Adipose issue-derived stem cells in tissue engineering and cell therapy

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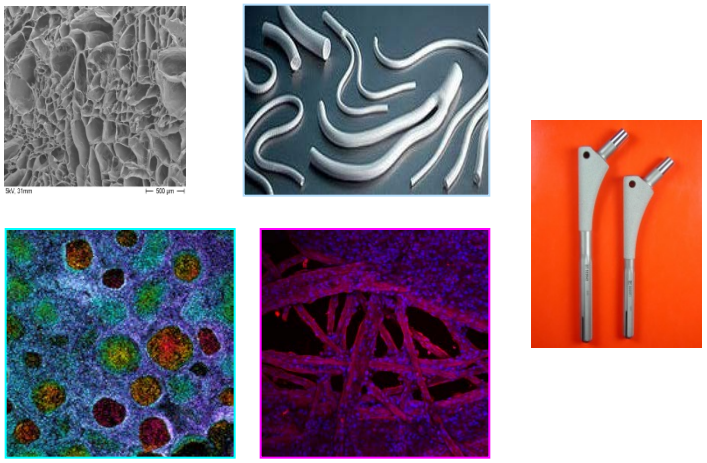
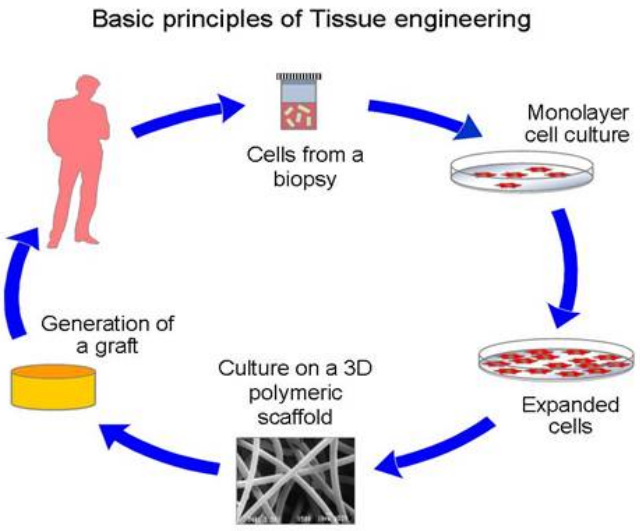
Biomaterials and Tissue Engineering

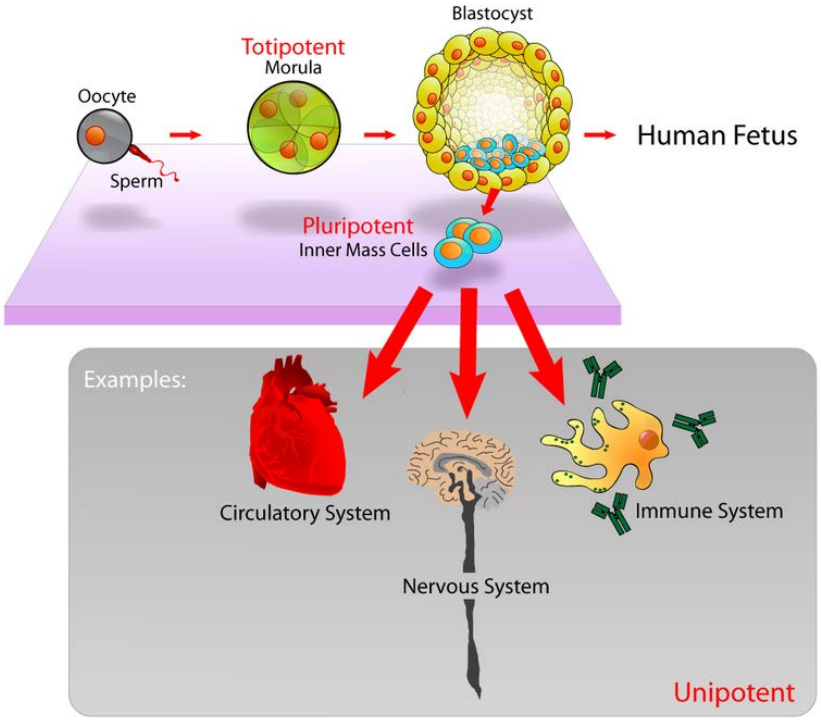
- **A biomaterial** is any matter, surface, or construct that interacts with living systems
  - Nature-derived (ECM proteins, polysaccharides...)
  - Prepared artificially (metallic alloys, synthetic polymers, ceramics, composite materials…)
- Biomaterials are used as cell carriers for **tissue engineering (TE)**
  - an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function (Langer and Vacanti, Science 260: 920-926, 1993)





Definition of Stem Cells

Stem cells are undifferentiated biological cells that can differentiate into specialized cells and can divide (through mitosis) to produce more stem cells.



Mike Jones: The source of pluripotent stems cells from developing embryos. https://commons.wikimedia.org/wiki/File:Stem_cells_diagram.png#/media/File:Stem_cells_diagram.png

Sources of Stem Cells

- **Embryonic**
  - Zygote
  - Morula
  - Blastula
  - Blastocyst
- **Adult organism**
  - Bone marrow
  - Blood (endothelial progenitor cells)
  - Skeletal muscle satellite cells
  - Skin
  - Organs (liver, heart, kidneys…)
  - Urinary stem cells
  - **Adipose tissue-derived stem cells**
- **Extrafetal tissues**
  - Placenta
  - Amniotic fluid
  - Umbilical cord
  - Umbilical cord blood
- **Induced pluripotent stem cells (iPS)**
  - Genetically manipulated and reprogrammed from adult differentiated somatic cells
  - Potential tumorigenicity
  - Immunogenicity

Why Stem Cells?

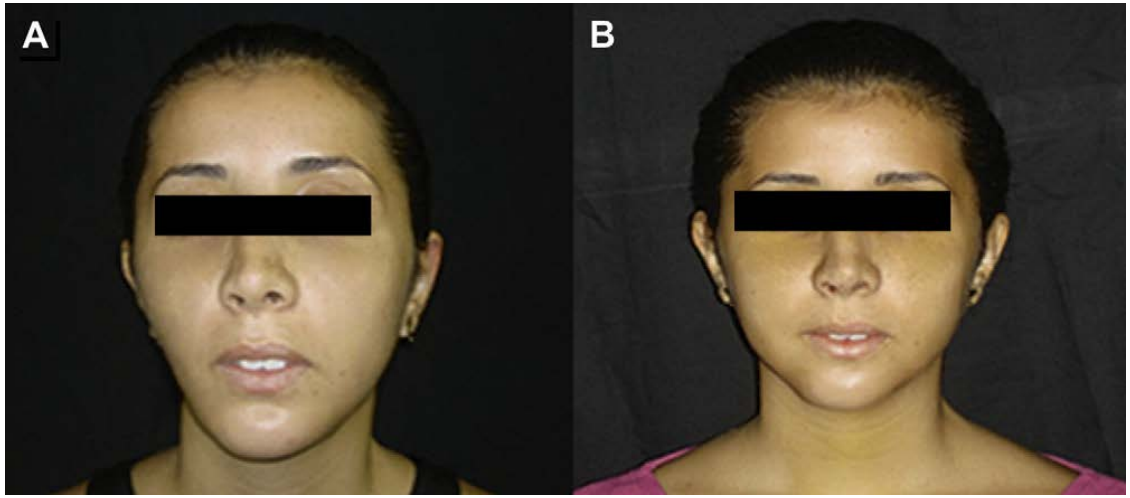
- Non-differentiated cells with a higher proliferation potential than in differentiated cells
- Can be isolated in larger quantities
- Expandable to larger quantities, withstand more passages, slower senescence
- Less immunogenic than differentiated cells
- Immunomodulatory and immunosuppressive function (treatment of inflammatory and autoimmune diseases)
- Have autocrine and paracrine functions
- Can be differentiated into desired cell phenotypes by appropriate culture conditions

Adipose Tissue-Derived Stem Cells (ASCs)

- Seem to be the most advantageous for cell therapies and tissue engineering
- Adipose tissue is abundant in many patients
- Subcutaneous localization, easily accessible
- Can be easily harvested with less discomfort, low donor-site morbidity and high amount compared to bone marrow-derived stem cells
- High amount of ASCs:
  - adipose tissue: is 1 cell per 50 cells
  - bone marrow: 1 cell per 10 000 cells
- Higher proliferation capacity compared to bone marrow stem cells (BMSCs)
- Undergo senescence later than BMSCs
- No calcification when used for cardiovascular tissue engineering

Clinical Applications of ASCs I

- Cell-assisted lipotransfer for tissue augmentation (CAL): autologous ASCs implanted together with an autologous fat graft in order to enhance its survival and to reduce its postoperative atrophy or resorption
  - **cosmetic breast enhancement**
  - **facial contouring** - Parry-Romberg syndrome (progressive atrophy of the right hemiface)



A. Preoperative view of a 19 year old woman with Parry-Romberg Syndrome.
B. Postoperative view of a 19 year old woman, one year after Cell Assisted Lipotransfer.

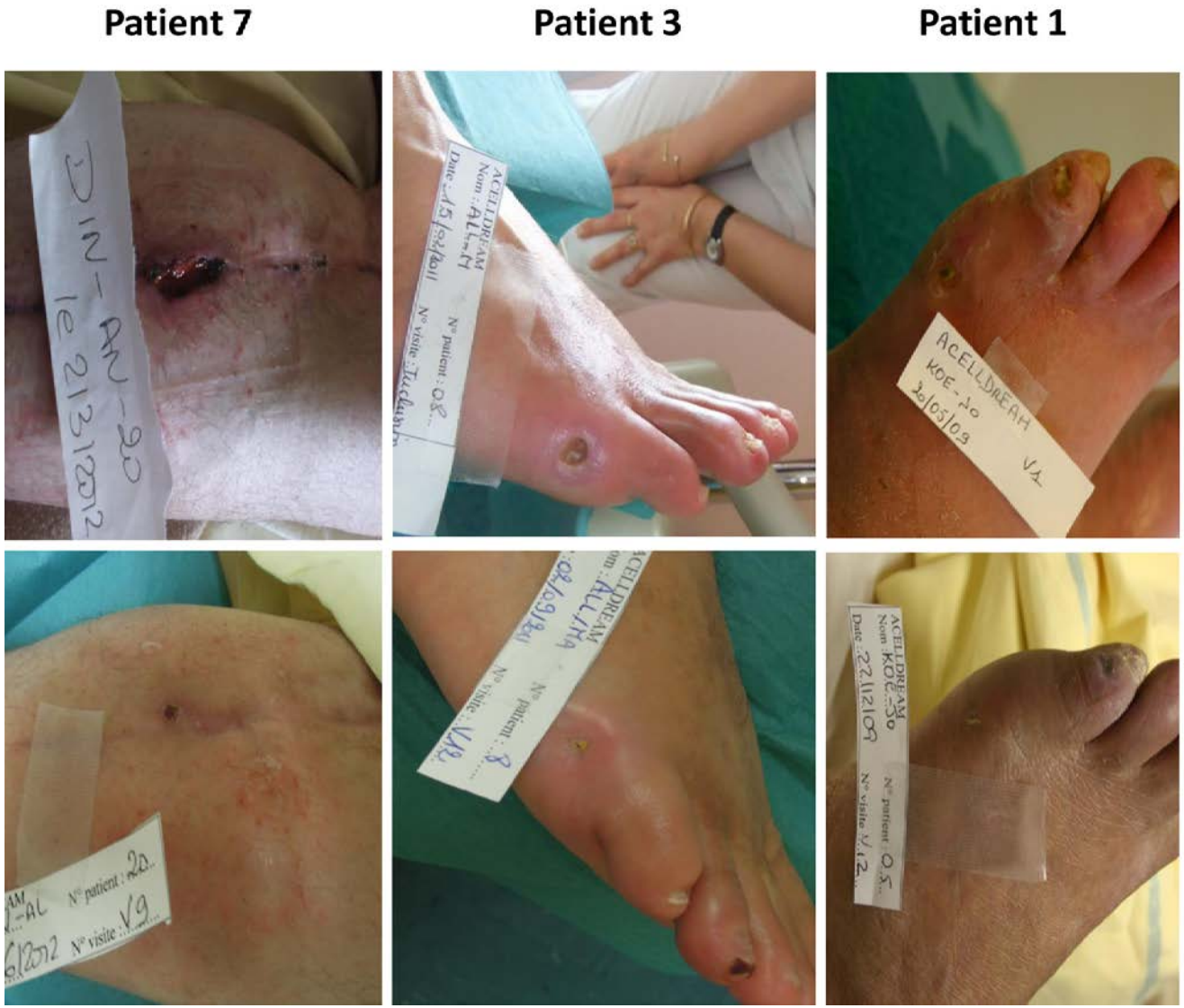
Sterodimas A. *et al.*: Journal of Plastic, Reconstructive & Aesthetic Surgery 63, 1886-1892, 2010

