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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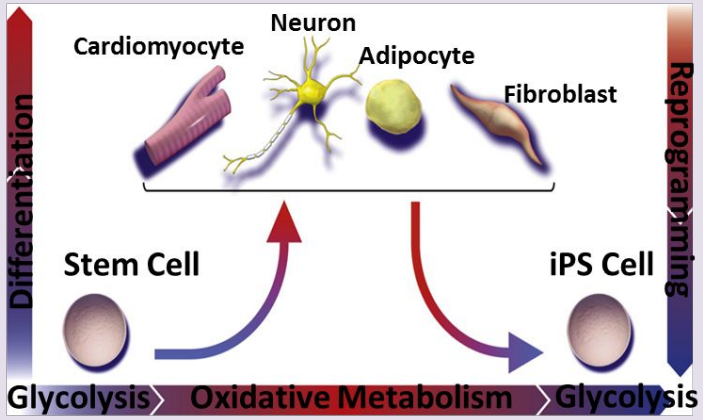
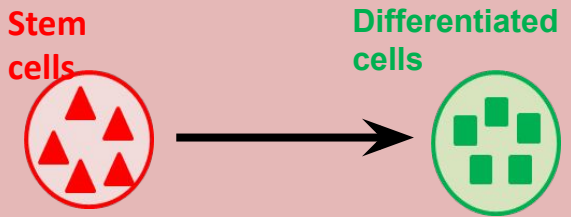
Functional-structural changes of MSCs during differentiation

Metabolism

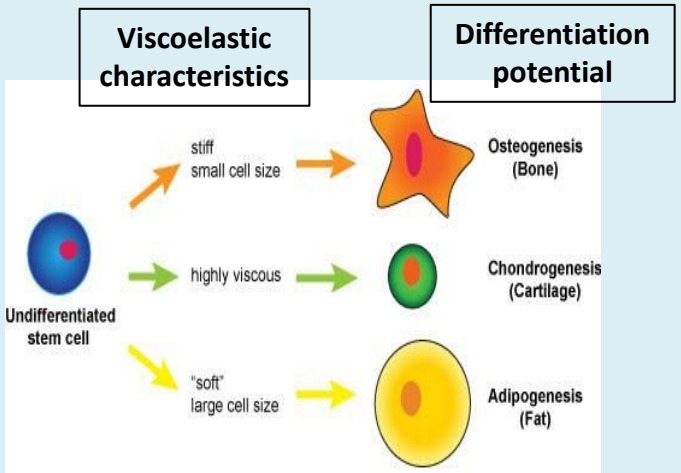


pH

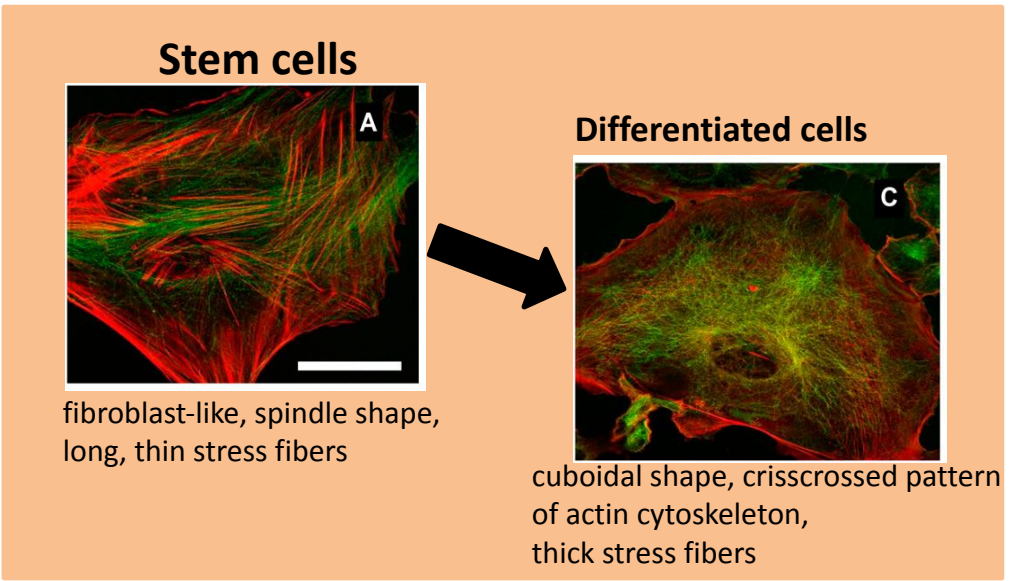


Mesenchymal Stem Cells

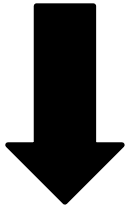
Viscosity



Cytoskeleton



Effective control of MSCs differentiation - great challenge



Complex analysis is required**!!!**

Methods of the stem cells morphology and physiology investigation

Feature	Method
Cell markers	• Flow cytometry • Immunocytochemistry • Magnetic-activated cell sorting
Genotype	• Polymerase chain reaction (PCR)
Differentiation potency	• Immunocytochemistry • Fluorescence Microscopy + fluorescence dyes /protein • **F**luorescence **L**ifetime **I**maging **M**icroscopy (**FLIM**) +exso/endogenous markers • **St**ochastic **O**ptical **R**econstruction **M**icroscopy (**STORM**) +fluorescence dyes/protein

