



Complex analysis of metabolic status, intracellular pH, viscosity and cytoskeleton of human mesenchymal stem cells during differentiation by fluorescent microscopy and FLIM

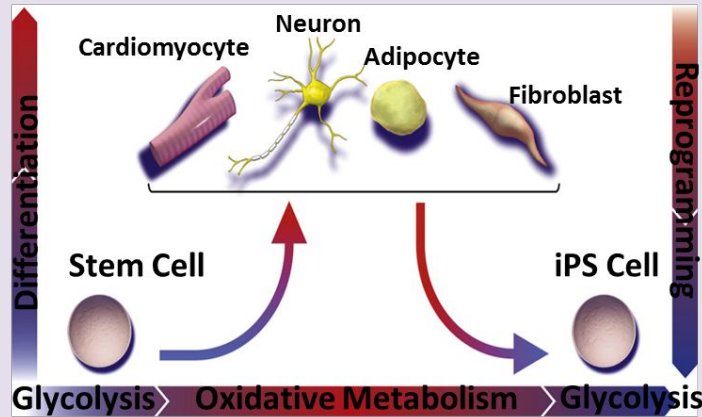
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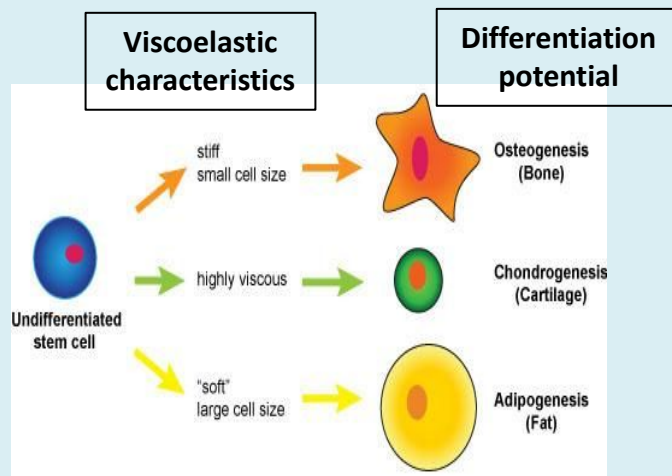
²Nizhny Novgorod State University, Nizhny Novgorod, Russia

Functional-structural changes of MSCs during differentiation

Metabolism



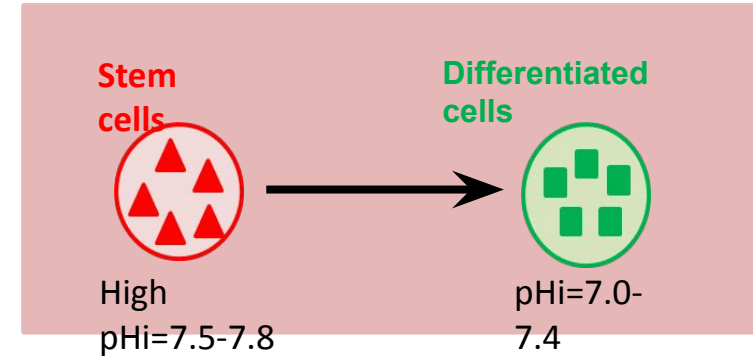
Viscosity



Mesenchymal Stem Cells

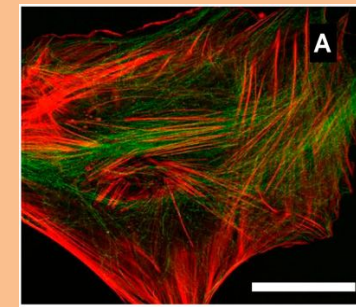


pH



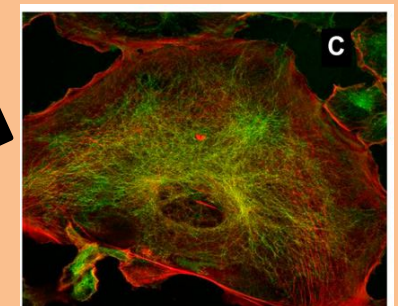
Cytoskeleton

Stem cells



fibroblast-like, spindle shape,
long, thin stress fibers

Differentiated cells



cuboidal shape, crisscrossed pattern
of actin cytoskeleton,
thick stress fibers

Effective control of MSCs differentiation - great challenge



Complex analysis is required!!!

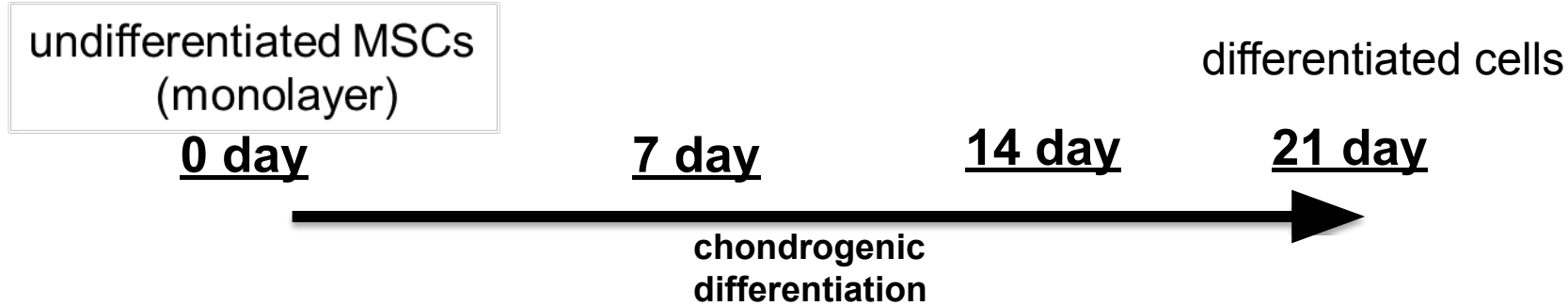
Methods of the stem cells morphology and physiology investigation



Feature	Method
Cell markers	<ul style="list-style-type: none">• Flow cytometry• Immunocytochemistry• Magnetic-activated cell sorting
Genotype	<ul style="list-style-type: none">• Polymerase chain reaction (PCR)
Differentiation potency	<ul style="list-style-type: none">• Immunocytochemistry• Fluorescence Microscopy + fluorescence dyes /protein• Fluorescence Lifetime Imaging Microscopy (FLIM) +exo/endogenous markers• Stochastic Optical Reconstruction Microscopy (STORM) +fluorescence dyes/protein

Outline of the experiment

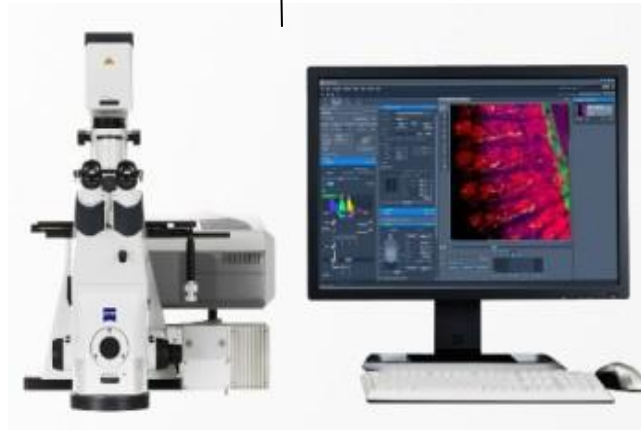
- **MSCs** – human mesenchymal stem cells bone marrow