Clinical Applications of ASC II

- Local injection of ASCs
  - healing wound after radiation therapy
  - skin rejuvenation
- Treatment of inflammatory and autoimmune diseases (by intravenous infusion of stem cells):
  - graft-versus-host disease
  - Crohn's disease
  - multiple sclerosis
- Orthopaedic applications
  - **Repair of maxilla**: ASCs + β-tricalcium phosphate and bone morphogenetic protein-2 (Mesimaki K *et al.*: Int J Oral Maxillofac Surg 2009;38:201–9)
  - **Repair of calvaria**: ASCs + fibrin glue in a 7-year-old girl with severe head injury (Lendeckel S *et al.*: J Craniomaxillofac Surg 2004;32:370–3)

Clinical Applications of ASC III

- Critical limb ischemia – „diabetic foot“



- ASCs from abdominal fat were grown for 2 weeks
- More than 200 million cells were obtained
- ASCs (10^8) were then intramuscularly injected into
- the ischemic leg of patients

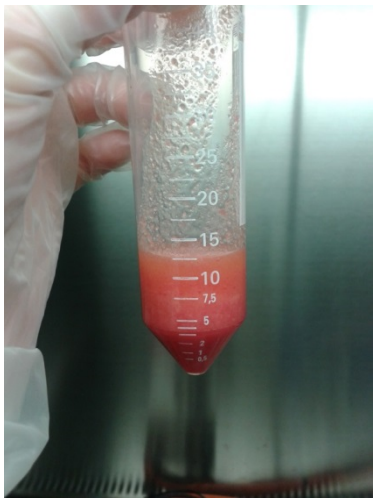
Bura A *et al.*: **Cytotherapy** 16(2): 245-257, 2014.

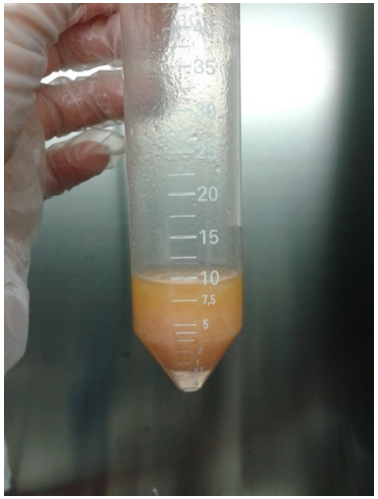
Paracrine Function of Stem Cells

- Able to produce various growth factors:
  - vascular endothelial growth factor (VEGF)
  - basic fibroblast growth factor (bFGF)
  - epidermal growth factor (EGF)
  - keratinocyte growth factor (KGF)
  - platelet-derived growth factor (PDGF)
  - hepatocyte growth factor (HGF)
  - transforming growth factor-beta (TGF-β)
  - insulin-like growth factor (IGF)
  - brain-derived neurotrophic factor (BDNF)
- Antioxidative effects
- Stem cells improve the growth and function of cells in the damaged tissues by paracrine manner

Isolation of Adipose Stem Cells

- Fat tissue obtained by liposuction (Dept. of Plastic Surgery, Bulovka Hospital, Prague), 10 ml of lipoaspirate
- Rinsed repeatedly with phosphate-buffered saline in order to remove blood cells
- Digested by collagenase I (1 hour at 37°C, shaking), centrifuged
- Filtered through Cell Strainer (100 μm pores, BD Falcon, U.S.A.)
- Seeded into polystyrene flasks (25 cm^2, TPP, Switzerland; 0.16 ml of the original lipoaspirate per cm^2)

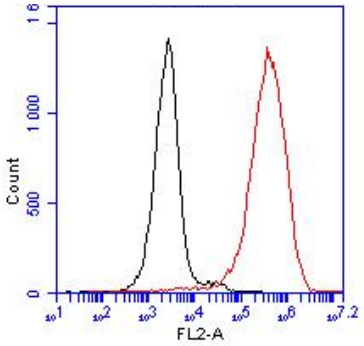




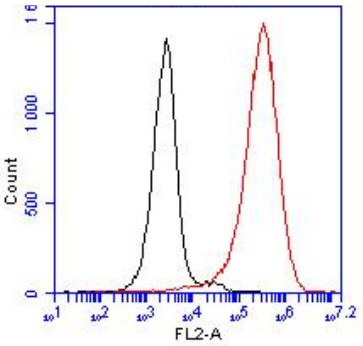
Estes BT *et al.*: Nat Protoc 5: 1294-1311, 2010

Characterization of ASCs by Flow Cytometry

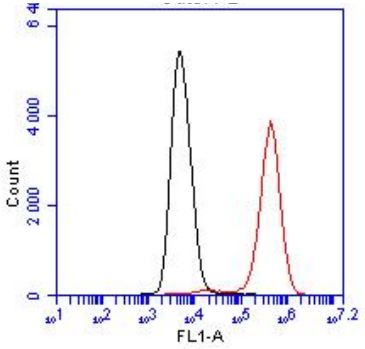
CD 105 (Endoglin, part of TGF beta receptor complex



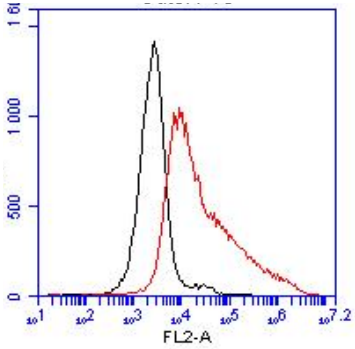
CD 73 (ecto-5'-nucleotidase, converts AMP to adenosine



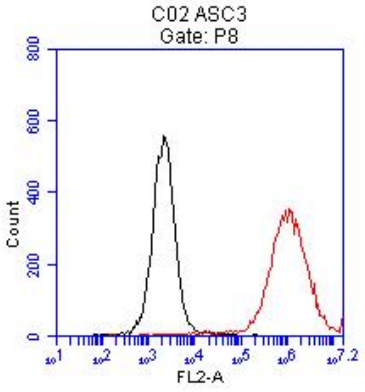
CD 29 (Integrin beta-1, fibronectin receptor)



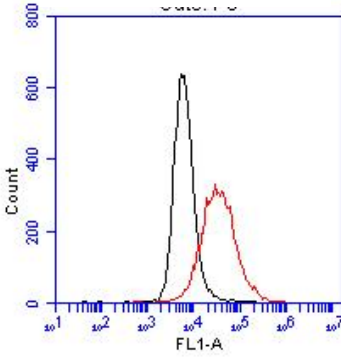
CD 146 (Melanoma cell adhesion molecule, receptor for laminin α 4



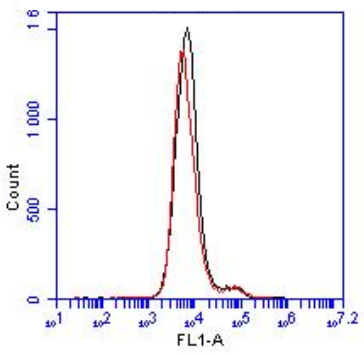
CD 90 (immunoglobuline Thy-1)

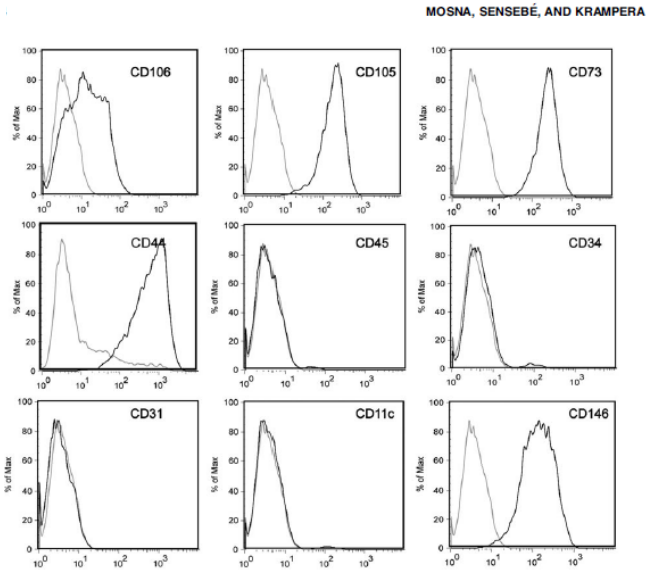


CD 34 (Hematopoietic progenitor cell antigen)



CD 31 (Platelet endothelial cell adhesion molecule, PECAM-1)





Stem Cells Dev. 2010; 19(10):1449-70

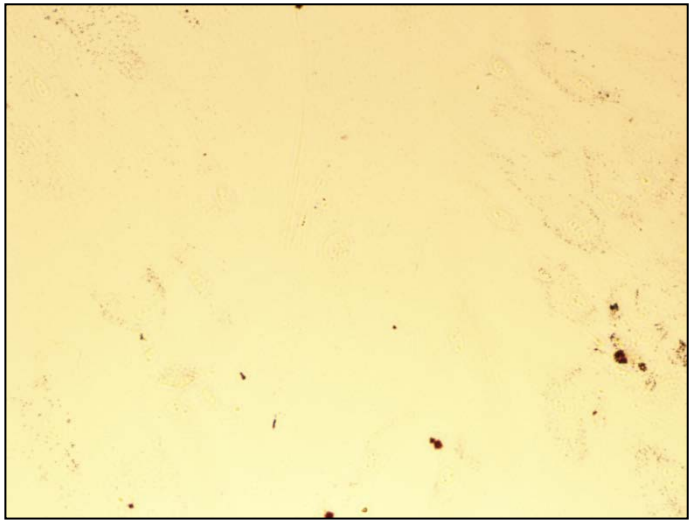
Differentiation of ASCs

- **Relatively easy**:
  - Adipocytes
  - Osteoblasts
  - Vascular smooth muscle cells
- **Difficult**:
  - Endothelial cells
  - Keratinocytes
- **Methods of differentiation**:
  - Composition of cell culture medium
  - Appropriate scaffolds
  - Mechanical stimulation in dynamic cell culture systems
  - Electrical stimulation

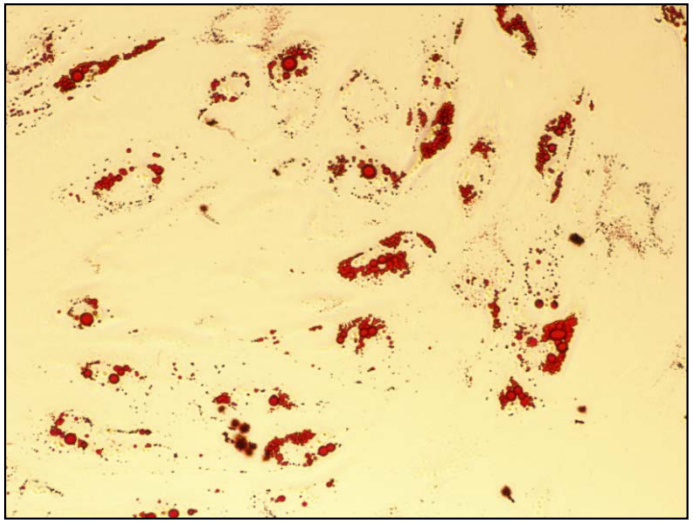
Adipogenic Differentiation

- Cultivation in DMEM medium (with FCS) supplemented with:
  - Dexamethasone (1 mM)
  - 3-isobutyl-1-methylxanthine (0.5 mM)
  - Indomethacin (60 mM)
  - Insulin (10 mg/ml)
  - Hydrocortisone (0.5 mM)
- Oil Red O (lipids stained in red):

