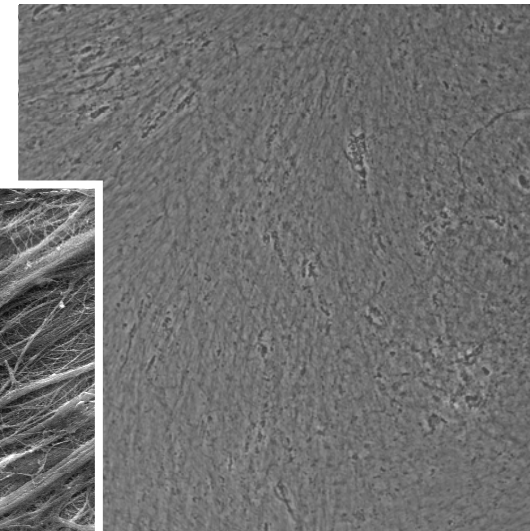
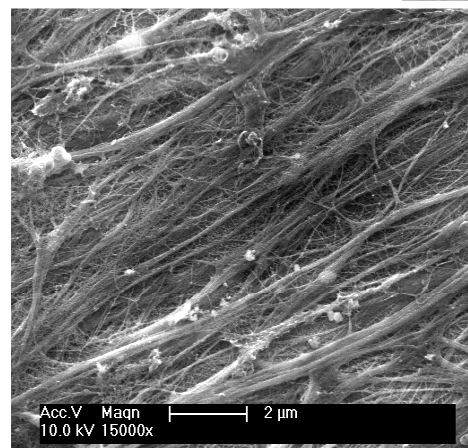
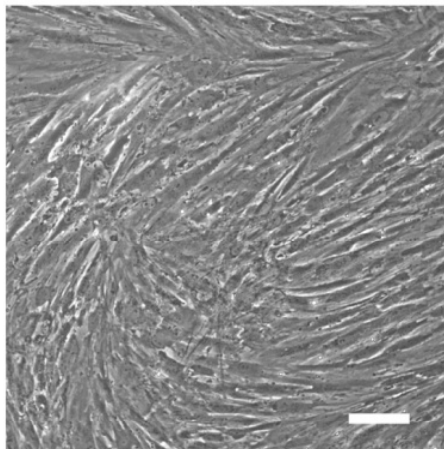
R = Extracellular matrix protein (i.e. Fibronectin)

MSC generated ECM



MSCs p1
ECM production

cell-free ECM

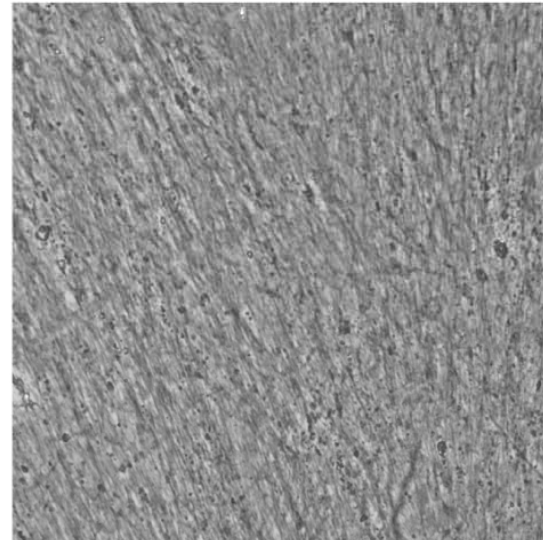


Two different types of cell-free ECM

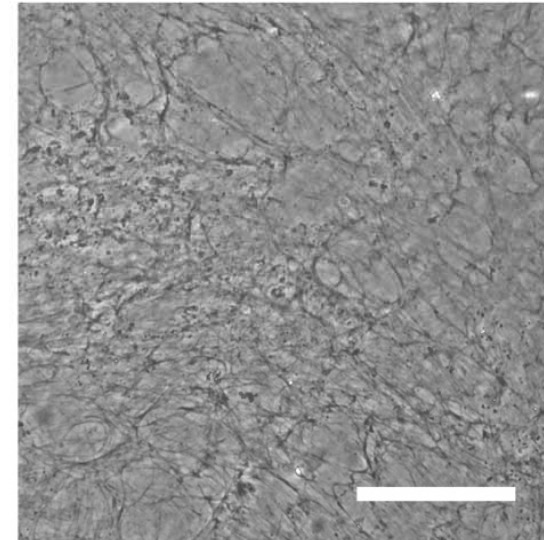


Bright field

aaECM



osteoECM

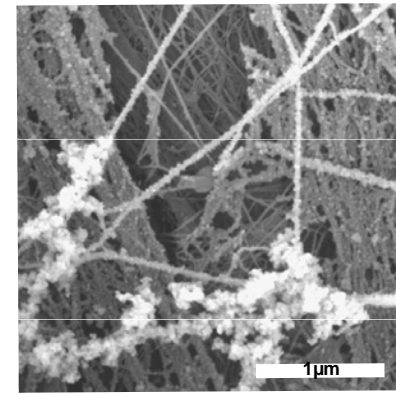
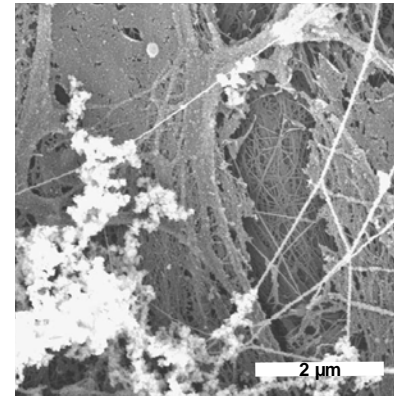
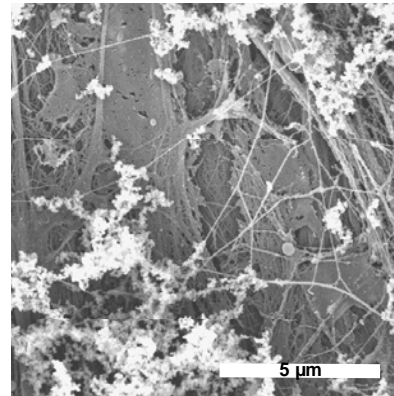
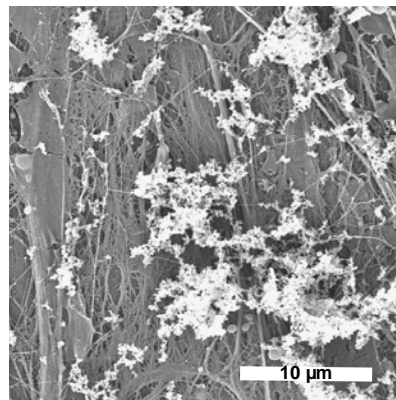


	aaECM	osteoECM
Feeding conditions for p1 MSC for ECM production (10 days).	50 μ g/ml ascorbic acid	50 μ M ascorbate 2-phosphate 10mM β -glycerol phosphate 100nM dexamethasone
dry hight	390 \pm 110 nm	97 \pm 43 nm

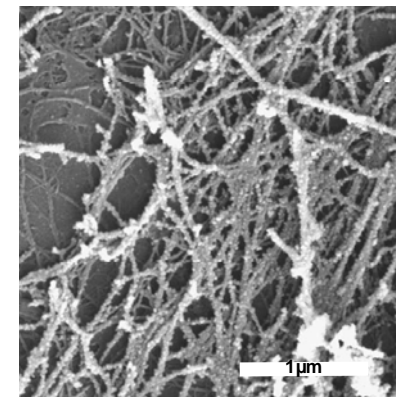
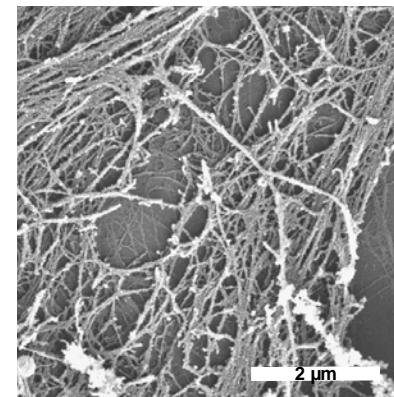
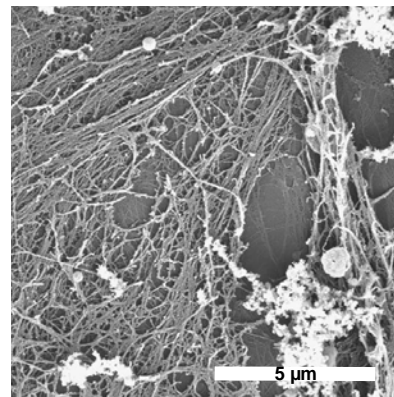
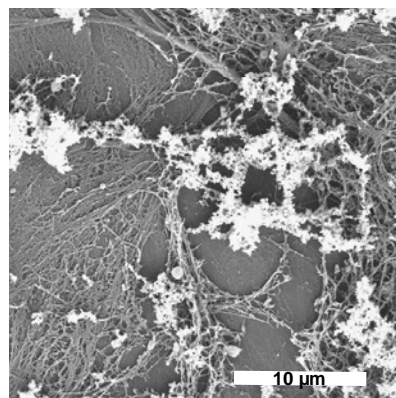
Appearance of MSC-generated ECM