Metabolism: fluorescence microscopy and FLIM of NAD(P)H and FAD

redox ratio FAD/NAD(P)H
Lifetimes

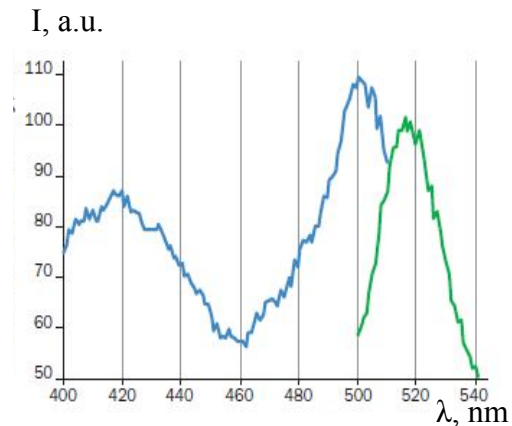
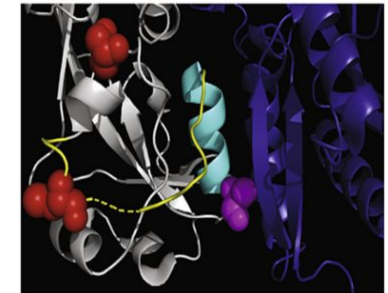
Nicotinamide adenine dinucleotide, **NADH**:
excitation - 750 nm ,detection - 455-500 nm
Flavine adenine dinucleotide, **FAD**:
excitation - 900 nm , detection – 500-550 nm



LSM 710 laser scanning confocal microscope (Carl Zeiss, Germany)
FLIM system based on Simple Tau 152 TCSPC system (Becker & Hickl GmbH)

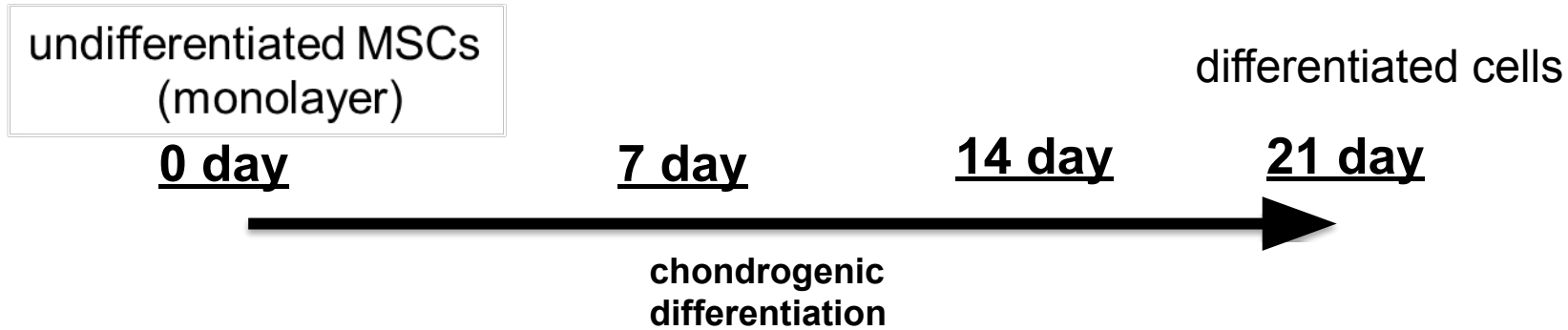
pH: fluorescence microscopy and SypHer-2

- YFP, monomer
- two peaks of fluorescence **excitation** (420 nm and 500 nm), peak **emission 516 nm**
- at **alkaline pH values**, the excitation peak at **420 nm decreases**, and at **500 nm - increases**, while for **acidic - on the contrary**

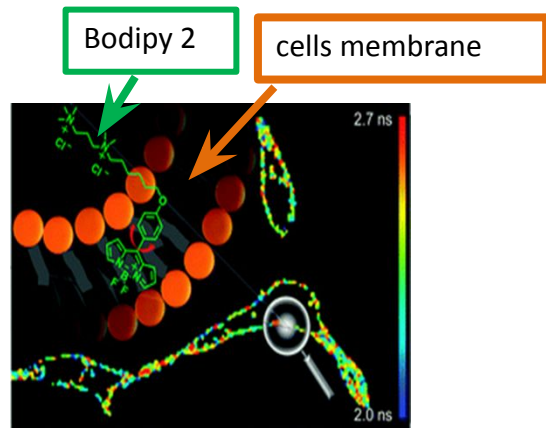


Outline of the experiment

- **MSCs** – human mesenchymal stem cells bone marrow



Viscosity: FLIM and Bodipy 2



ex = 800 nm,
detection range = 409-660 nm



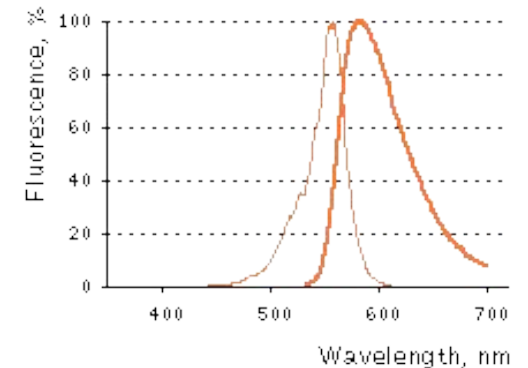
LSM 710 laser scanning confocal microscope (Carl Zeiss, Germany)
FLIM system based on Simple Tau 152 TCSPC system (Becker & Hickl GmbH)