Outline of the experiment

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

undifferentiated MSCs (monolayer) → differentiated cells

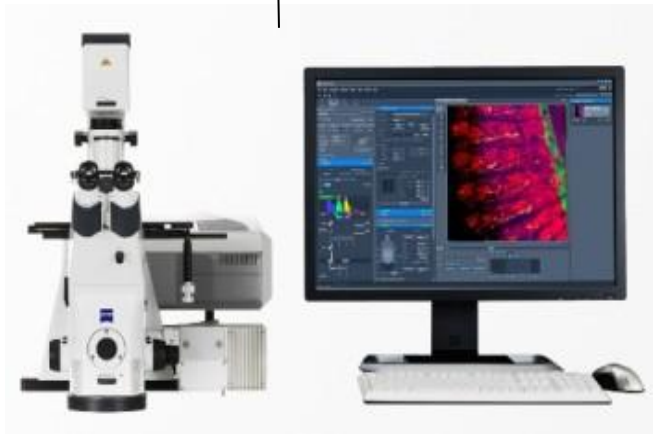
0 day — **7 day** — **14 day** — **21 day**

chondrogenic differentiation

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

redox ratio FAD/NAD(P)H
Lifetimes

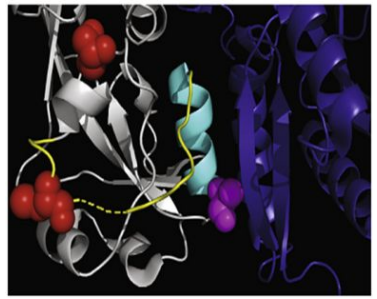
Nicotinamide adenine dinucleotide, **NADH:**
excitation - 750 nm ,detection - 455-500 nm
Flavine adenine dinucleotide, **FAD:**
excitation - 900 nm , detection – 500-550 nm

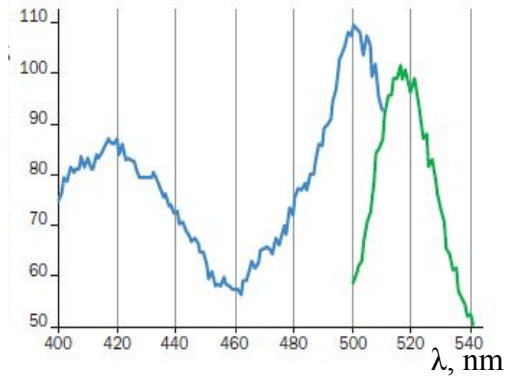


LSM 710 laser scanning confocal microscope (Carl Zeiss, Germany)
FLIM system based on Simple Tau 152 TCSPC system (Becker & Hickl GmbH)

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

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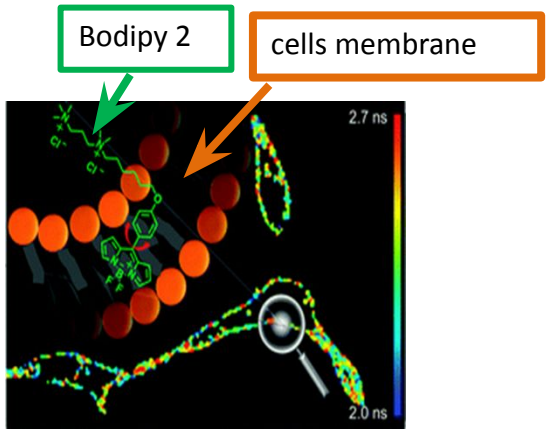
0 day **7 day** **14 day** **21 day**

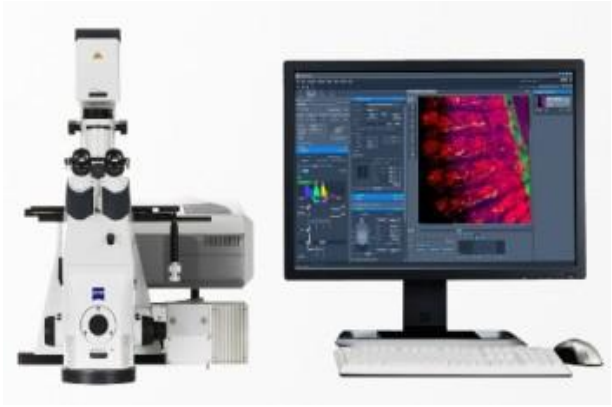
differentiated cells

chondrogenic differentiation

Viscosity: FLIM and Bodipy 2



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

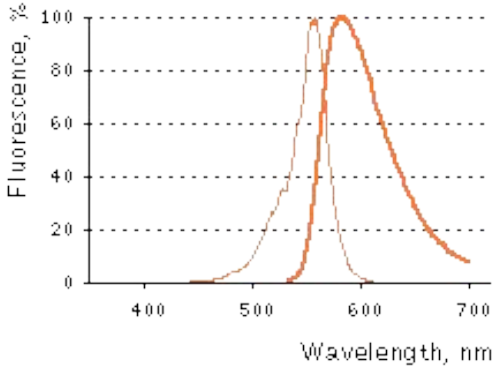


LSM 710 laser scanning confocal microscope (Carl Zeiss, Germany)
FLIM system based on Simple Tau 152 TCSPC system (Becker & Hickl GmbH)