aaECM



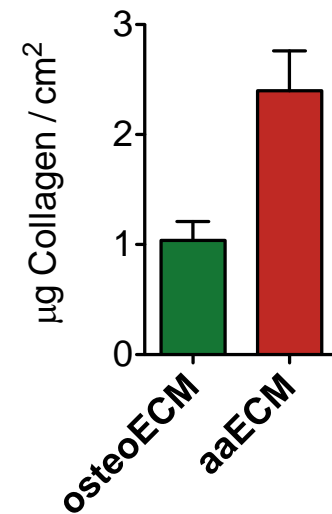
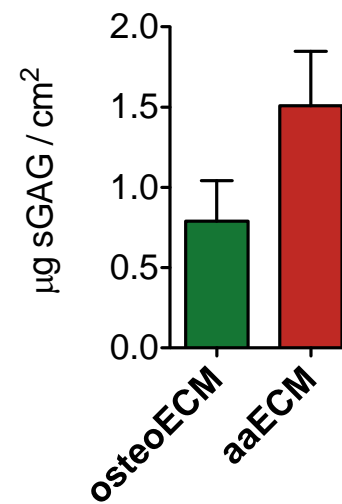
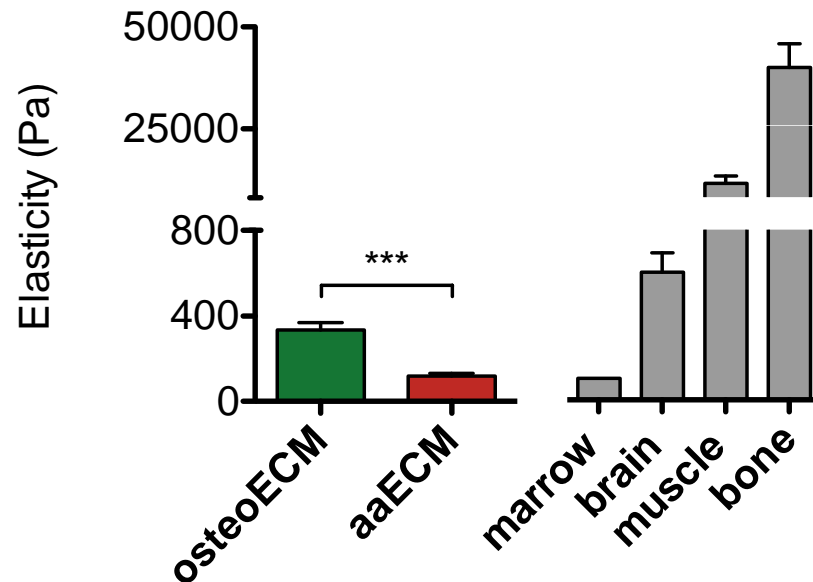
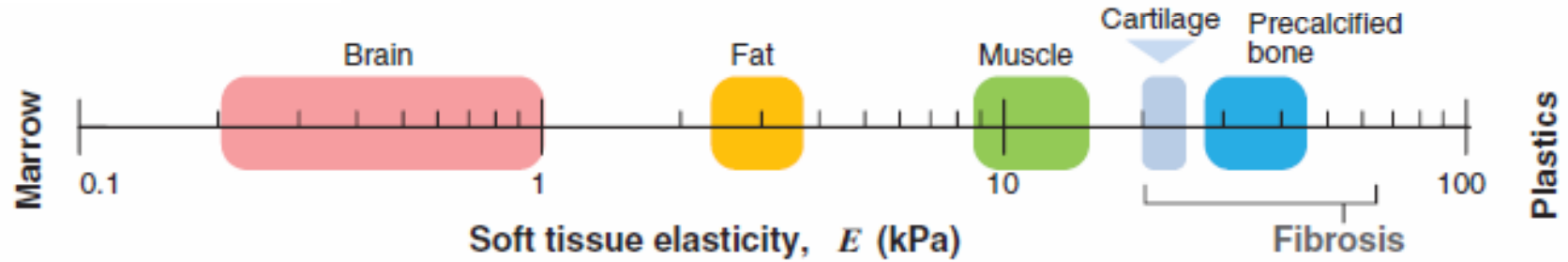
osteoECM



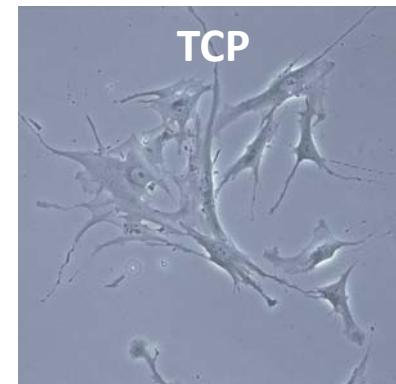
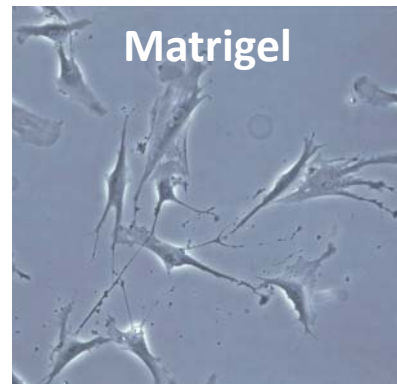
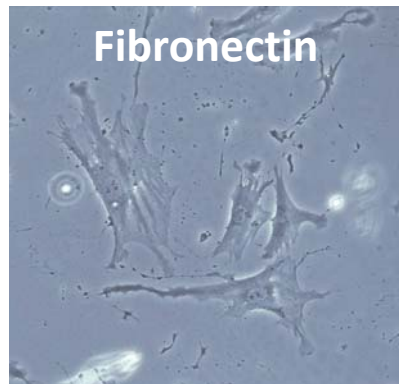
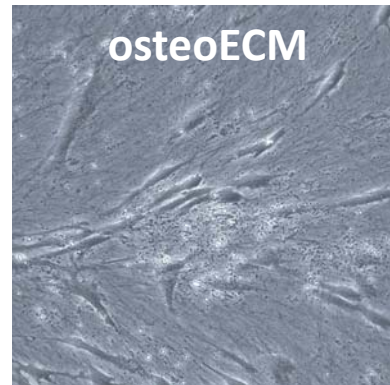
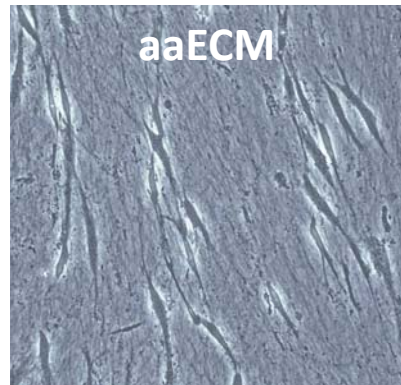
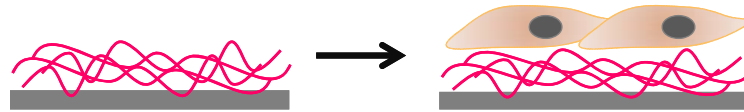
Mechanical characteristics of the cell-free ECM