Cytoskeleton: STORM and TagRFP



EclipseTi (Nikon, Japan),
module **N-STORM**, system PSF

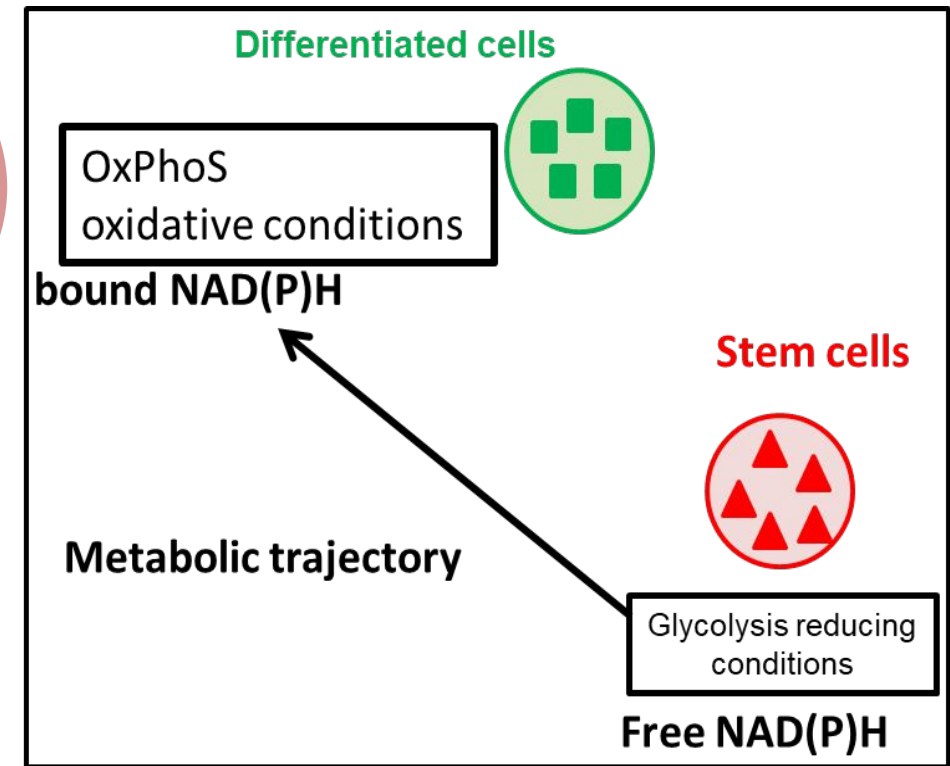
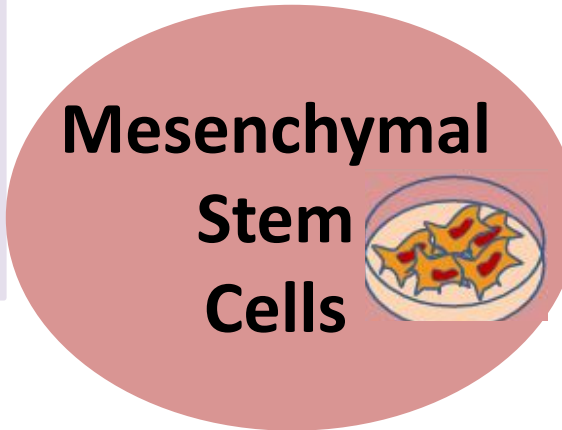
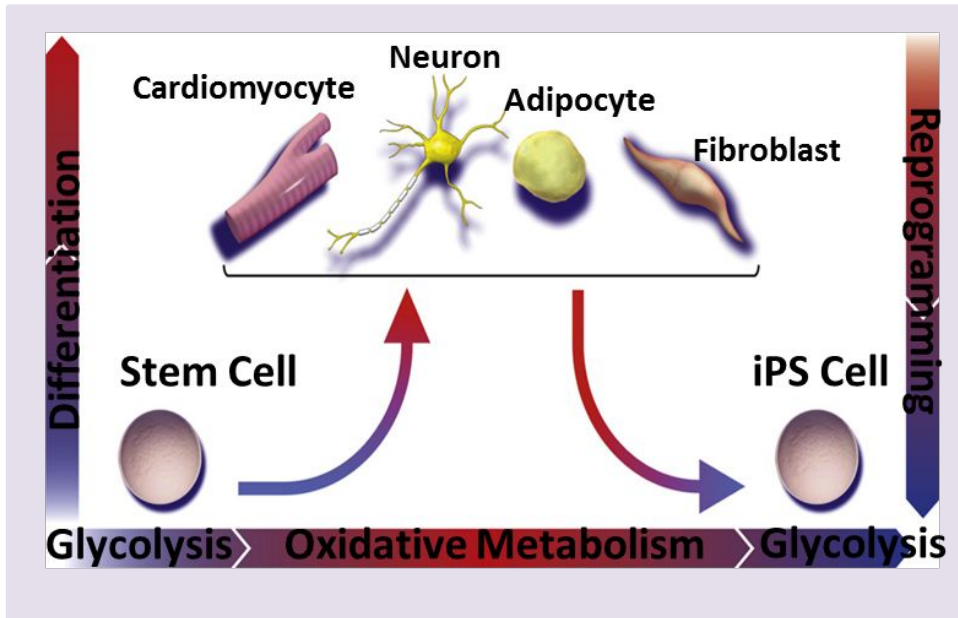
TagRFP



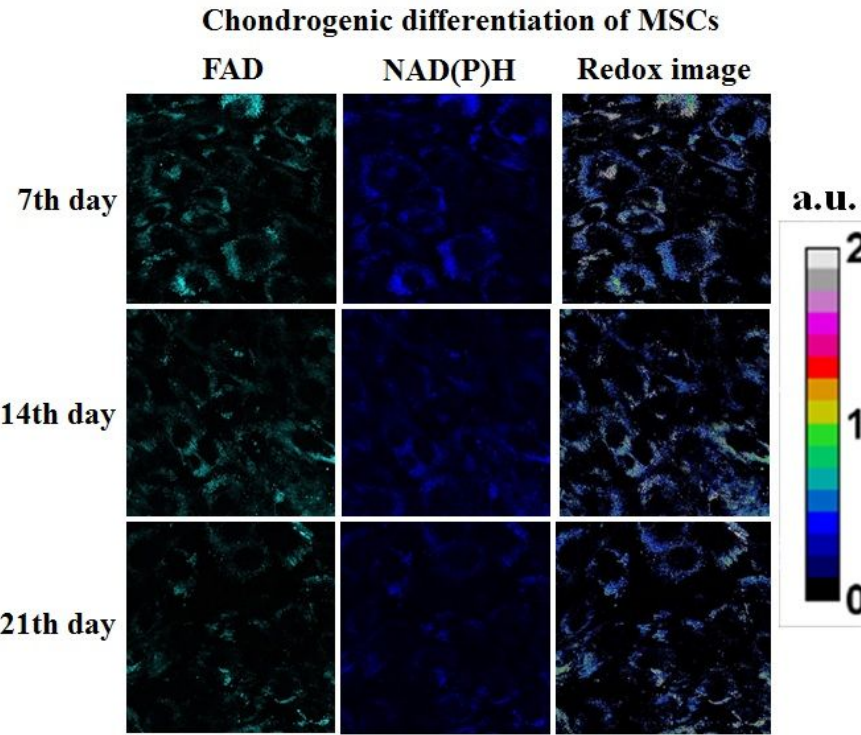
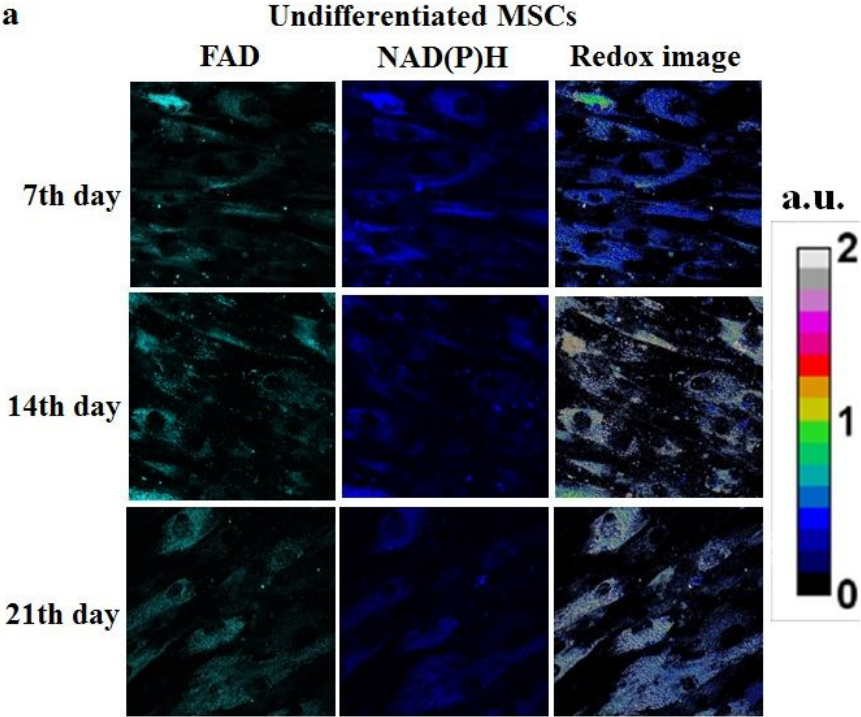
em=550nm
detection= 584nm