Cells in standard medium



Cells in adipogenic medium



Osteogenic Differentiation

- Cultivation in DMEM medium (with 10% of FCS) supplemented with:

  Dexamethasone …….. 10^{-8} M (393 ng/ml)

  β-glycerolphosphate……10 mM (2.16 mg/ml)

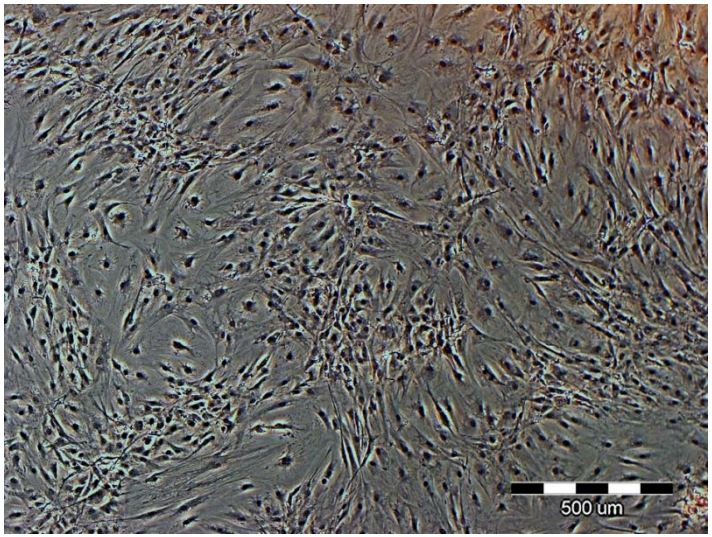
  L-glutamine …… 2 mM (292 μg/ml)

  Ascorbic acid……. …….50 μg/ml

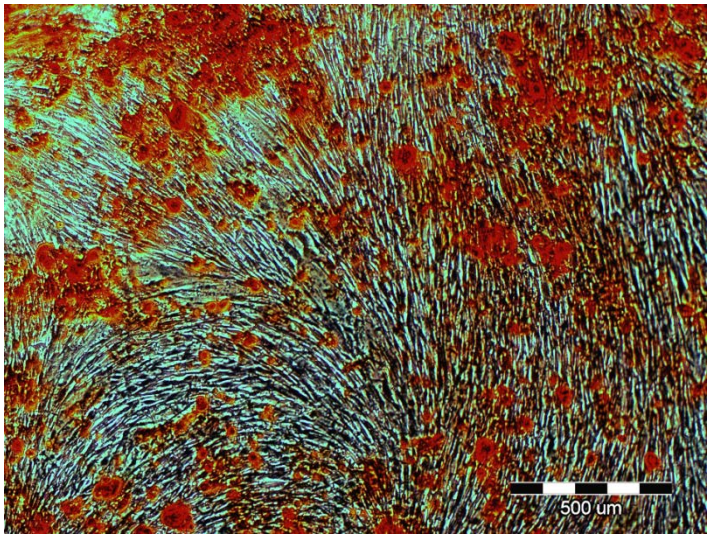
  Dihydroxyvitamin D_3…….. ..10^{-6} M (385 ng/ml)

- Alizarin Red (mineral deposits stained in red):

Cells in standard medium



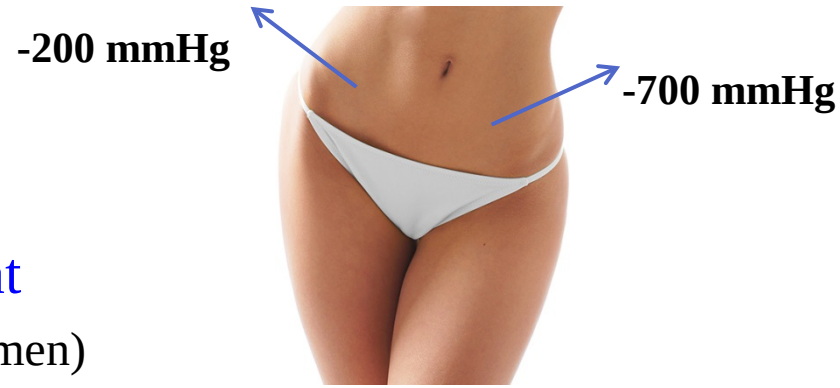
Cells in osteogenic medium



Influence of Liposuction Parameters on the Quantity and Quality of ASCs

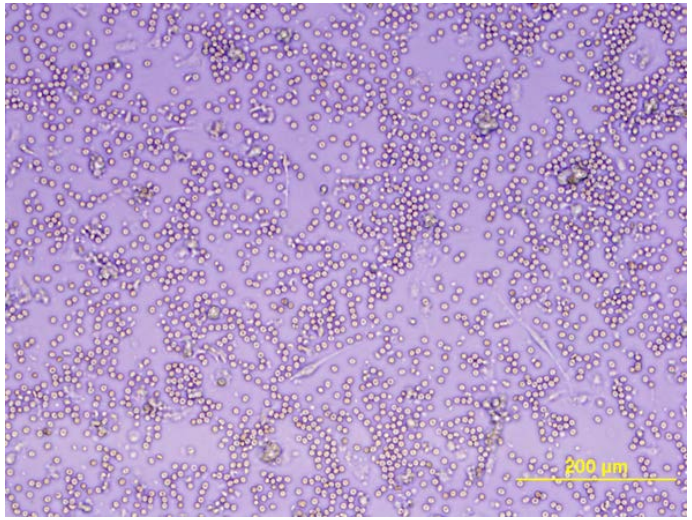
- Manual liposuction or vacuum machine
- Anaesthesia (general, local)
- Composition of tumescent solution:
  - Isotonic NaCl
  - Lidocain (2%): anaesthesia, emulsification of fat tissue
  - Adrenalin (1:200000): in order to stop bleeding
- Negative pressure:
  - 200 mm Hg
  - 700 mm Hg
  - Taken from the same patient

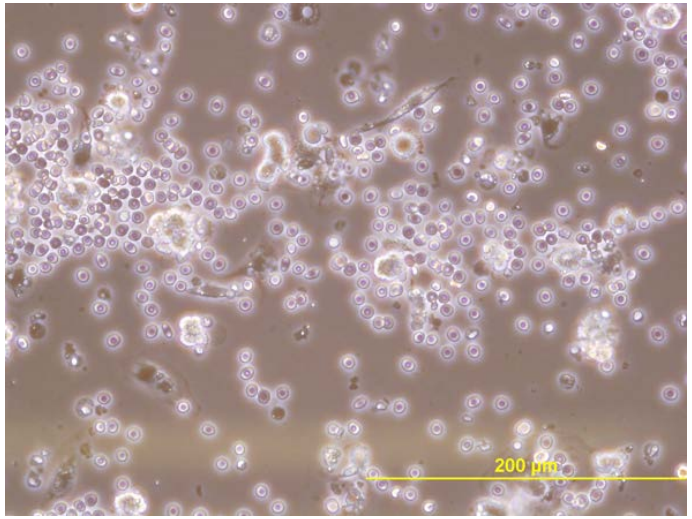
    (left and right parts of the abdomen)



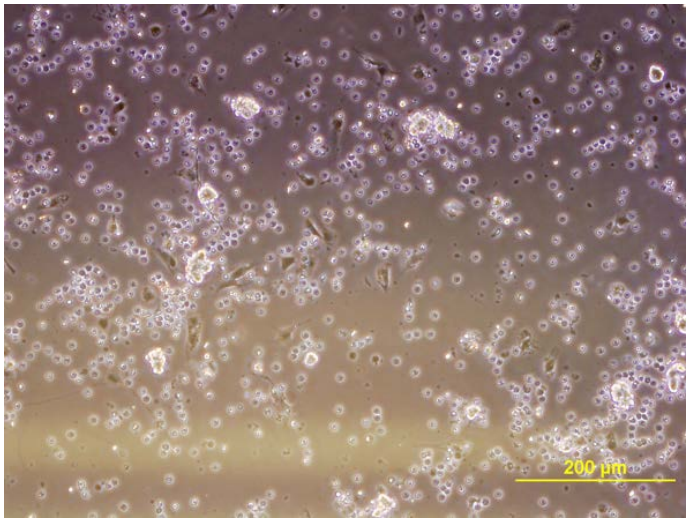
Patient 1: Day 1 after isolation – before medium change

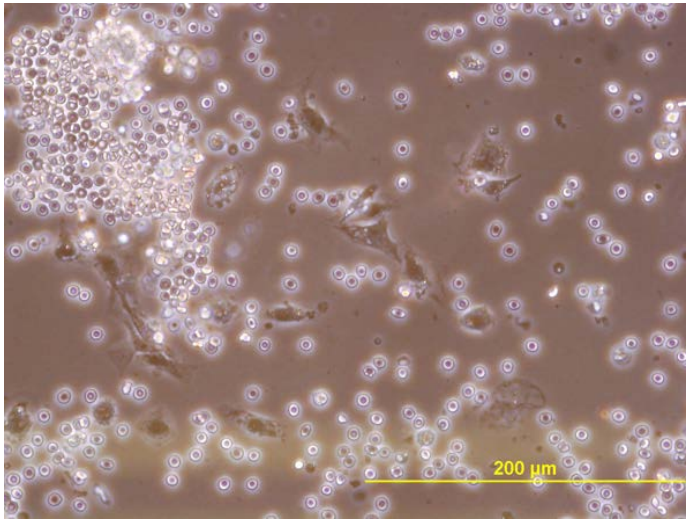
Low negative pressure (-200 mmHg)





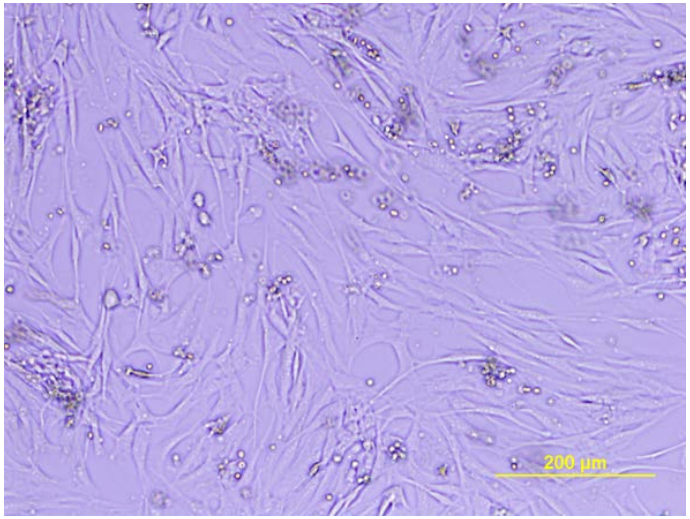
High negative pressure (-700 mmHg)