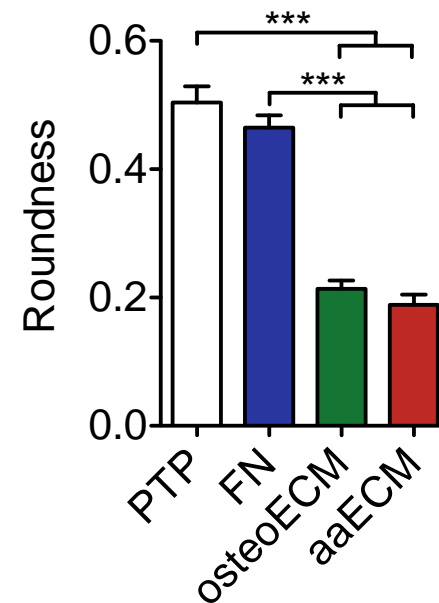
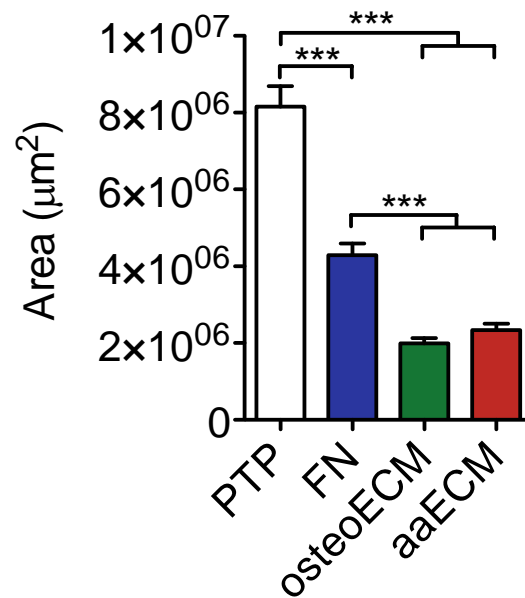
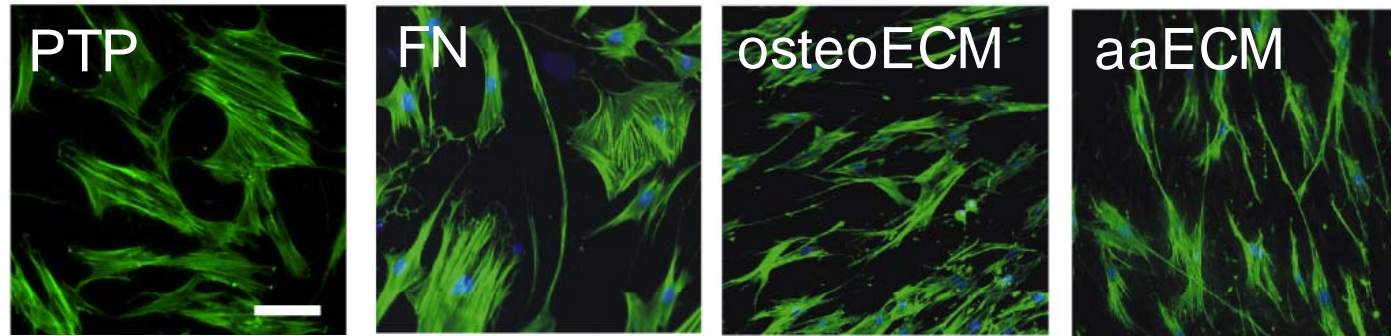
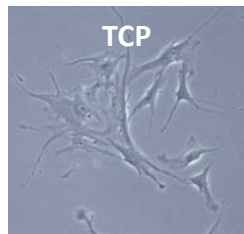
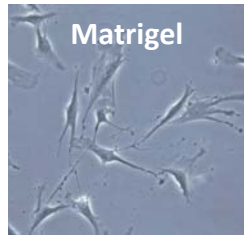
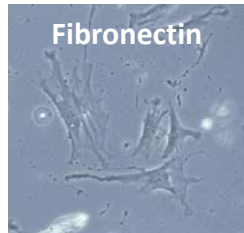
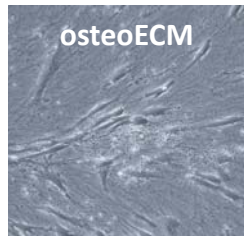
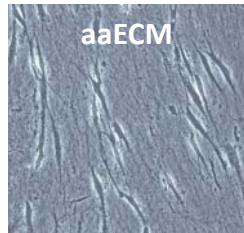
Growth Factors, Matrices, and Forces Combine and Control Stem Cells
 Dennis E. Discher, et al.
 Science 324, 1673 (2009);
 DOI: 10.1126/science.1171643



How do MSCs react to their own matrix?



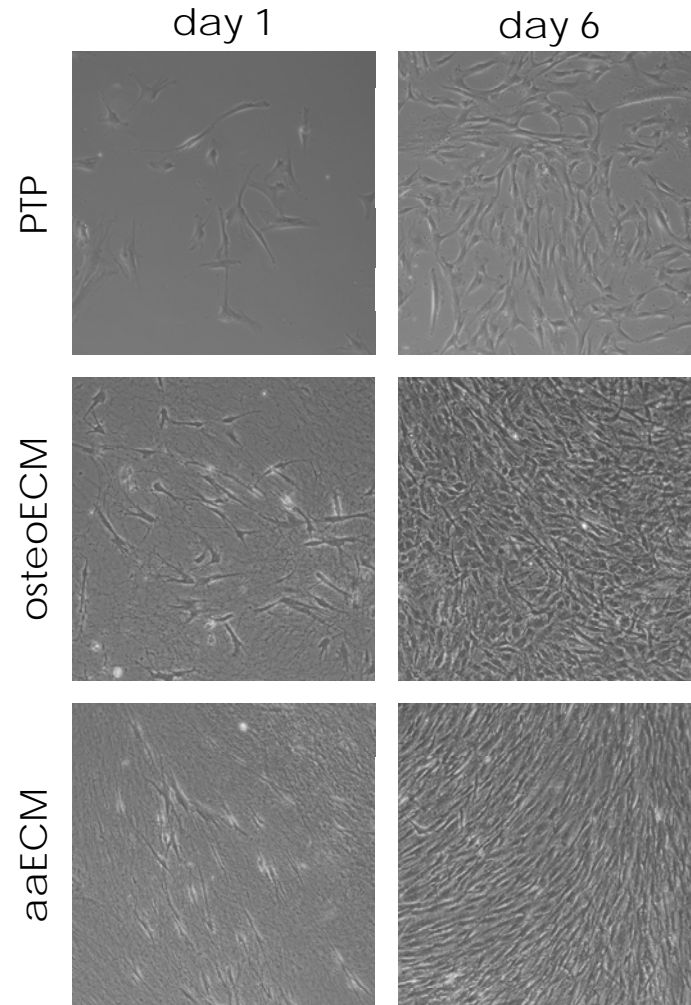
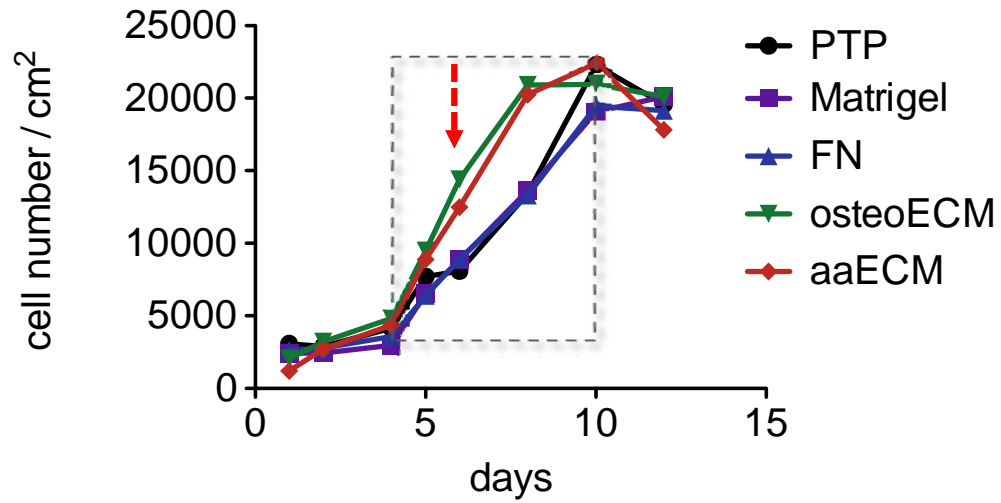
Change in cell morphology