Functional-structural changes of MSCs during differentiation

Metabolism



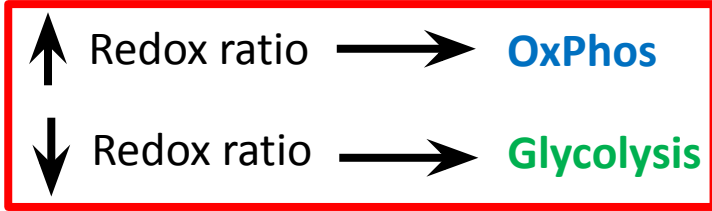
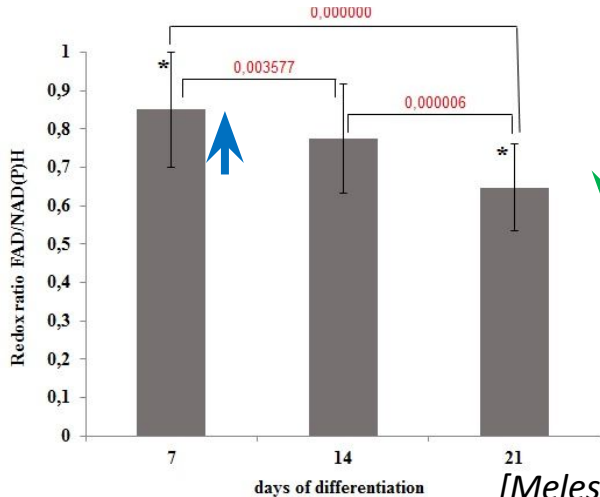
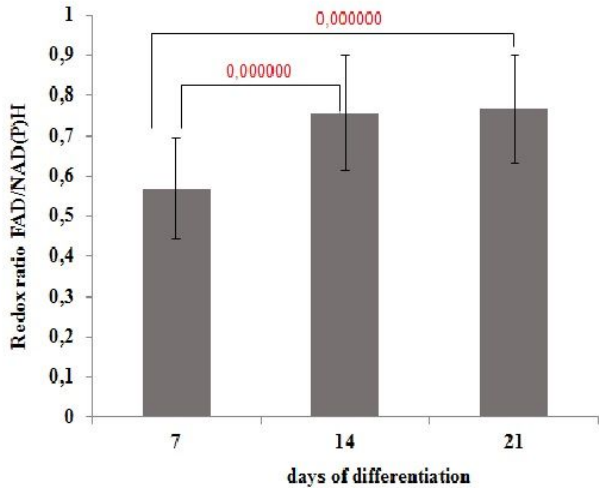
Optical redox ratio of FAD/NAD(P)H changes during chondrogenic differentiation



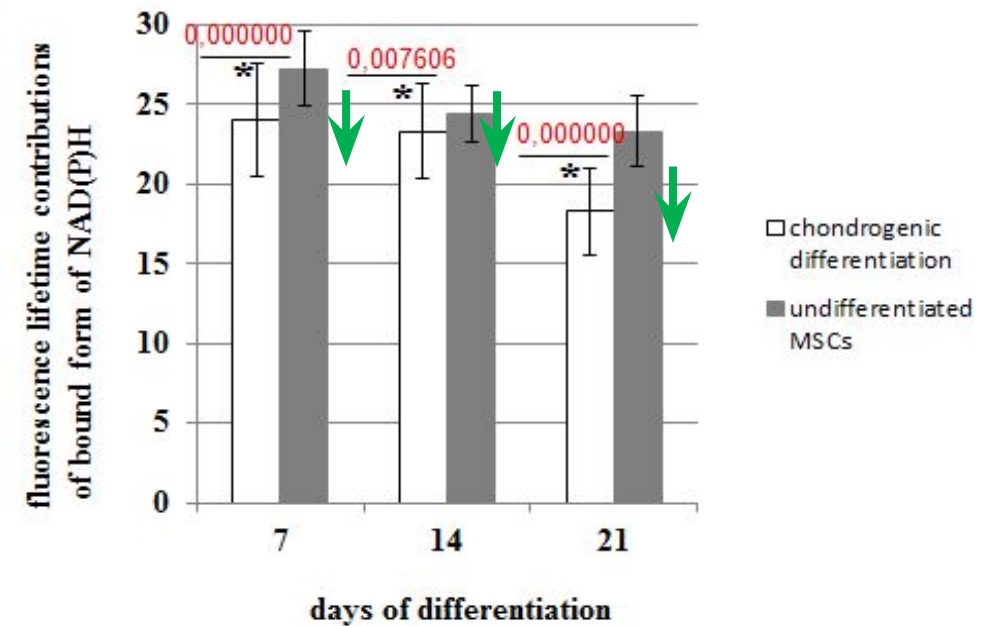
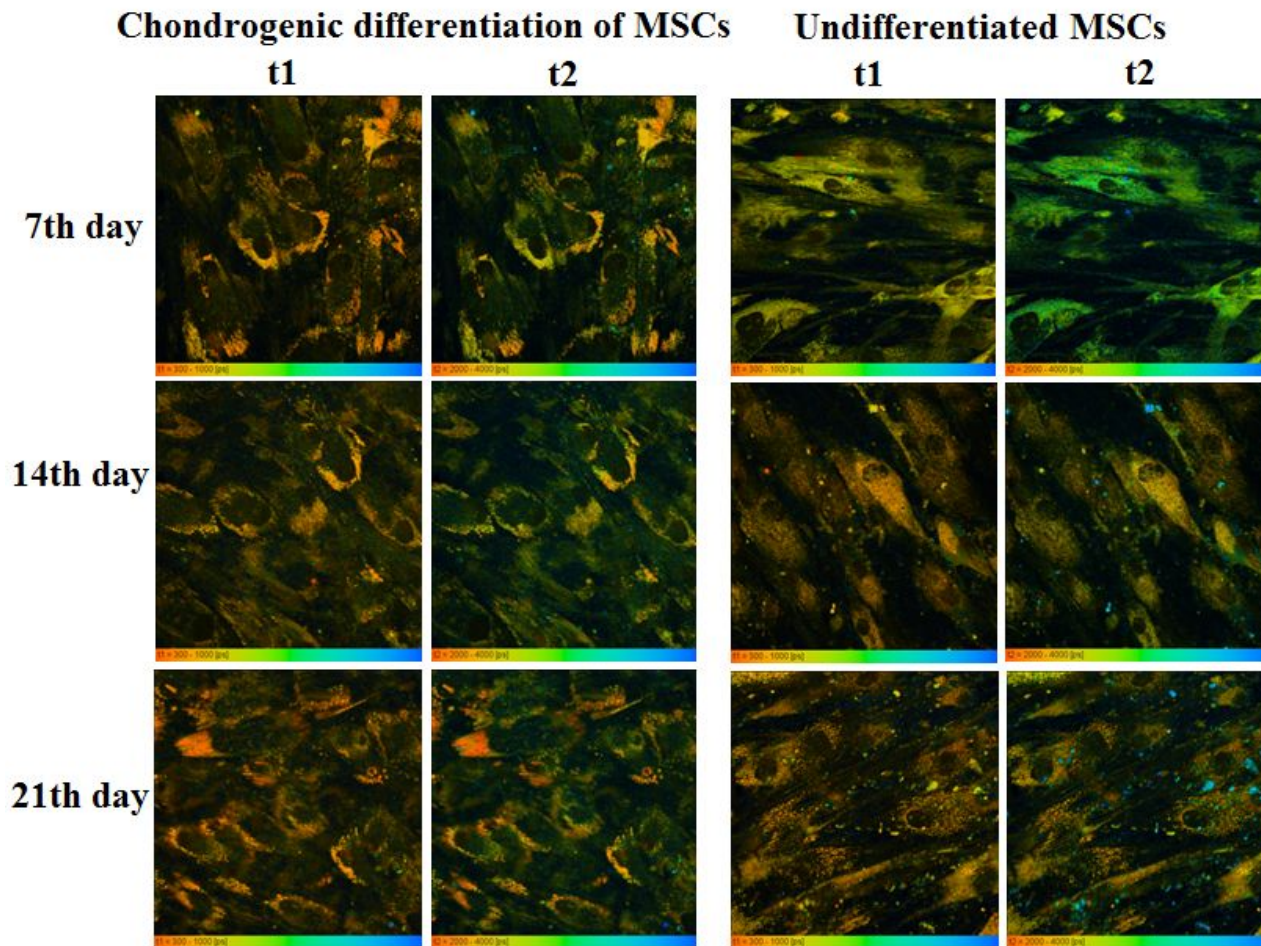
FAD:
excitation - 900 nm (5mW) ,
detection - 500-550 nm