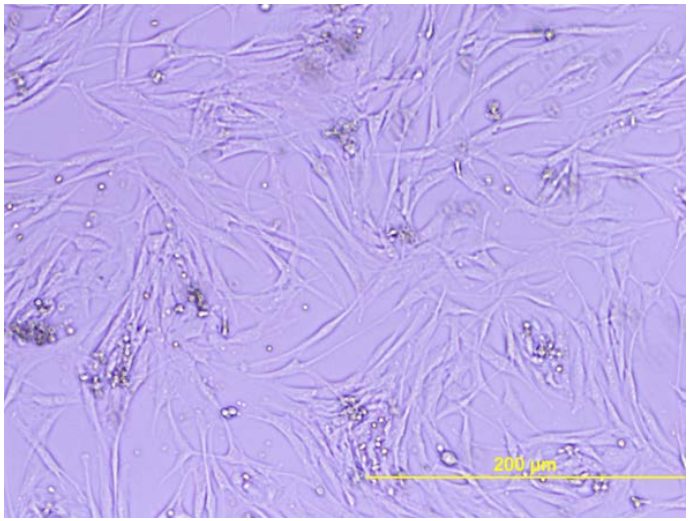




Patient 1: Primoculture, day 5 after isolation

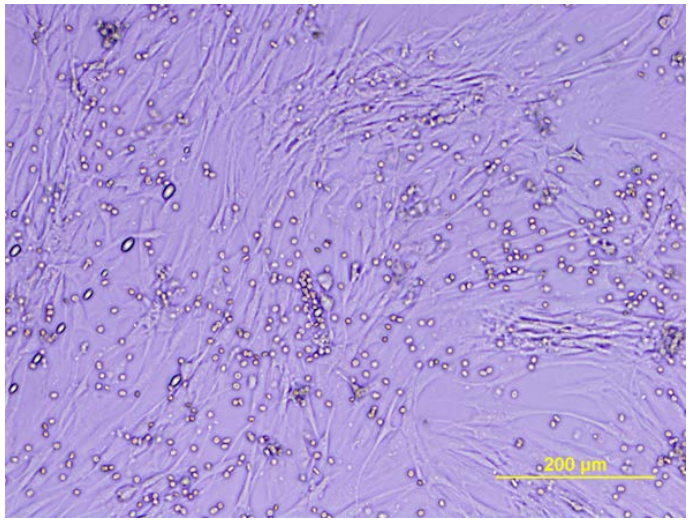
Low negative pressure (-200 mmHg)

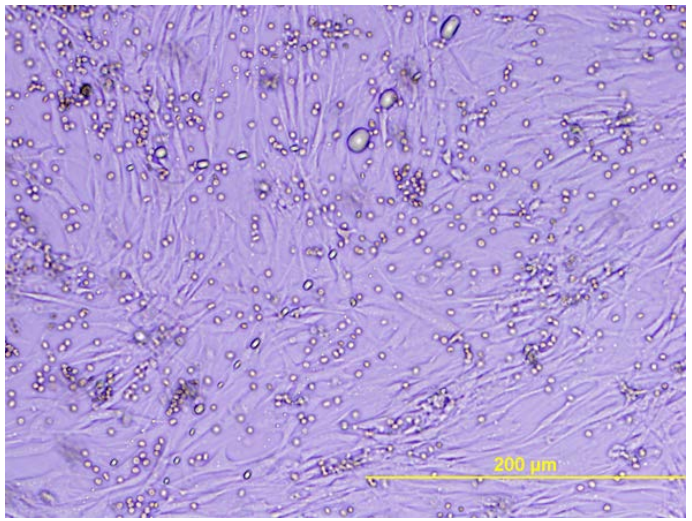




39 800 ± 3 600 cells/cm^2

High negative pressure (-700 mmHg)

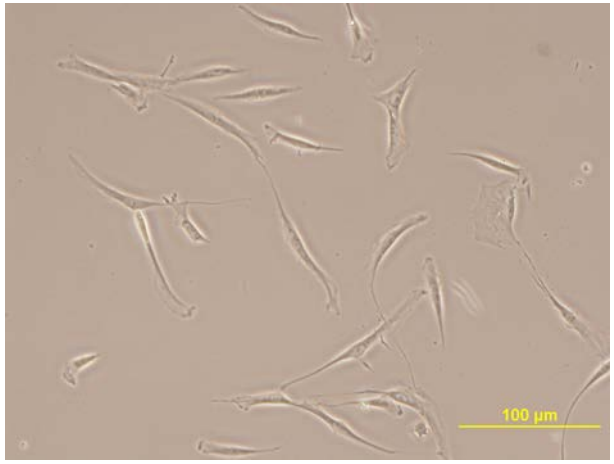


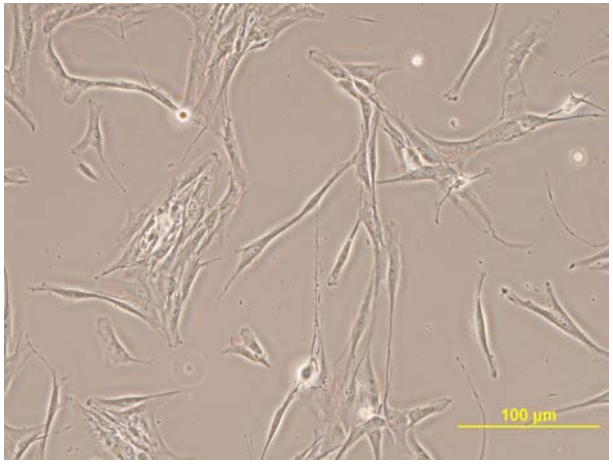


62 900 ± 3 100 cells/cm^2

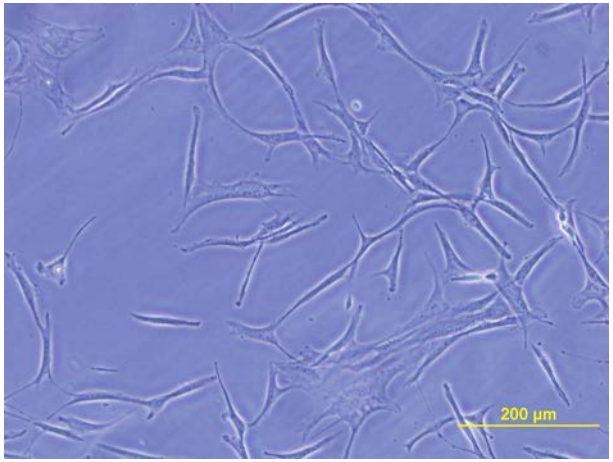
Patient 1: Passage 1 (4 000 cells/cm^2)

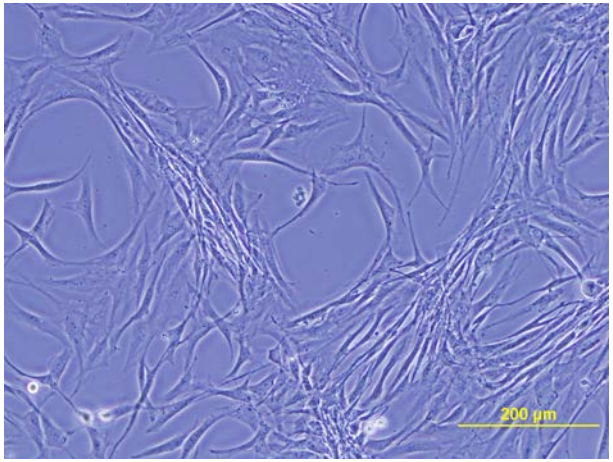
Low negative pressure (-200 mmHg) High negative pressure (-700 mmHg)





Day 2



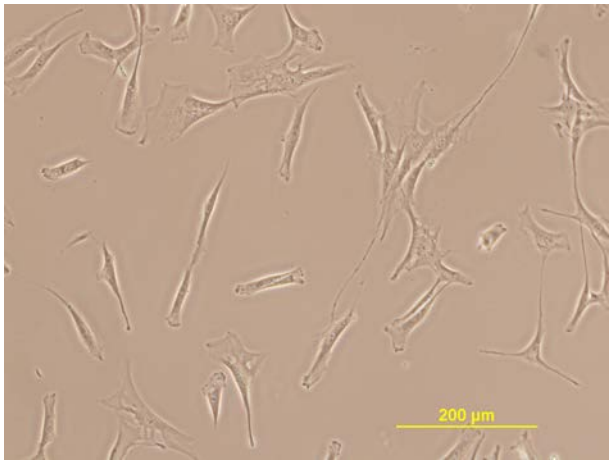


Day 4

18 500 ± 600 cells/cm^2 27 900 ± 2 600 cells/cm^2

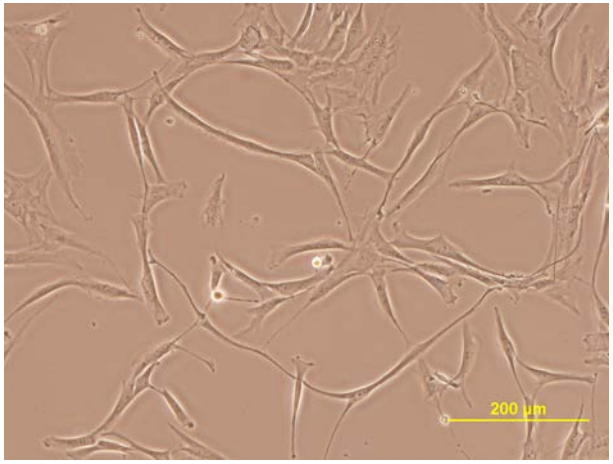
Patient 1: Passage 2 (4 000 cells/cm^2)

Low negative pressure (-200 mmHg)



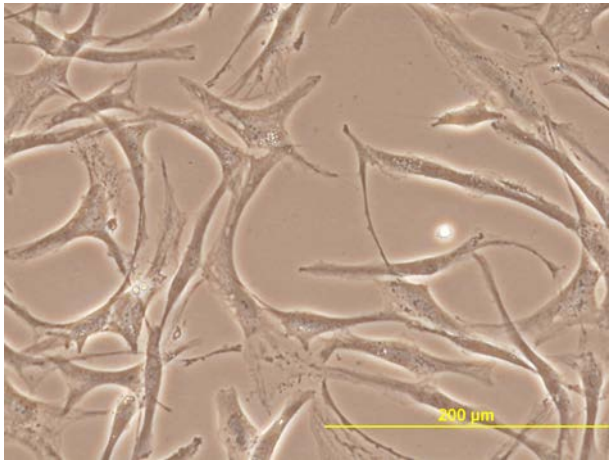
8 300 ± 400 cells/cm^2

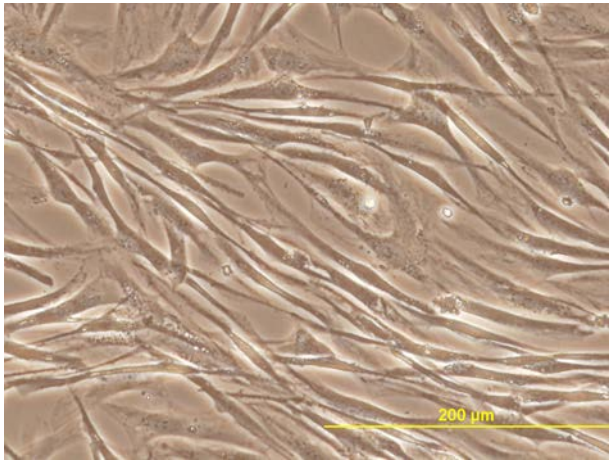
High negative pressure (-700 mmHg)