MSC Proliferation and Expansion



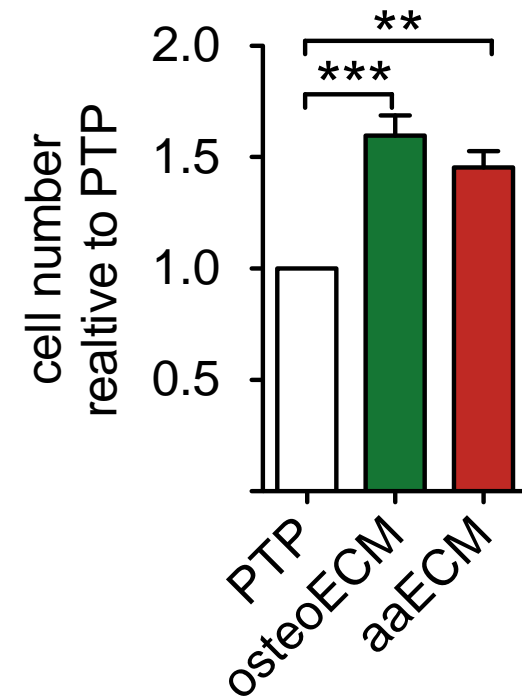
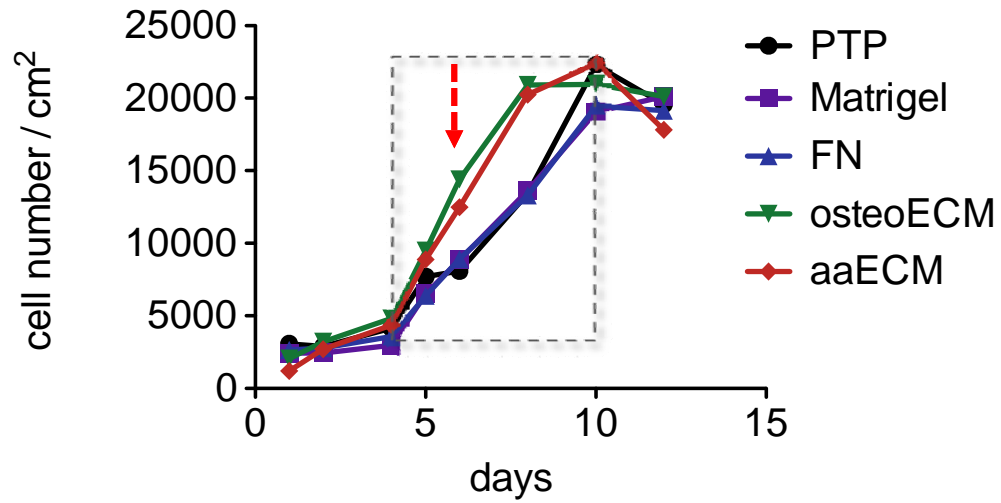
Short term growth curve over one passage.



MSC Proliferation and Expansion



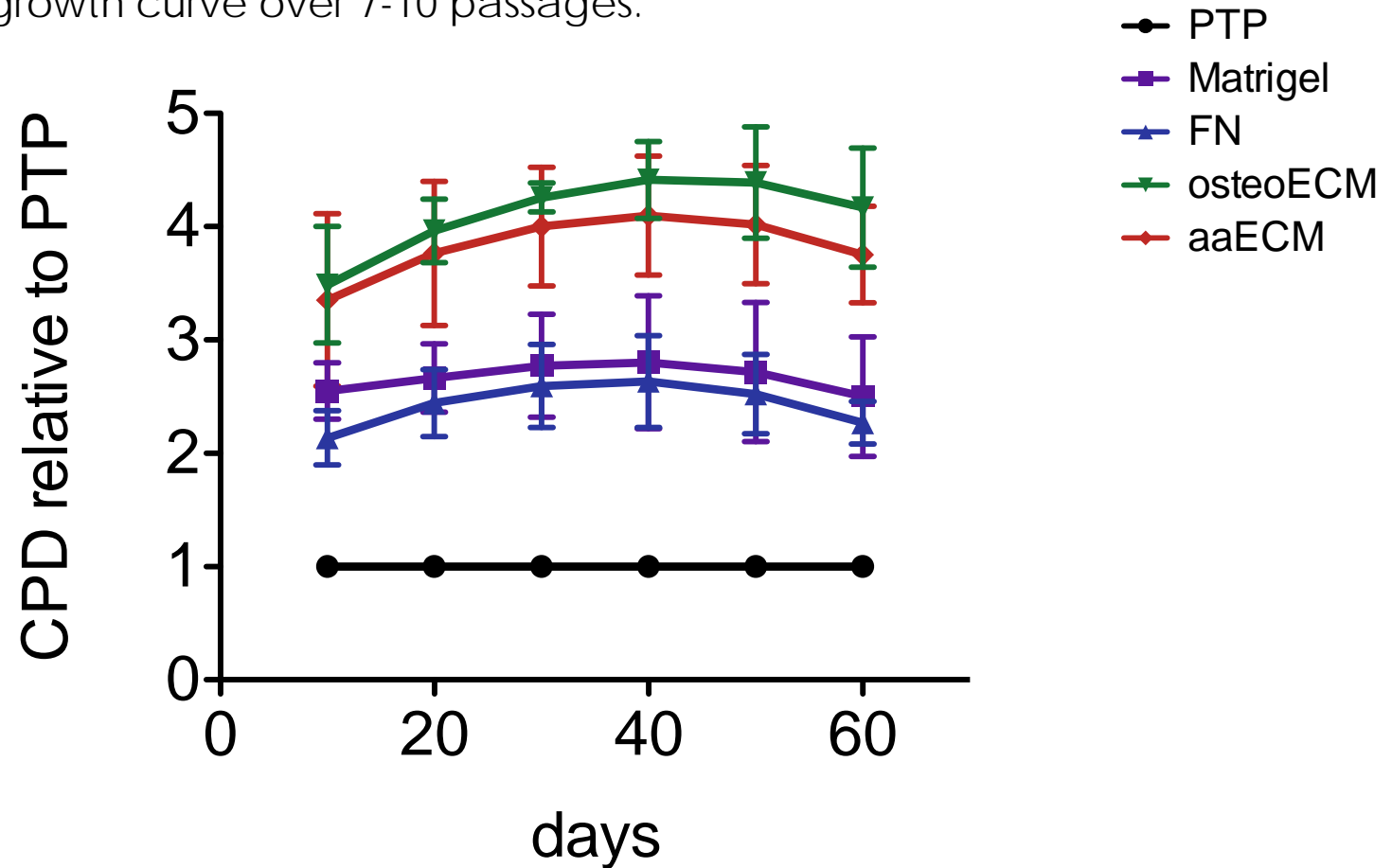
Short term growth curve over one passage.