NADH:
excitation - 750 nm (5 mW)
detection - 455-500 nm

image size is 213 × 213 μm
(1024 × 1024 pixels)



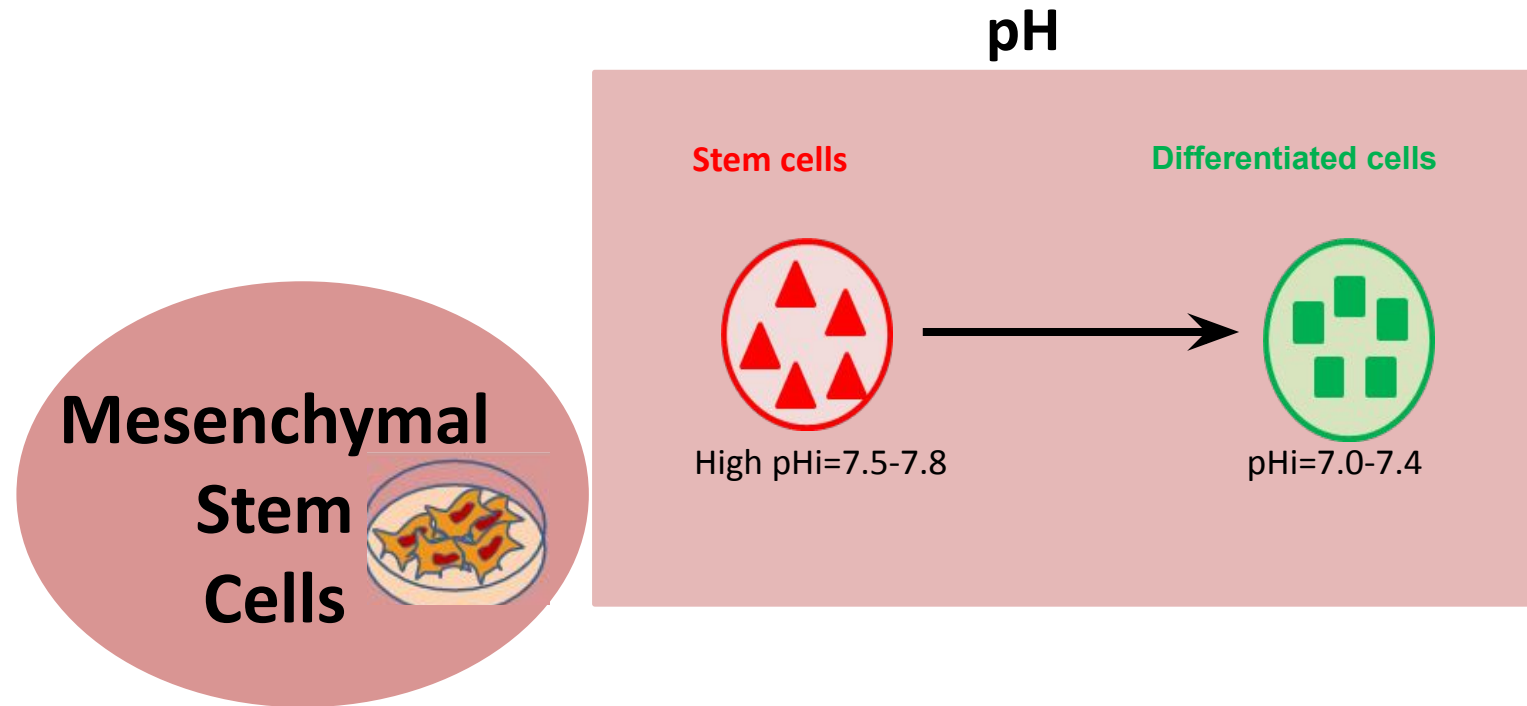
Dynamic of bound NAD(P)H in MSCs during chondrogenic differentiation



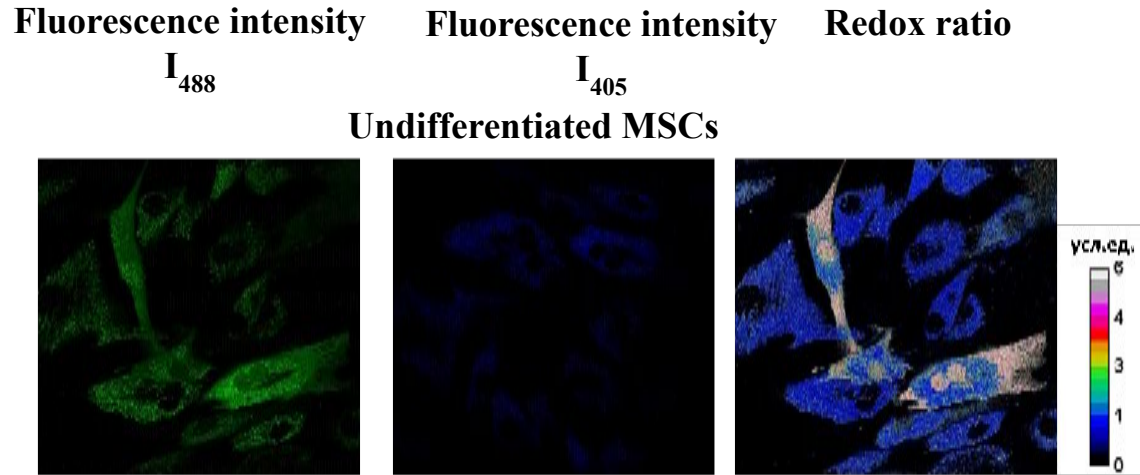
Pseudocolor-coded FLIM images of the free (t1) and protein-bound (t2) forms of NAD(P)H.