13 000 ± 600 cells/cm^2

Day 3



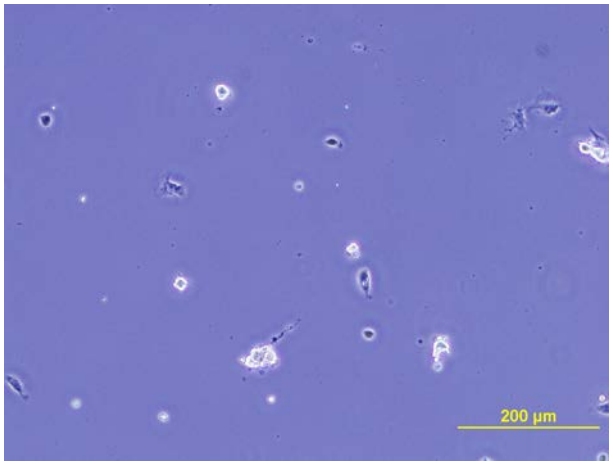


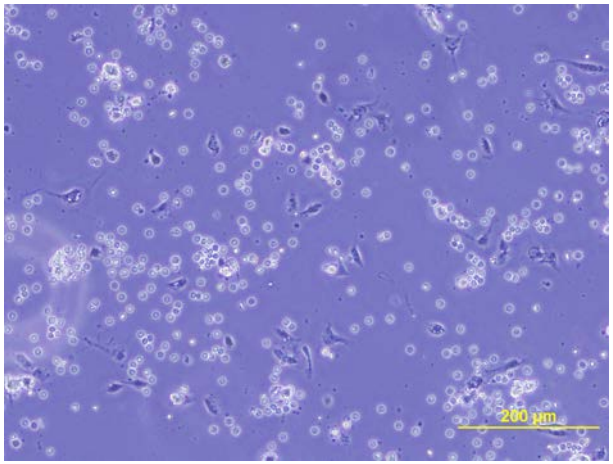
Day 7

Patient 2 - Primoculture

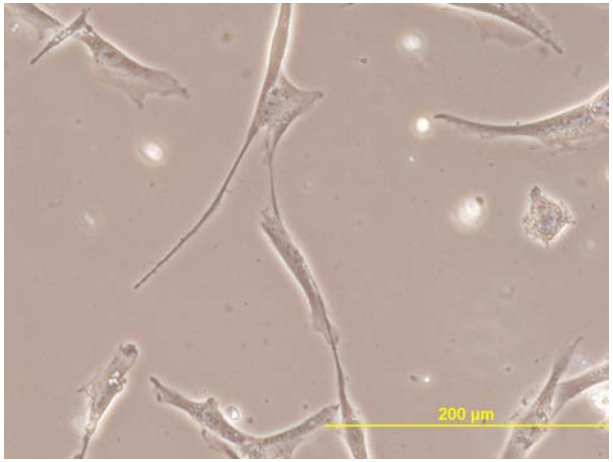
Low negative pressure (-200 mmHg)

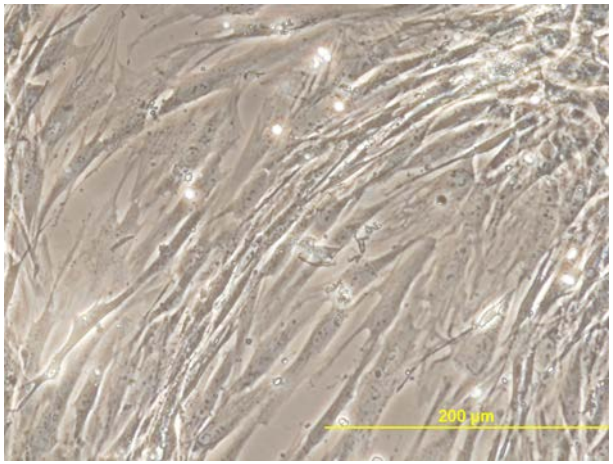
High negative pressure (-700 mmHg)





Primoculture day 1





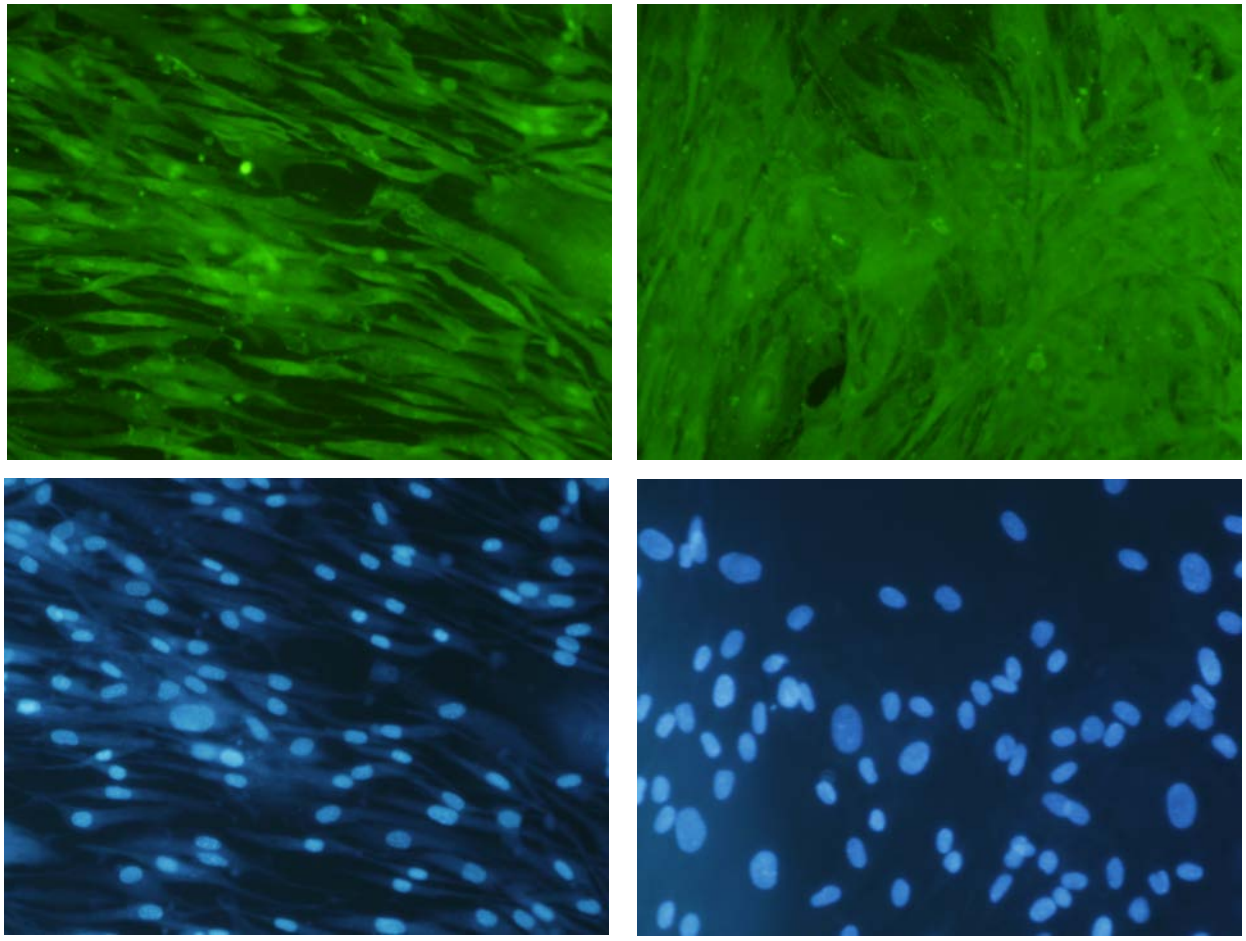
Primoculture day 6

Osteogenic Cell Differentiation

Measured by the intenzity of fluorescence after immunofluorescence staining of collagen I, alkaline phosphatase and osteocalcin

Pat. 1, pas. 2, low pressure, after freezing, day 5

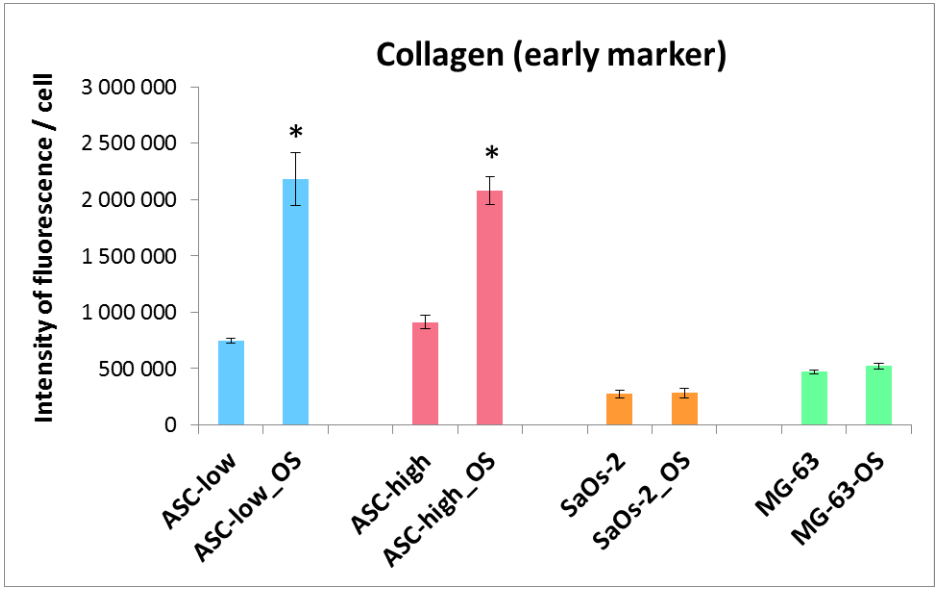
Standard medium **Osteogenic medium**

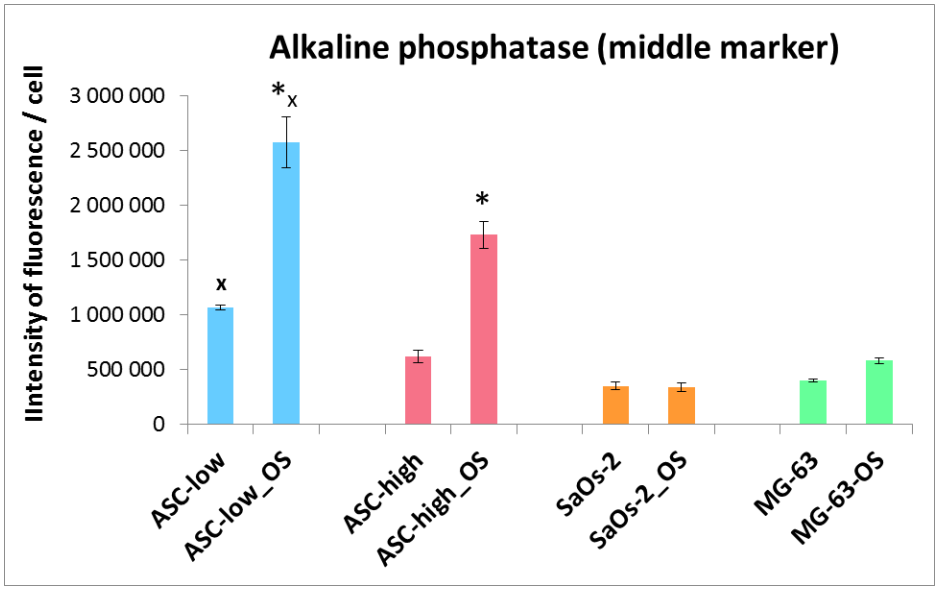


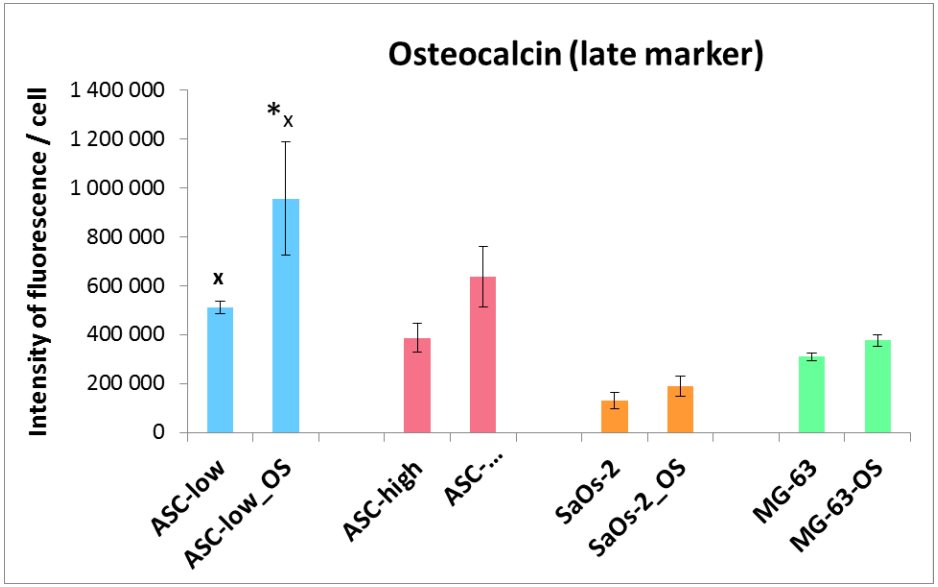
- Fluorescent Image Analyser software (version 1.0)
- The fluorescence intensity was normalized per cell
- The fluorescence intensity of control samples stained without primary antibodies was subtracted