Cummulative population doubling over long-term culture

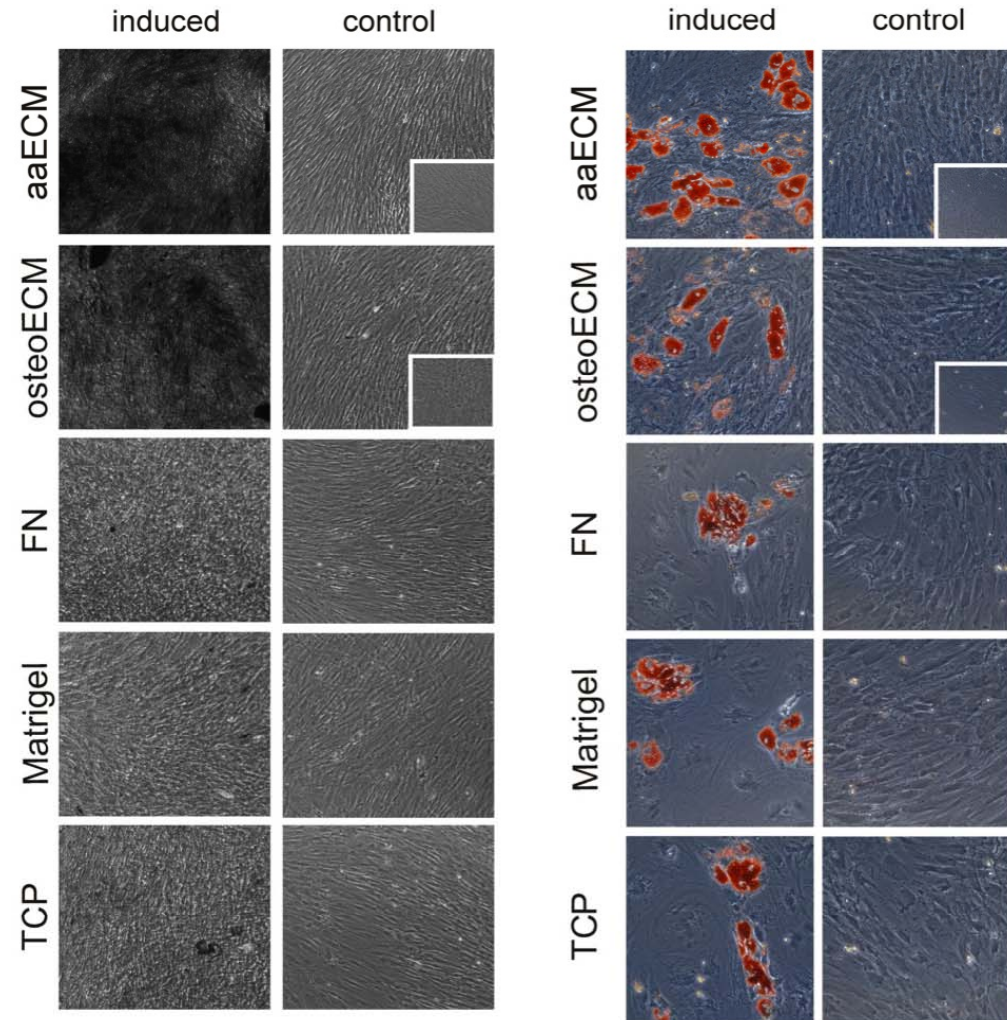
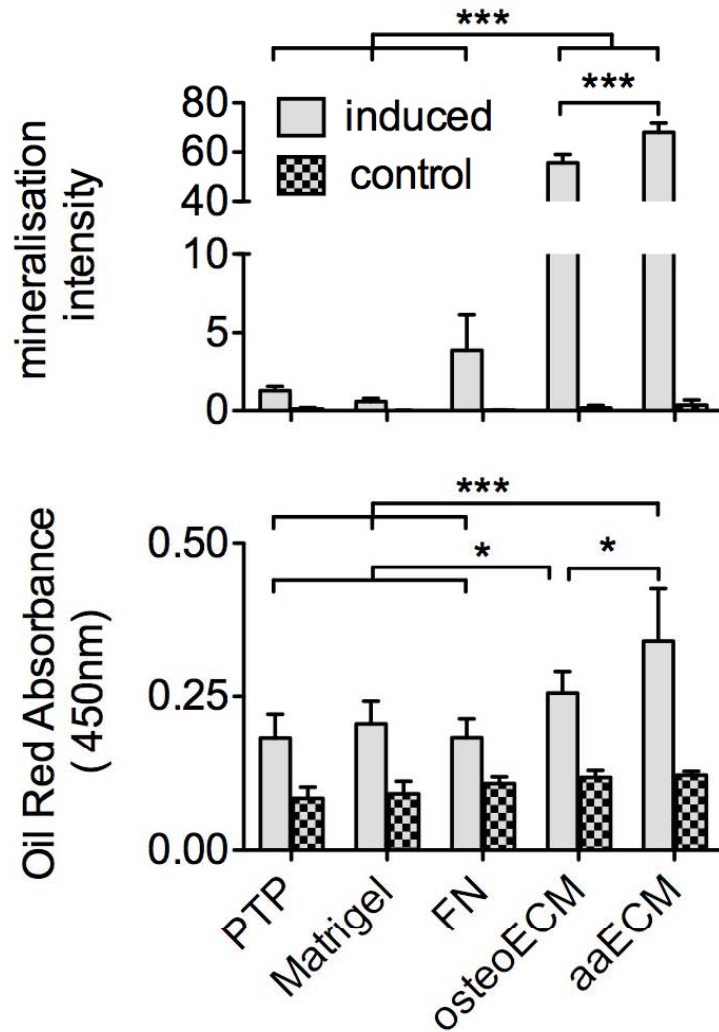


Long term growth curve over 7-10 passages.



4-fold greater expansion of MSCs on ECM substrates.

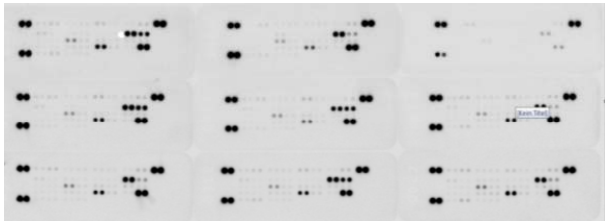
Differentiation potential of MSCs



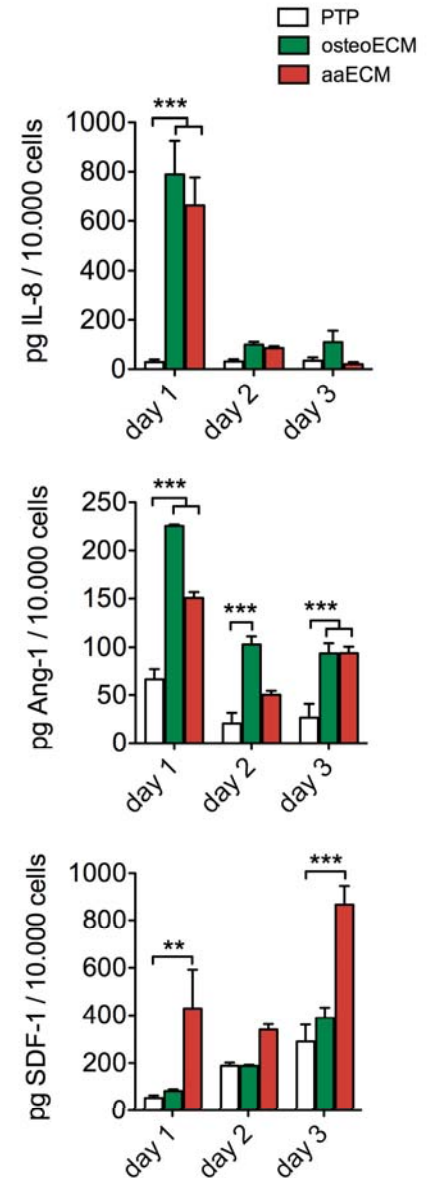
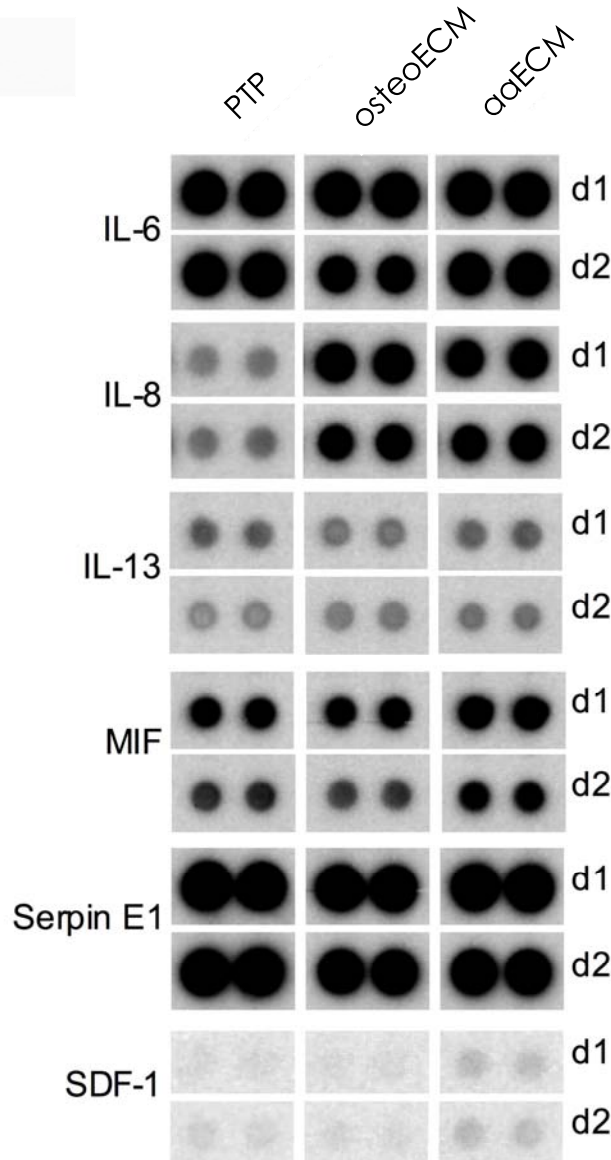
Secretory profile of MSCs