For NAD(P)H: excitation - 750 nm, detection - 455–500 nm. Field of view 213*213µm (512*512 pixels)

Functional-structural changes of MSCs during differentiation



Intracellular pH analysis in MSCs during differentiation by fluorescence microscopy and SypHer-2

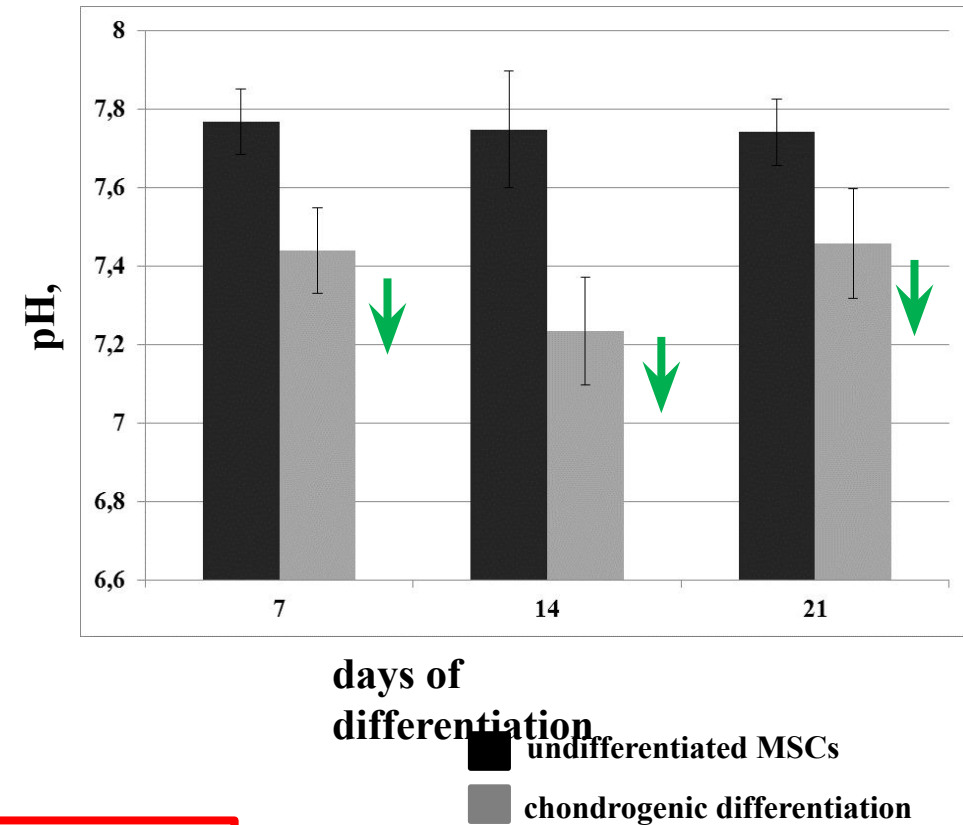


Chondrogenic differentiation of MSCs



ex = 405 nm and 488 nm, detection range = 500-550 nm

$$pH = I_{488}/I_{405} + 1,0219/0,2688$$

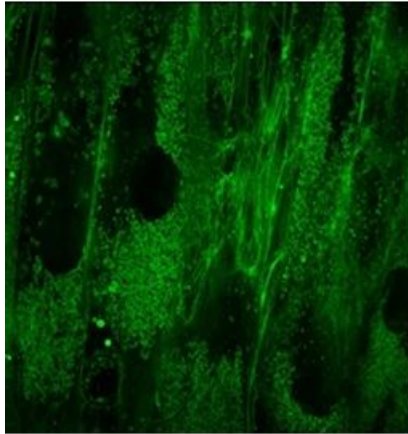


bias to acidic pH values

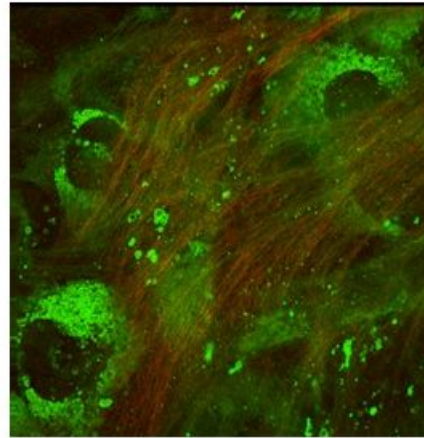
[unpublished data]

Analysis of collagen formation during chondrogenic differentiation using SHG

Undifferentiated MSCs

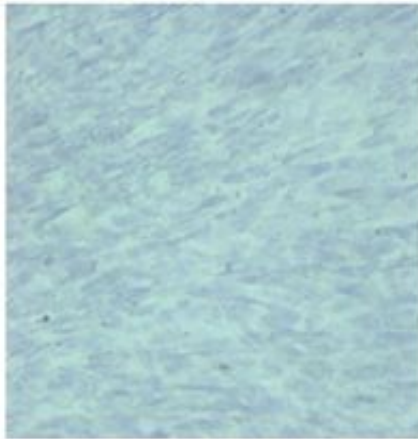


Chondrogenic differentiation of MSCs



green –
cell autofluorescence

red- collagen fiber



Hematoxylin
staining

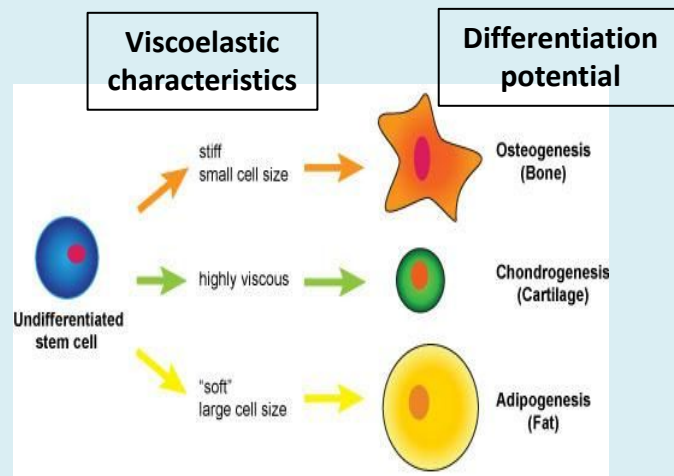


Alcian blue staining
on acidic polysaccharides

SHG of collagen was excited at wavelength of 750 nm and detected in the range 373-387 nm
the image size is 130×130 μm (512 × 512 pixels)