Matejka, R., ALICE: Fluorescent Image Analyser (ver 1.0); http://alice.fbmi.cvut.cz

Osteogenic Differentiation (pat. 1, pas. 2, after freezing, d. 5)



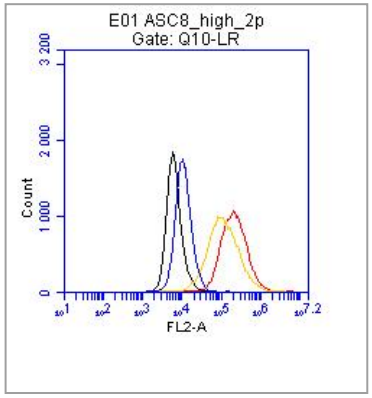




Flow-cytometric characterization of ASCs

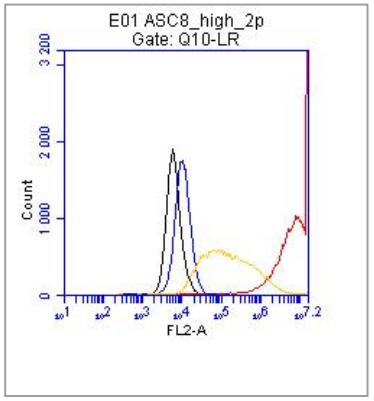
black: ASC-H – control/ **blue:** ASC-L – control/ **red:** ASC-H - stained/ **yellow:** ASC-L -stained

CD105 (endoglin, part of TGF-β receptor complex)



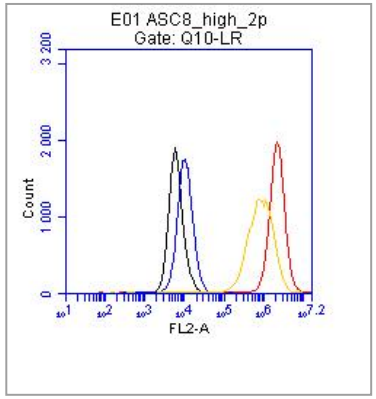
ASC-H: 88 %
ASC-L: 70 %

CD90 (immunoglobuline Thy-1)



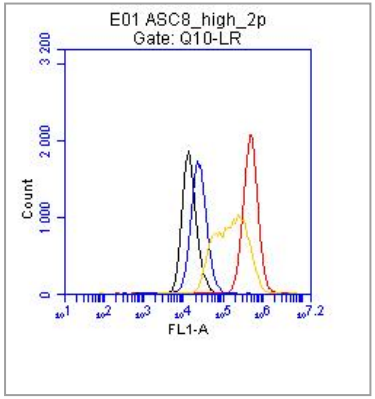
ASC-H: 100 %
ASC-L: 90 %

CD73 (ecto-5'-nucleotidase)



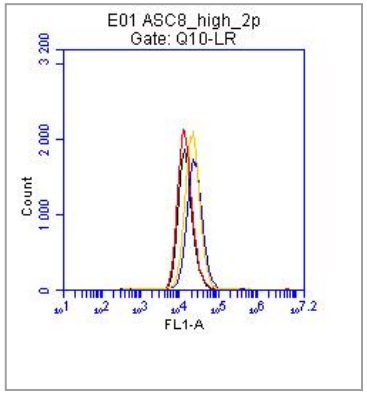
ASC-H: 100 %
ASC-L: 99 %

CD29 (integrin β_1), fibronectin receptor



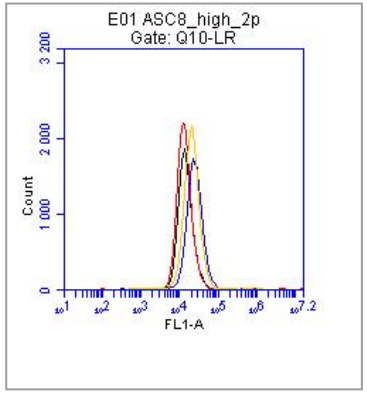
ASC-H: 100 %
ASC-L: 60 %

CD34 (hematopoietic progenitor cell antigen)



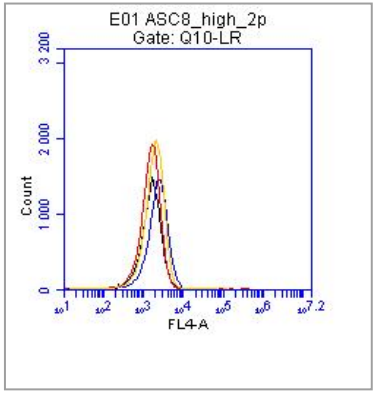
Not detected

CD31 (platelet-endothelial cell adhesion molecule, PECAM-1)



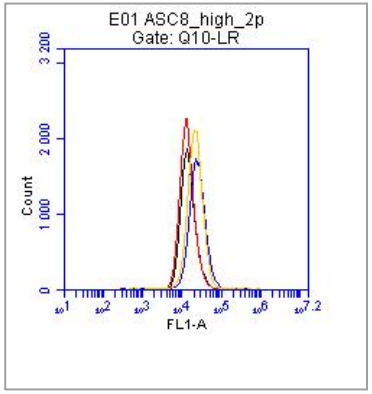
Not detected

CD45 (member of the protein tyrosine phosphatase family



Not detected

CD146 (melanoma cell adhesion molecule, receptor for laminin α_4)



Not detected

Conclusion

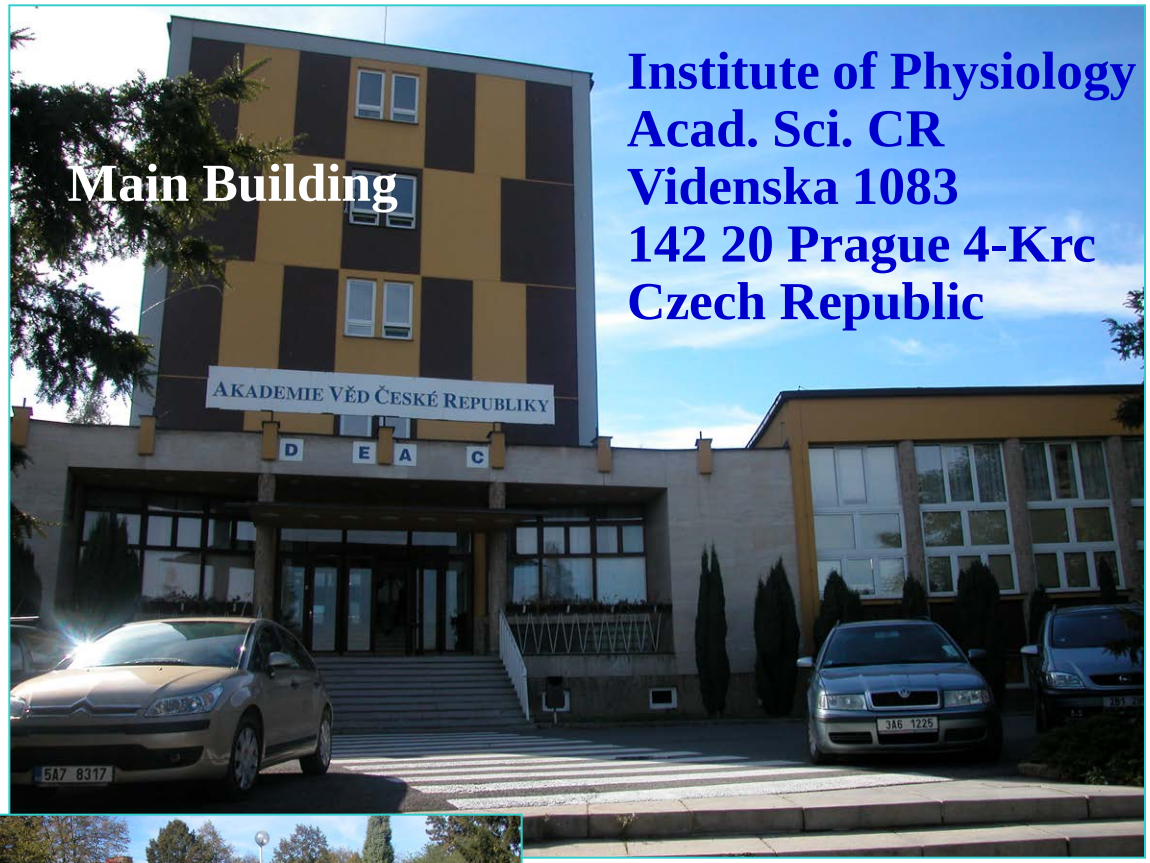
Parameter	Lower pressure (-200 mm Hg)	Higher pressure (-700 mm Hg)
Amount of adhering ASCs after isolation	↓	↑
Growth of ASCs (measured by cell number)	↓	↑
Osteogenic differentiation of ASCs (AF, Ocn)	↑	↓

These differences could be, at least partly, explained by phenotypic differences between the two ASC populations revealed by flow cytometry

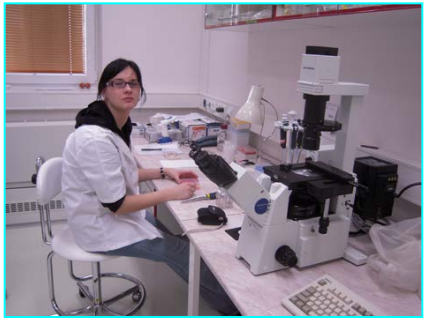
Supported by the grant „ Application of adipose tissue-derived stem cells obtained by liposuction in tissue engineering“, No. 15-33018A, Ministry of Health of the CR

Team members:

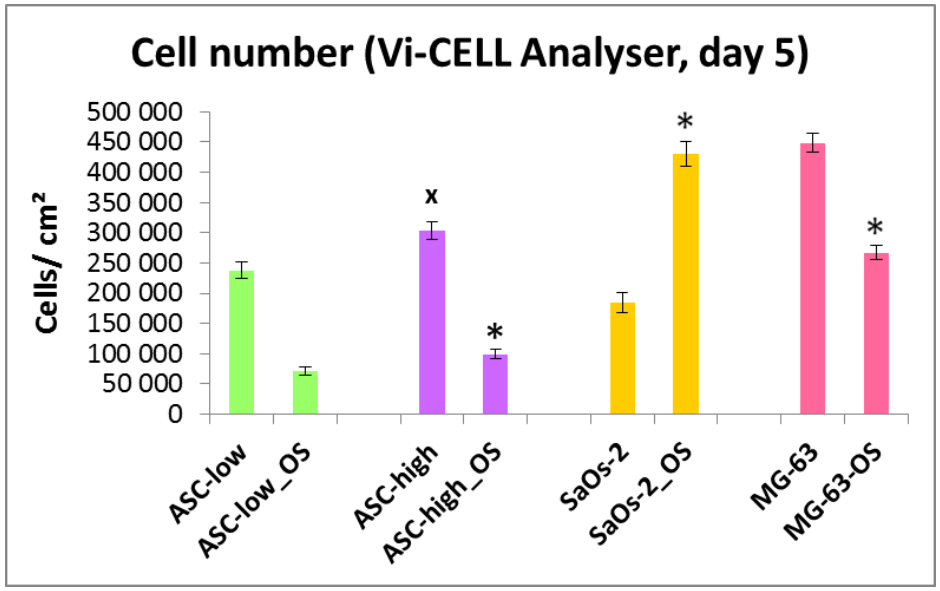
Thank you very much for your attention!