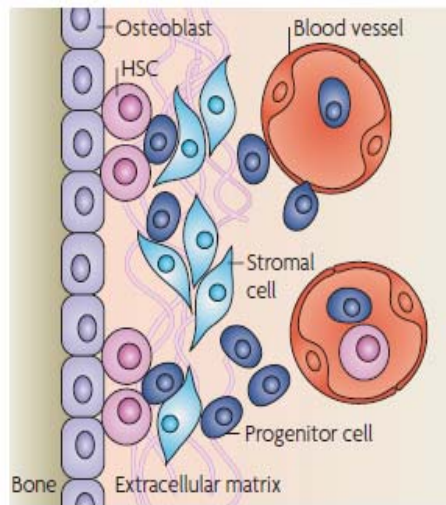
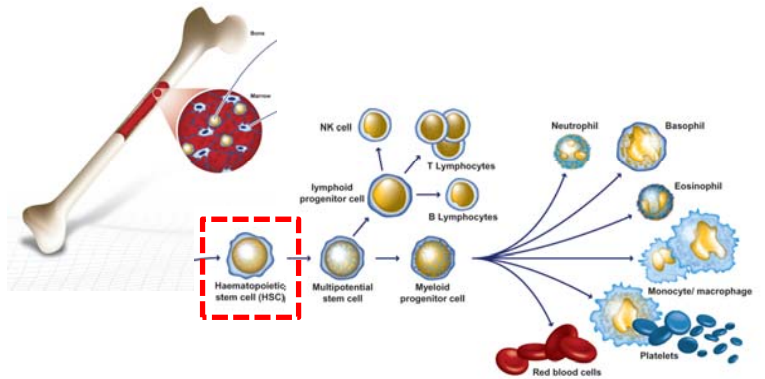
Dot Blot Proteome Profiler
Human Cytokine Array



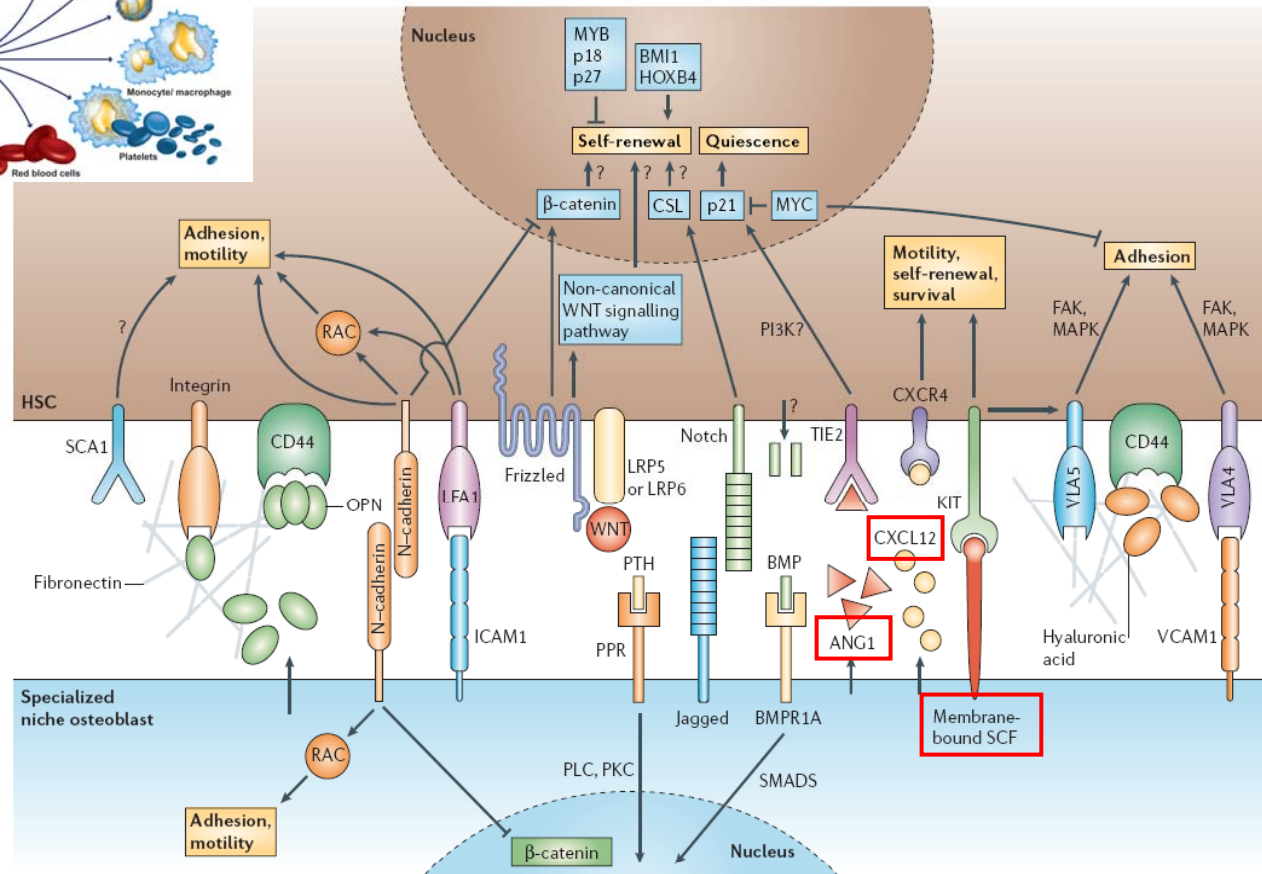
Pooled cell culture supernatants from 3 MSC donors normalized to cell number before incubation with array membrane.



Influence of MSC- ECM on other Bone Marrow Stem Cells

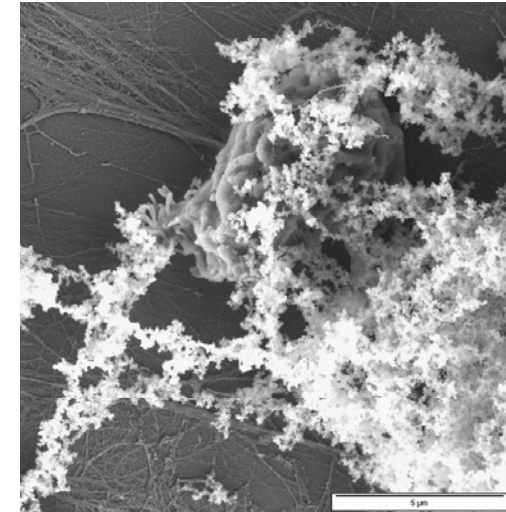
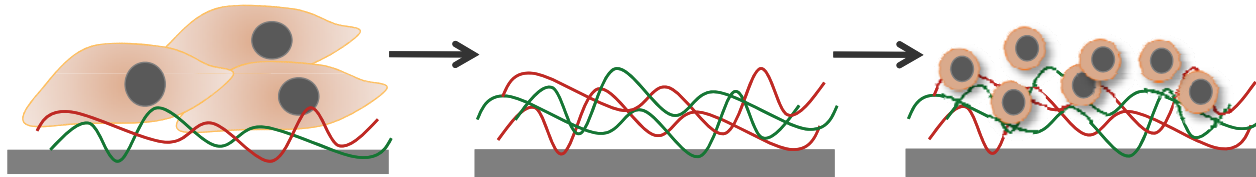


Nature Rev Mol Cell Biol (2008) 9 11



Nature Reviews Immunology, 2006, 6, 93-106

Human CD34+ HPCs culture on ECM substrates



Cytokine wash to prepare blank ECM substrates

CD34+ human Hematopoietic progenitor cells
(from Leukapheresis)