Functional-structural changes of MSCs during differentiation

Viscosity

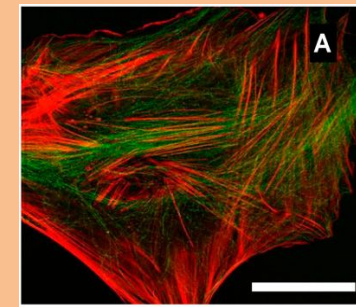


Mesenchymal Stem Cells



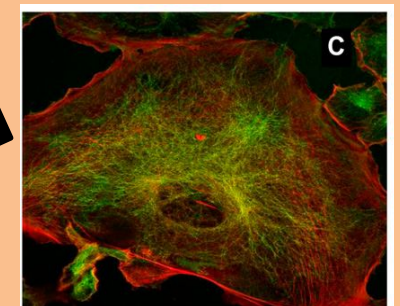
Cytoskeleton

Stem cells



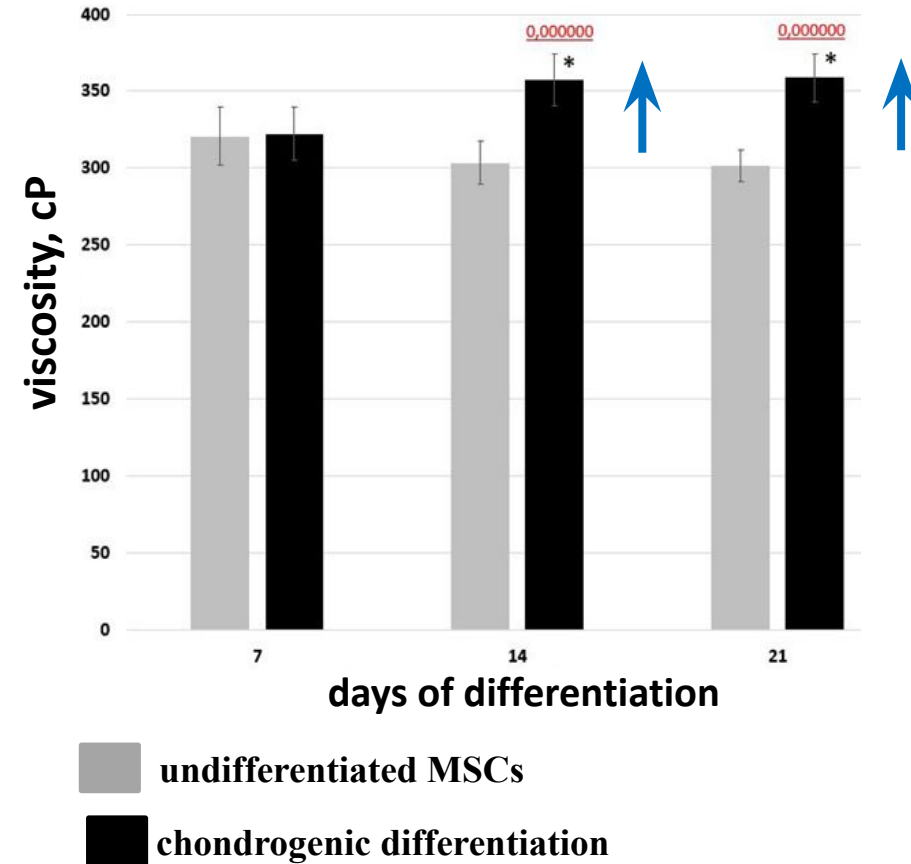
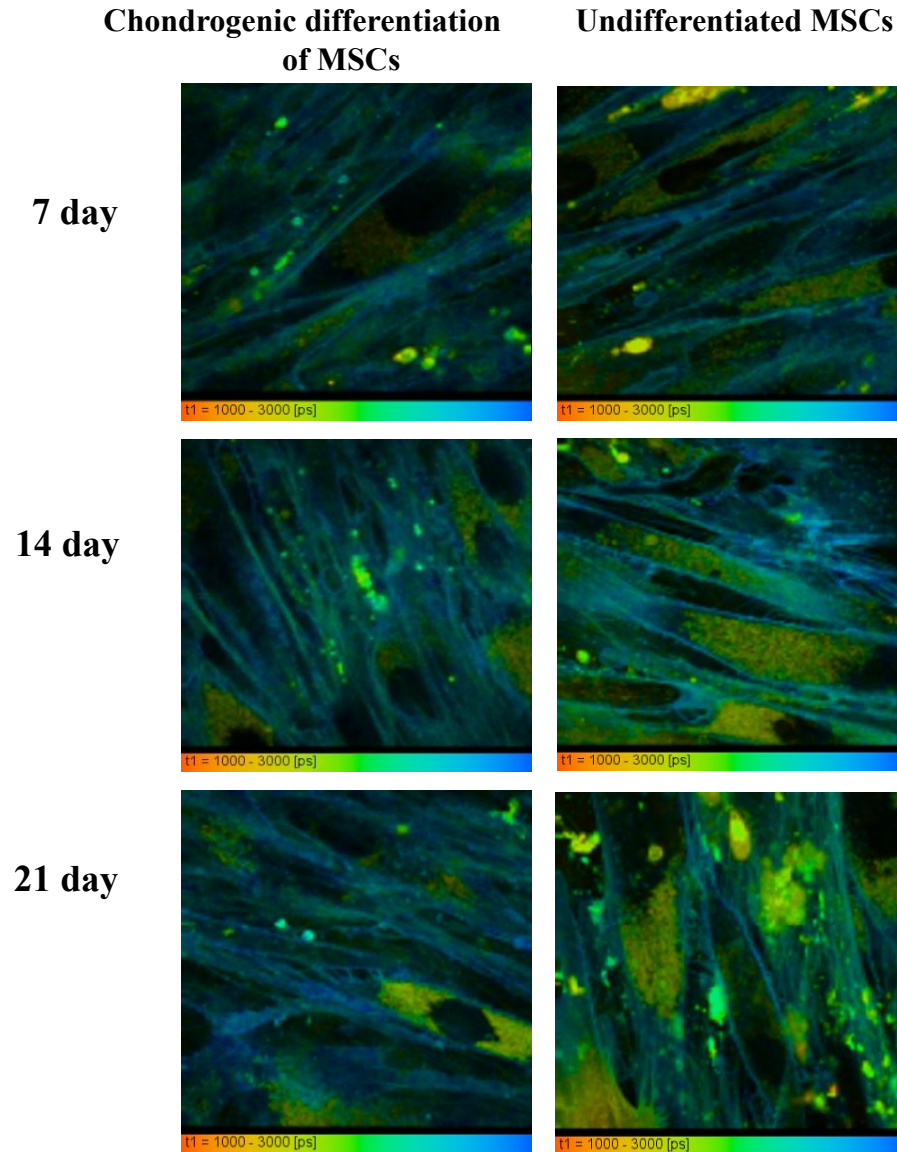
fibroblast-like, spindle shape,
long, thin stress fibers