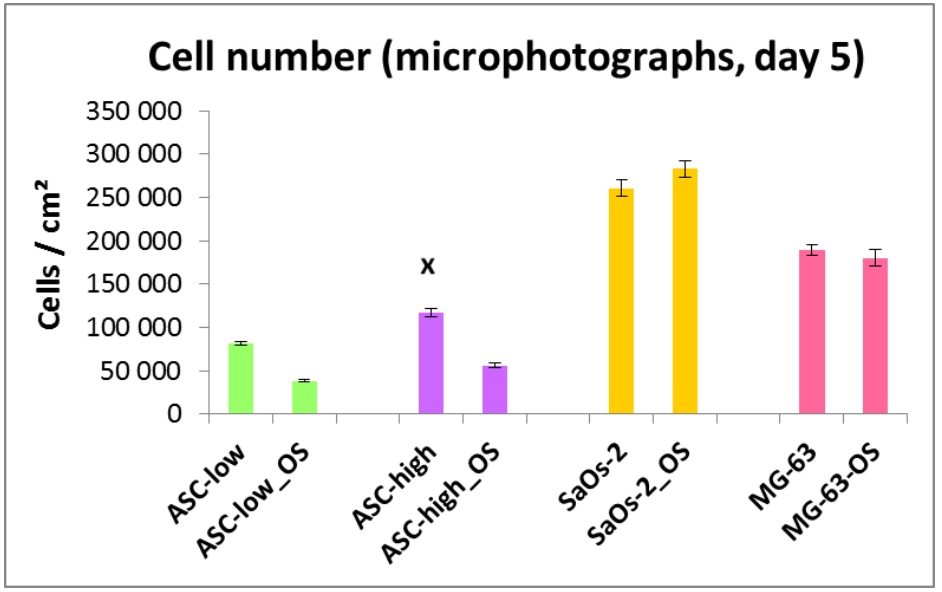


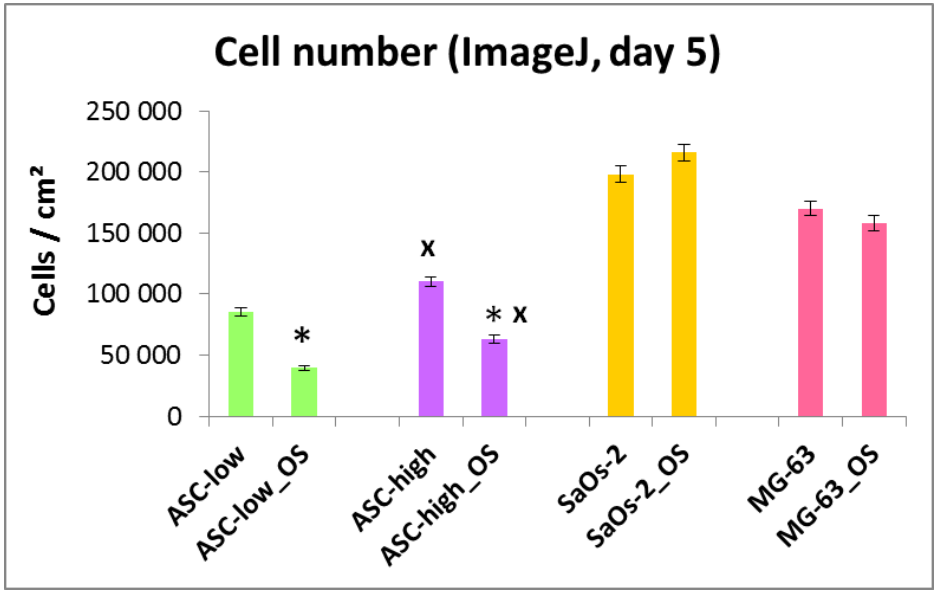
Cell Staining & Microscopy



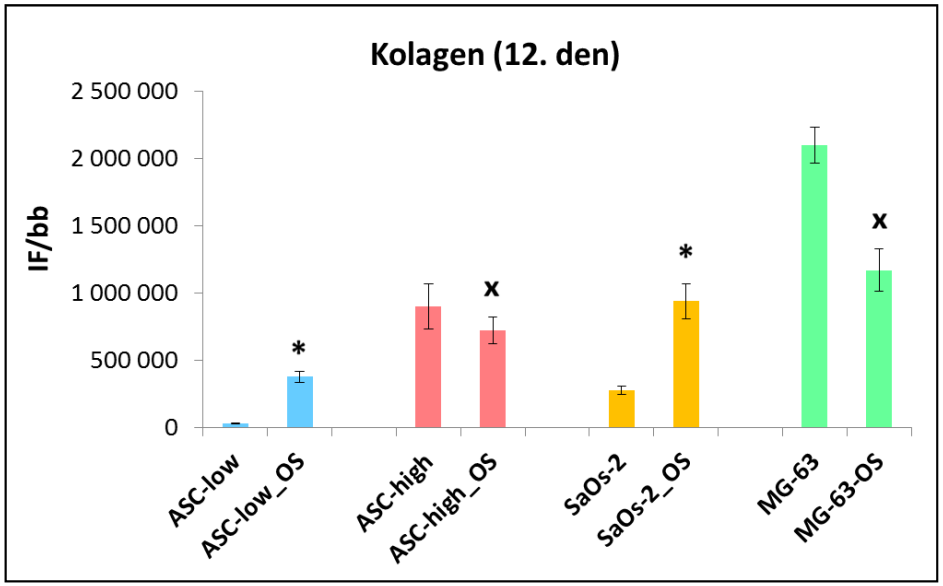
Cell Number (patient 1, pas. 2, after freezing, day 5)

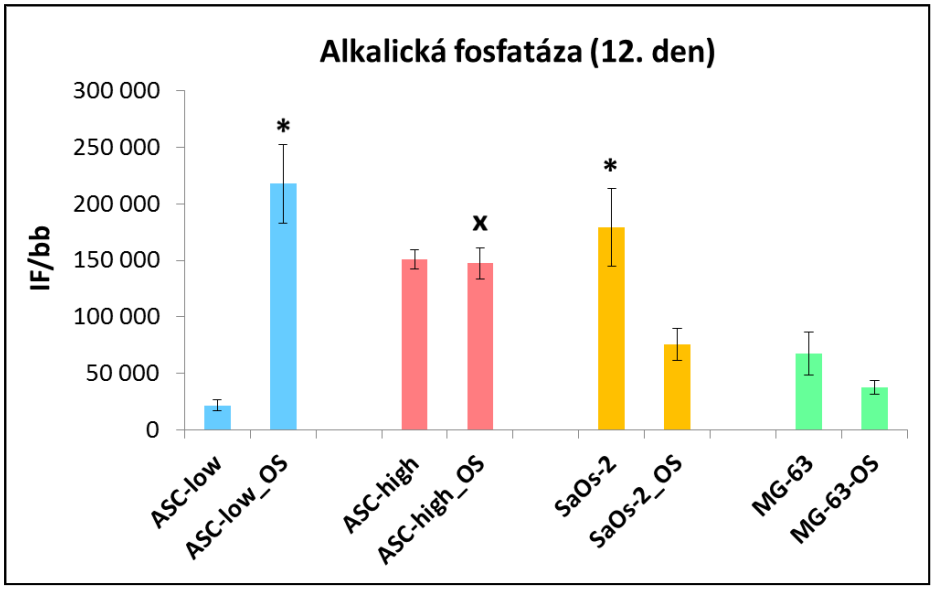


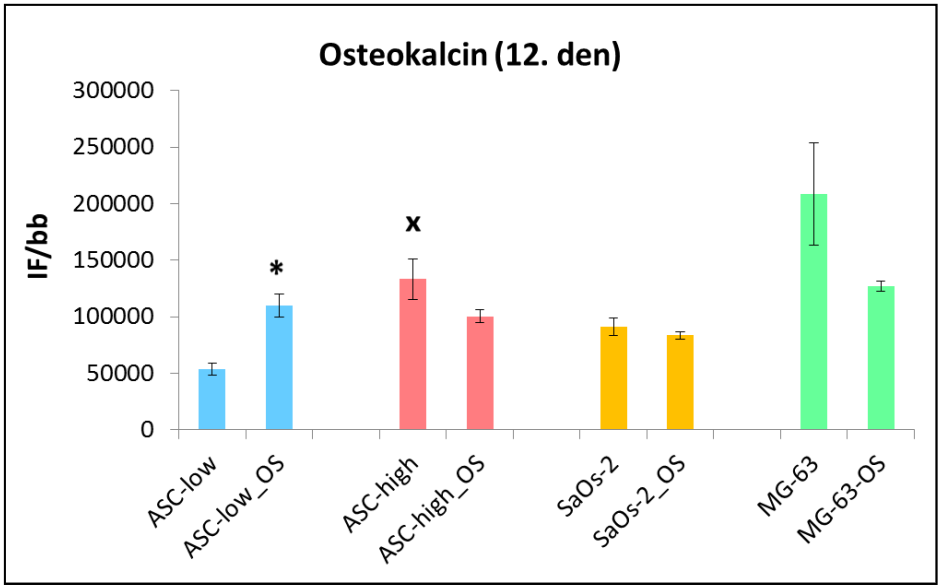




Osteogenic Differentiation (pat. 1, pas. 2, after freezing, d. 12)







Ti-6Al-4V Alloy with Various Surface Modifications

Prepared in collaboration with VUHZ Joint-Stock Co. (Dr. Roman Gabor) and Medin Joint-Stock Co. (Dr. Jaroslav Marvan)

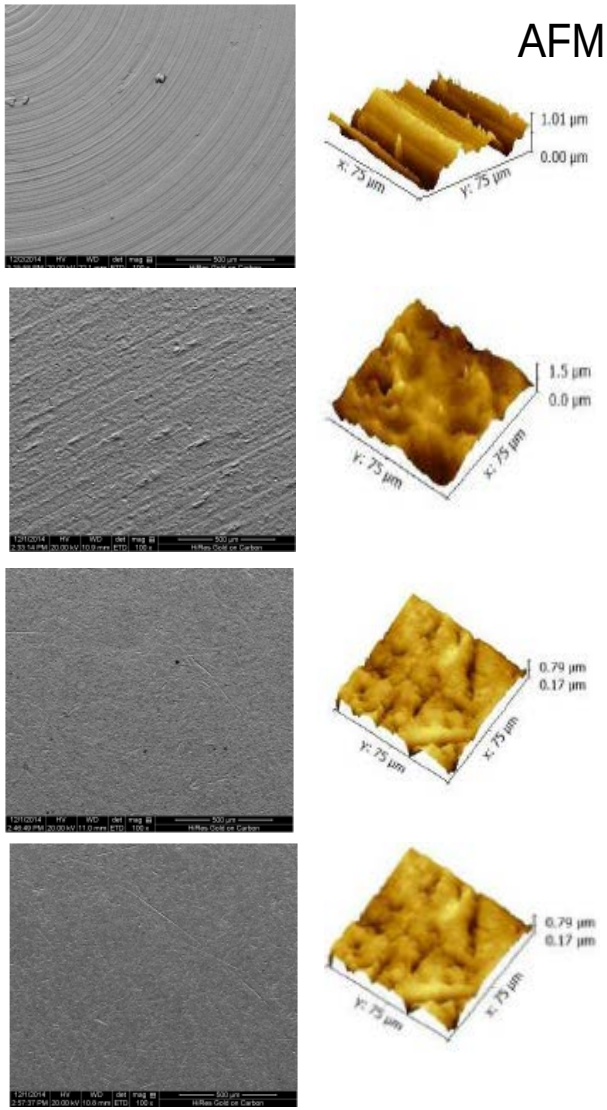
K ……. Control unmodified samples (disc, diameter 14 mm, thickness 2 mm, Ra = 280 nm)