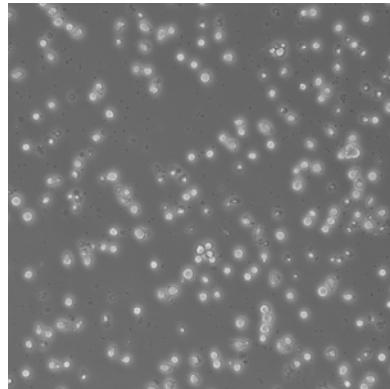
1 week culture under serum-free conditions
Supplemented with 10ng/ml cytokines (SCF, Tpo, Flt-3)

Flow Cytometry Analysis for stem cell marker expression

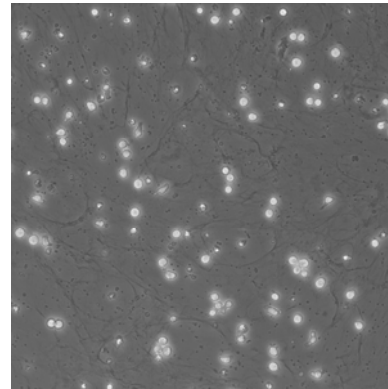
Human CD34⁺ HPCs culture on ECM substrates



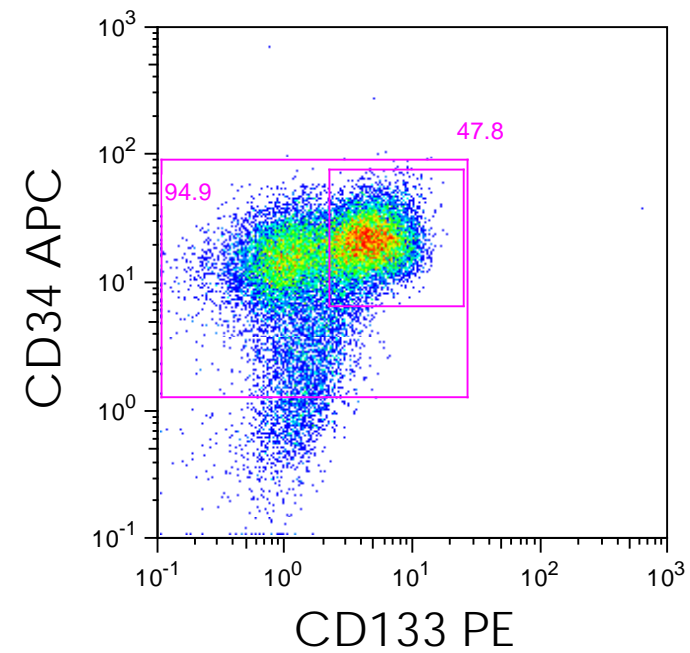
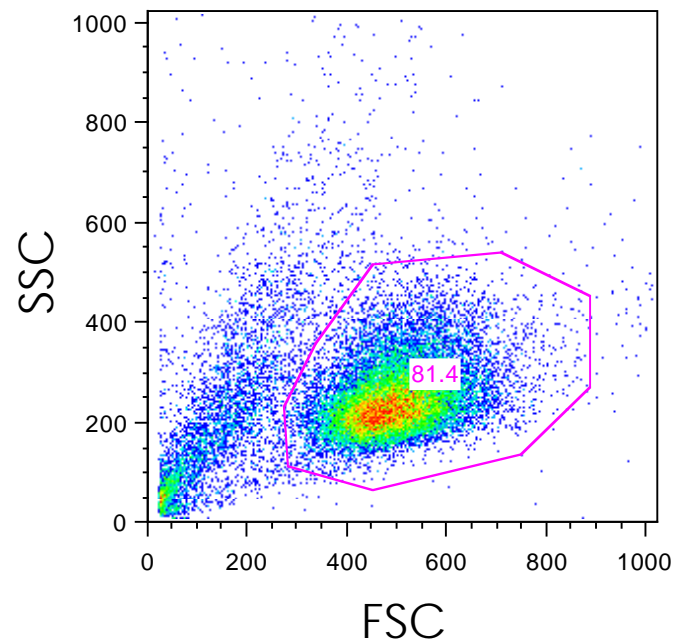
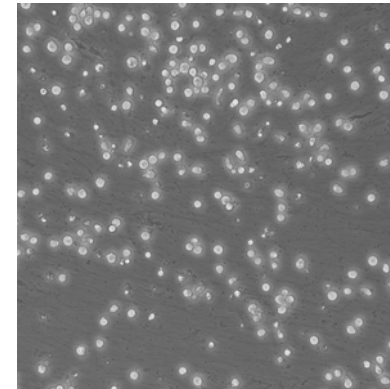
PTP



osteoECM



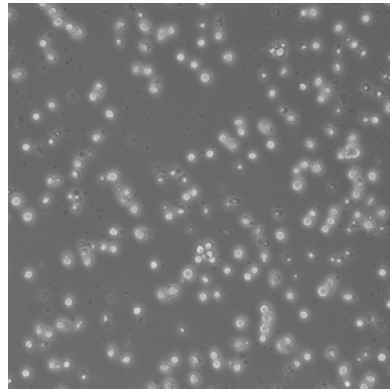
aaECM



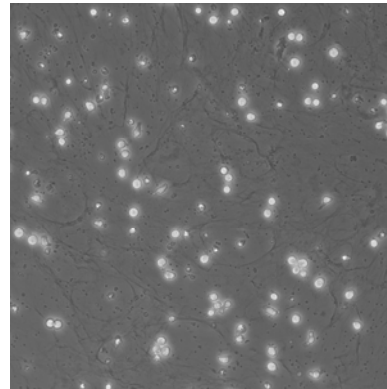
Human CD34+ HPCs culture on ECM substrates



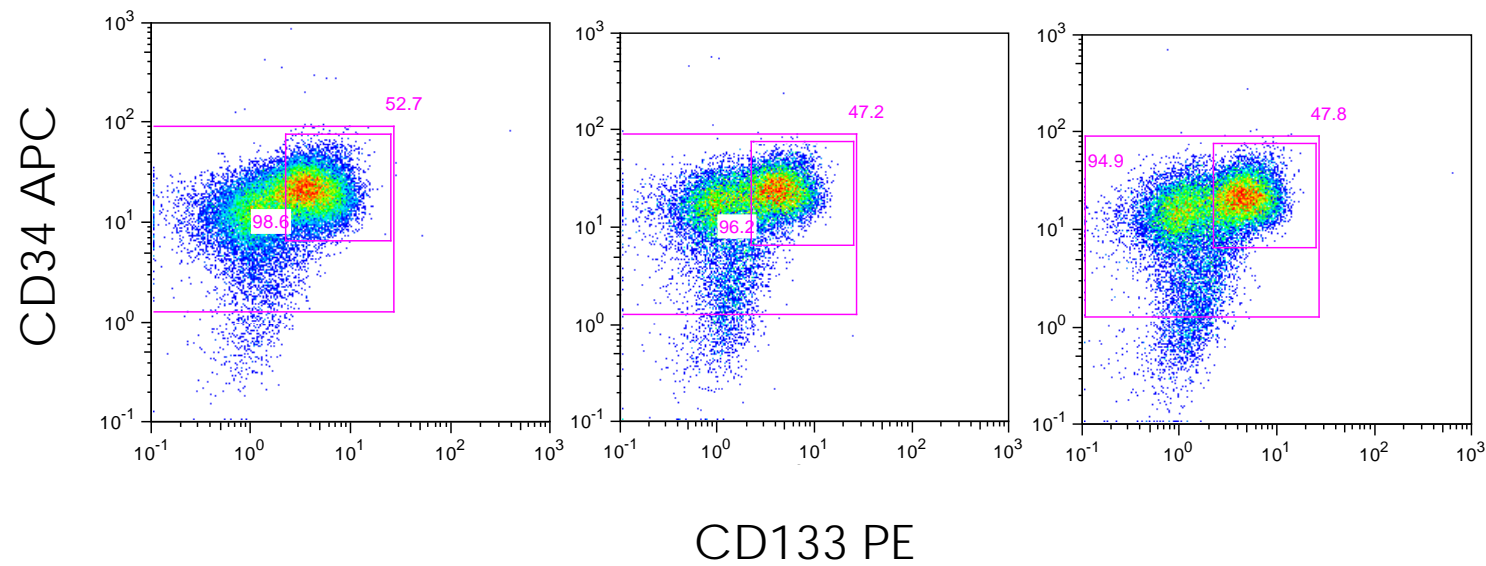
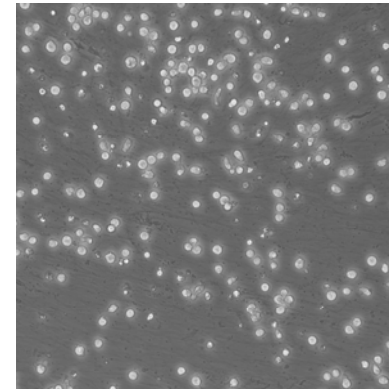
TCP