Differentiated cells



cuboidal shape, crisscrossed pattern
of actin cytoskeleton,
thick stress fibers

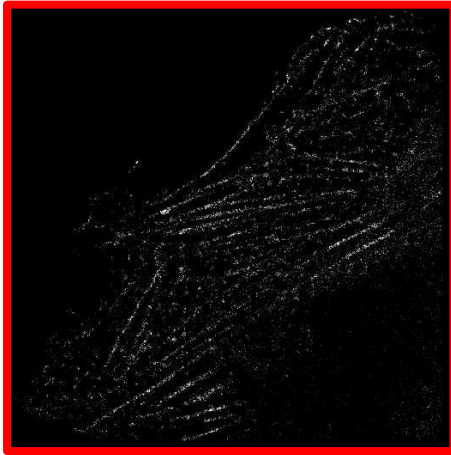
MSCs viscosity analysis during differentiation using FLIM and Bodipy 2



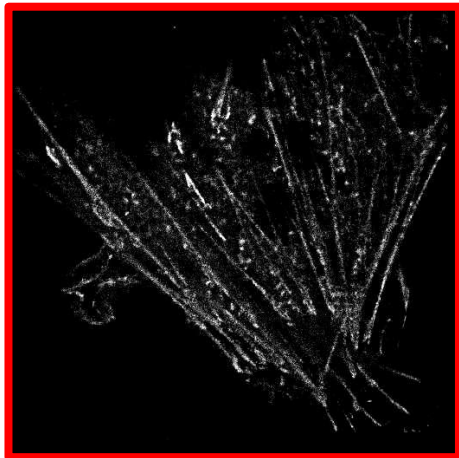
viscosity increase – cholesterol accumulation

Analysis of cytoskeleton organization in MSCs during differentiation by STORM and TagRFP

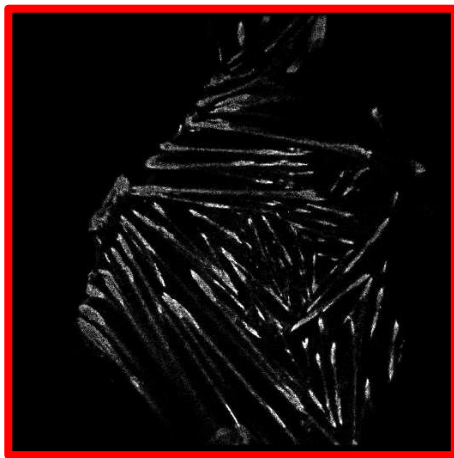
Undifferentiated MSCs



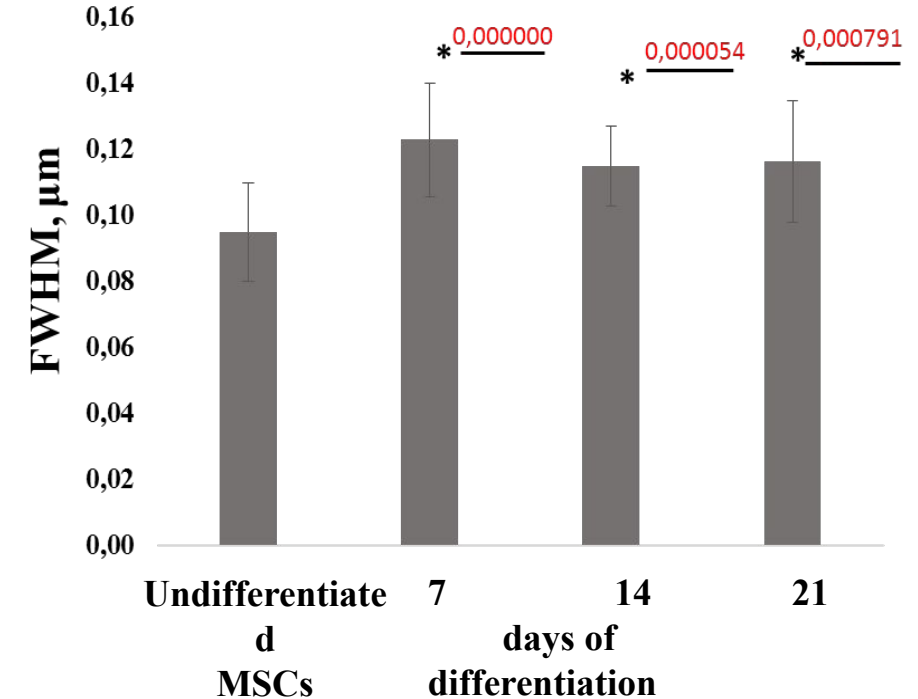
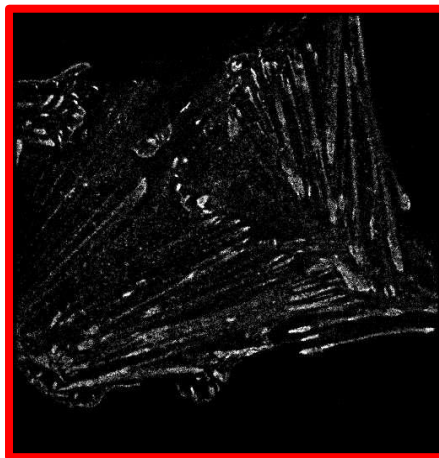
7 day



14 day



21 day



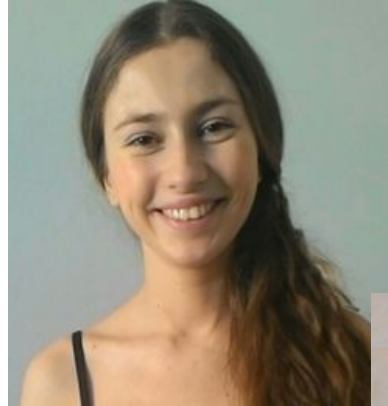
Increase of actin fibers thickness

take home message

1. **Metabolic plasticity of MSCs** during chondrogenic differentiation: glycolysis – more glycolytic state
2. **Intracellular pH**
bias of pH values towards a more acidic pH
3. **Membrane viscosity**
viscosity increase – cholesterol accumulation
4. **Cytoskeleton organization**
undifferentiated MSCs having a fibroblast-like morphology, the actin fibers are represented by long, parallel fibrils extending through the cytoplasm of the cells. Chondrocytes have increased the thickness of end parts of actin fibers. In addition, chondrocytes have changed their orientation: actin fibrils crossed cells in different directions

Acknowledgements

This work has been financially supported by Russian Science Foundation (grants No. 14-15-00536)



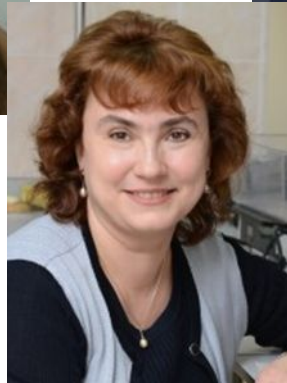
V.V. Dudenkova



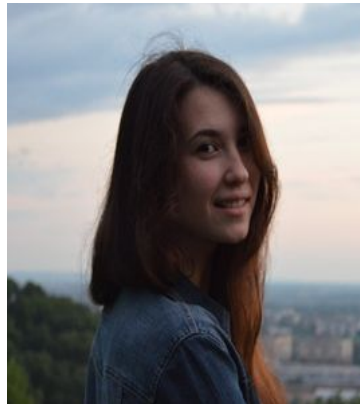
M.K. Kuimova



M.V. Shirmanova



E.V. Zagaynova



A.S. Bystrova



N.V. Klementieva



F.A. Kulagin



O. Furman



Thank you for your attention!