A ……. Modified by shot blasting and tarnishing (tryskání a matování), Ra = 200 nm

B ……. Vibratory finishing (omílání), Ra = 100 nm

C ……. Vibratory finishing, shot blasting and polishing (omílání, tryskání a leštění), Ra = 80 nm

SEM AFM

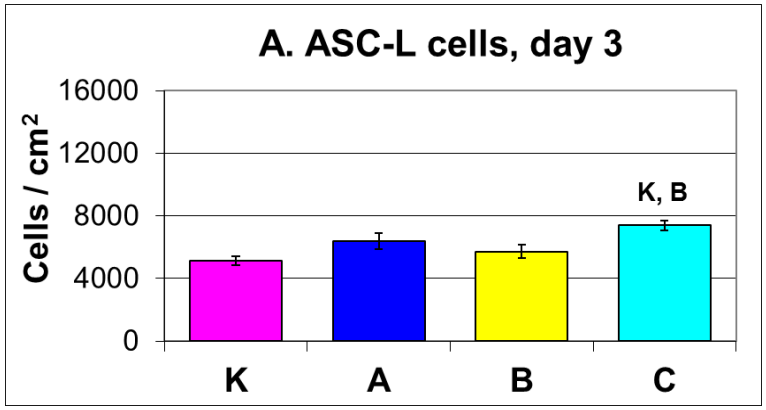


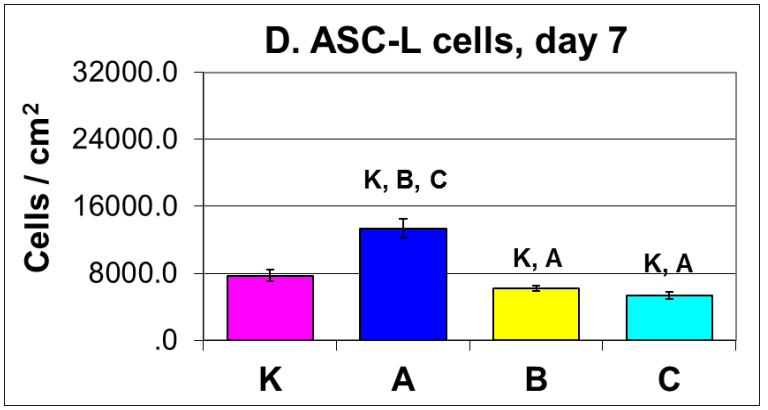
Contact Angle and Surface Free Energy

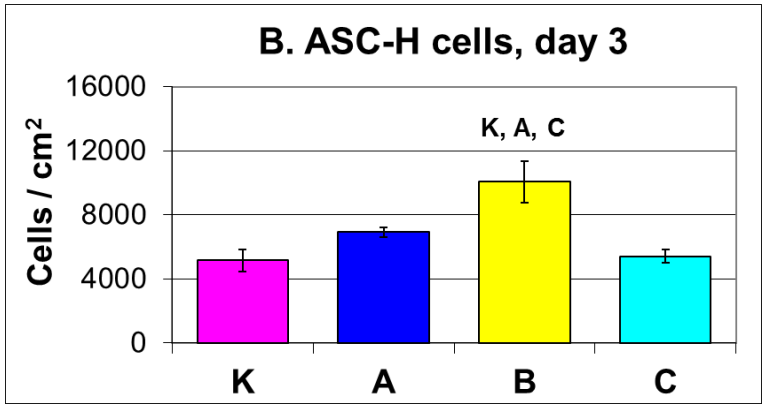
Sample	Contact angle [°]		Surface free energy [mN/m]		
	Water	Polyethylene glycol	Total	Disperse part	Polar part
K	85.6 ± 4.9	63.4 ± 4.1	25.4 ± 13.85	19.5 ± 8.47	6 ± 5.38
A	56.8 ± 2.1	12.4 ± 2.9	50.9 ± 2.72	45.3 ± 1.8	5.7 ± 0.9
B	52.7 ± 4.7	33.0 ± 0.7	47.0 ± 10.98	12.8 ± 3.42	34.2 ± 7.55
C	44.0 ± 2.3	30.4 ± 3.0	55.4 ± 5.97	8.7 ± 1.75	46.7 ± 4.22

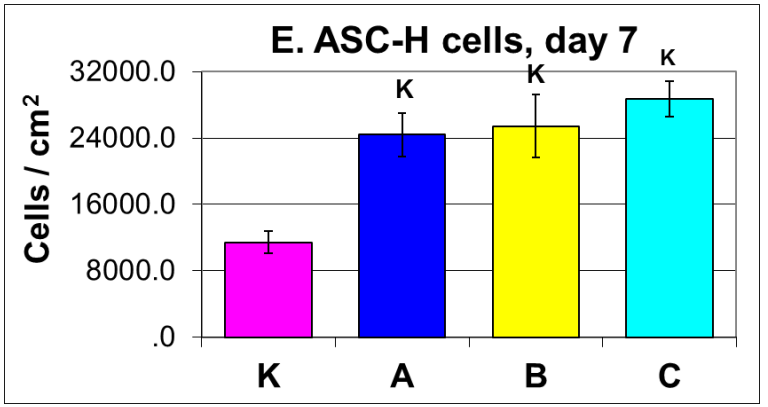
Mean ± S.D. from 7 measurements (Inst. Chemical Technology; Dr. Nikola Kasálková Slepičková)

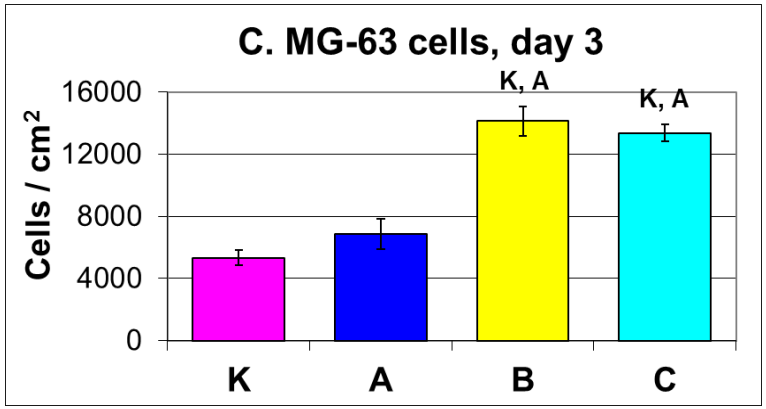
Cell Number on Ti-6Al-4V samples

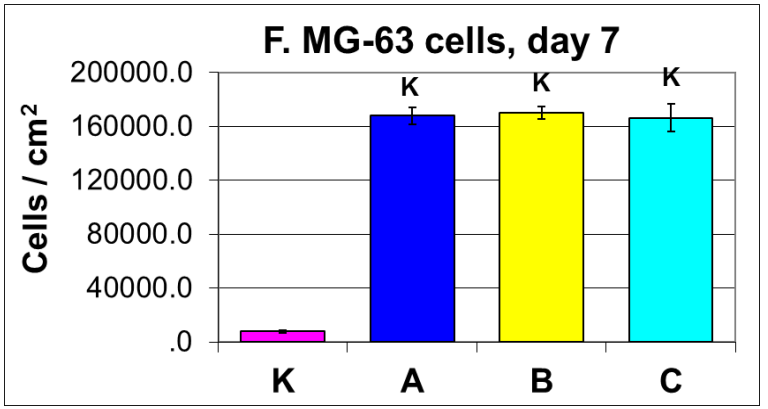










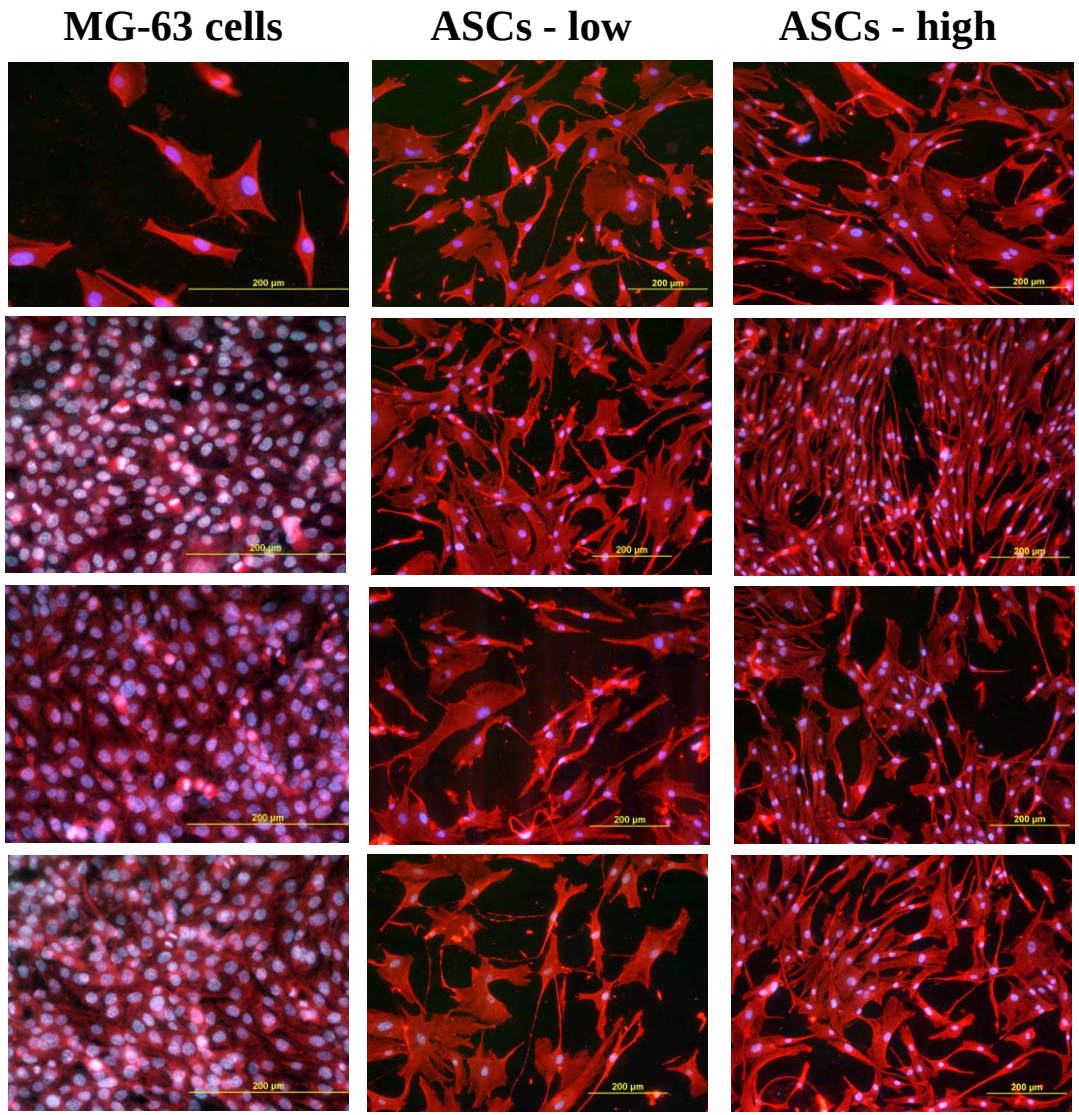


Mean ± S.E.M. from 20 measurements

ANOVA, Student-Newman-Keuls method.

$p \leq 0.05$ compared to the groups mentioned above the columns.

Cell Morphology on Ti-6Al-4V Samples



Day 7 after seeding

Control non-modified

Shot blasting and tarnishing (tryskání a matování)

Vibratory finishing (omílání)

Vibratory finishing, shot blasting and polishing (omílání, tryskání a leštění)

Texas Red C_2-Maleimide and Hoechst 33258. Olympus IX 51 microscope, DP 71 digital camera, objective 10x, bar = 200 µm