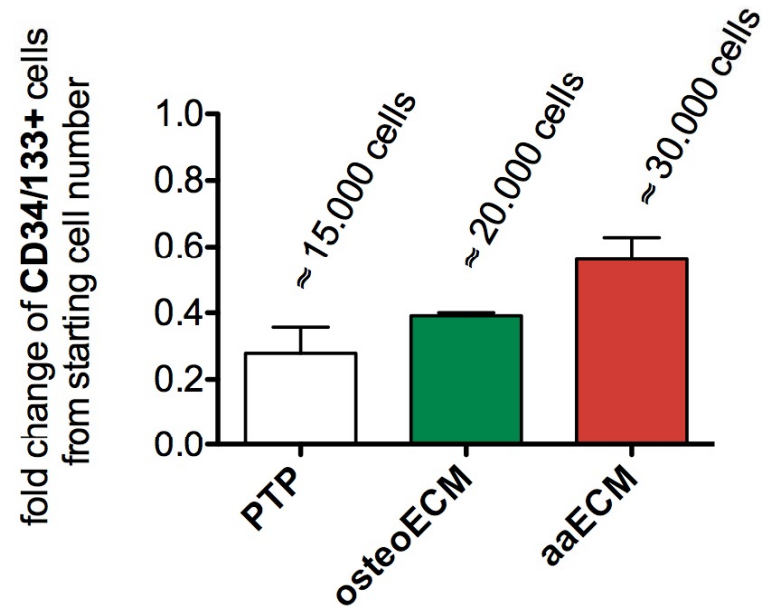
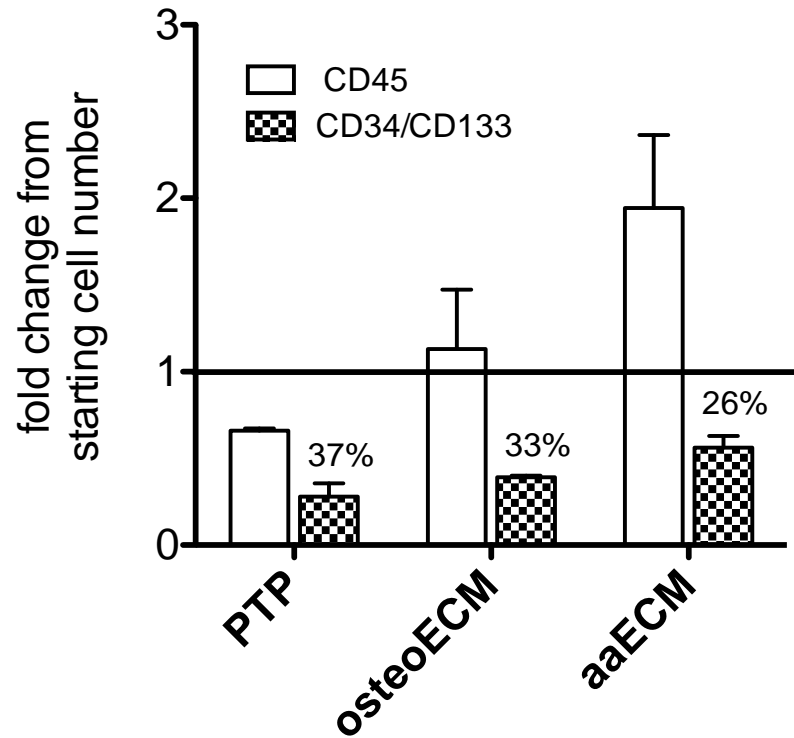
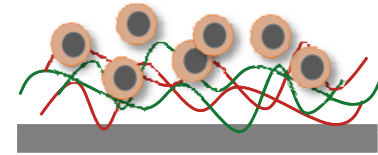
osteoECM



aaECM

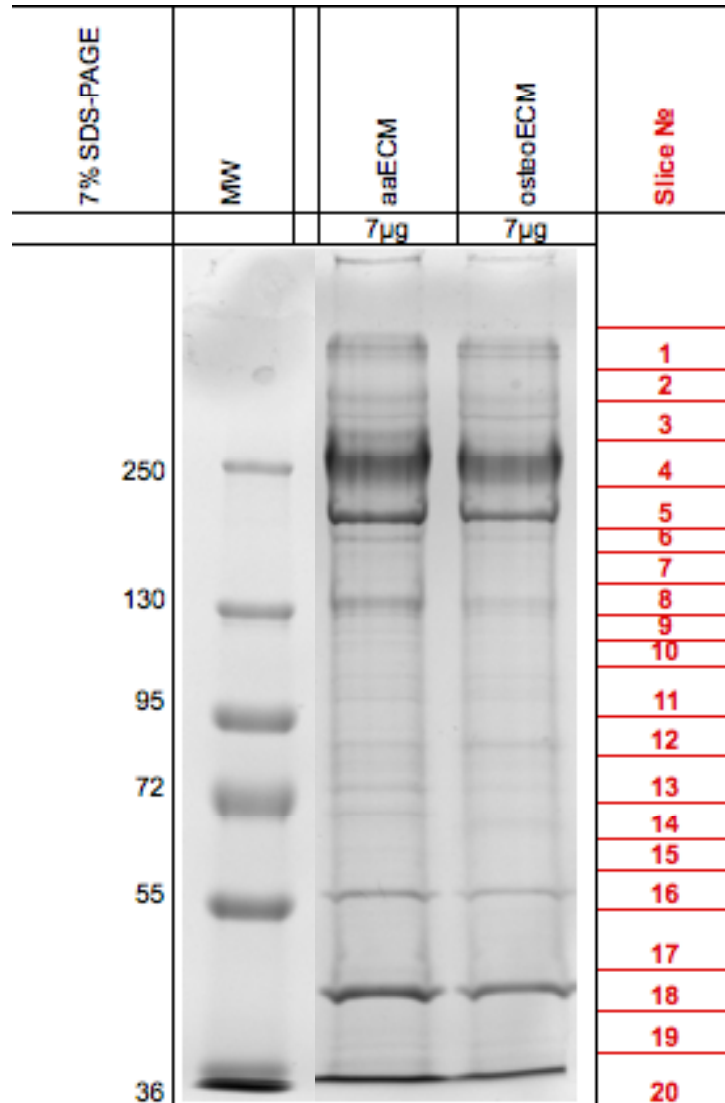


Human CD34+ HPCs culture on ECM substrates



Expansion of CD34+ progenitor cells and maintenance of CD34/CD133 progenitor cells.

Proteomic Analysis of MSC-generated ECM



Protein extraction of ECM after decellularisation

Fragmentation of separated ECM Extracts (20 gel fragments)

MS-Analysis of ECM components

Better understanding of ECM composition allows for artificial design of defined culture conditions.

Summary & Outlook



- Decellularisation of confluent MSC layers creates an extracellular matrix substrate that resemble a fibrillar meshwork of matrix- and glyco-proteins
- Bone Marrow MSCs show up to 4-fold superior expansion on ECM substrates compared to standard tissue culture plastic
- Differentiation of MSCs into the adipogenic or osteogenic lineage to a higher extent on ECM than on PTP substrates
- Secretion of cytokines and growth factors shows significant difference on ECM substrates
- CD34+ Hematopoietic progenitor cells can be expanded on ECM substrates
- *In vivo* functional testing of expanded cells to validate the stem/progenitor potential

Vielen Dank für Ihre Aufmerksamkeit!



Prof. Carsten Werner
Prof. Martin Bornhäuser
Dr. Konstantinos Anastasiadis
Dr. Philipp Seib