Cytoskeleton: STORM and TagRFP

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

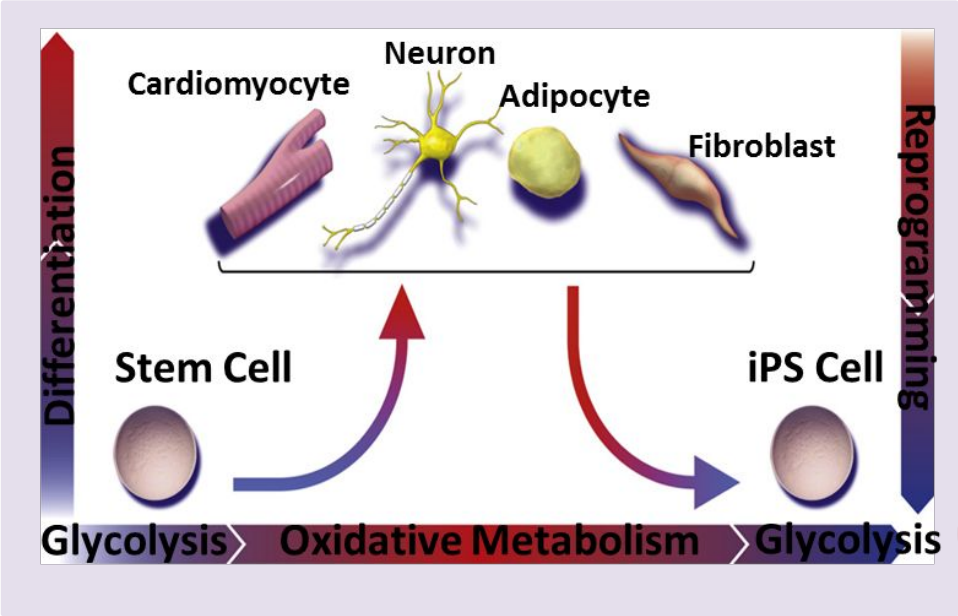
TagRFP



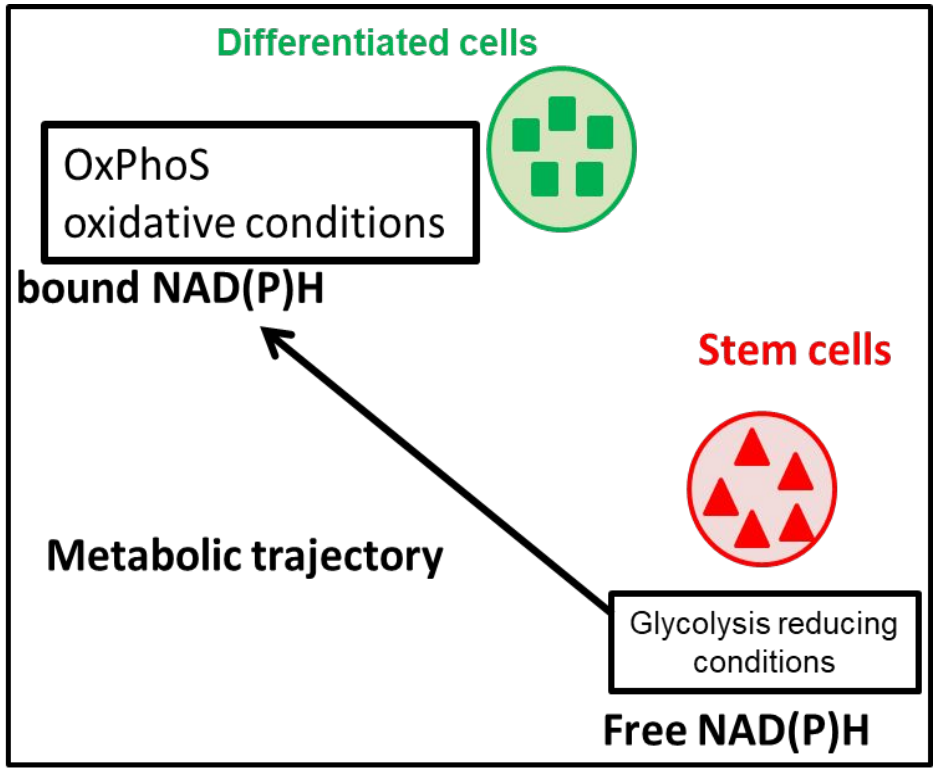
em=550nm
detection= 584nm

Functional-structural changes of MSCs during differentiation

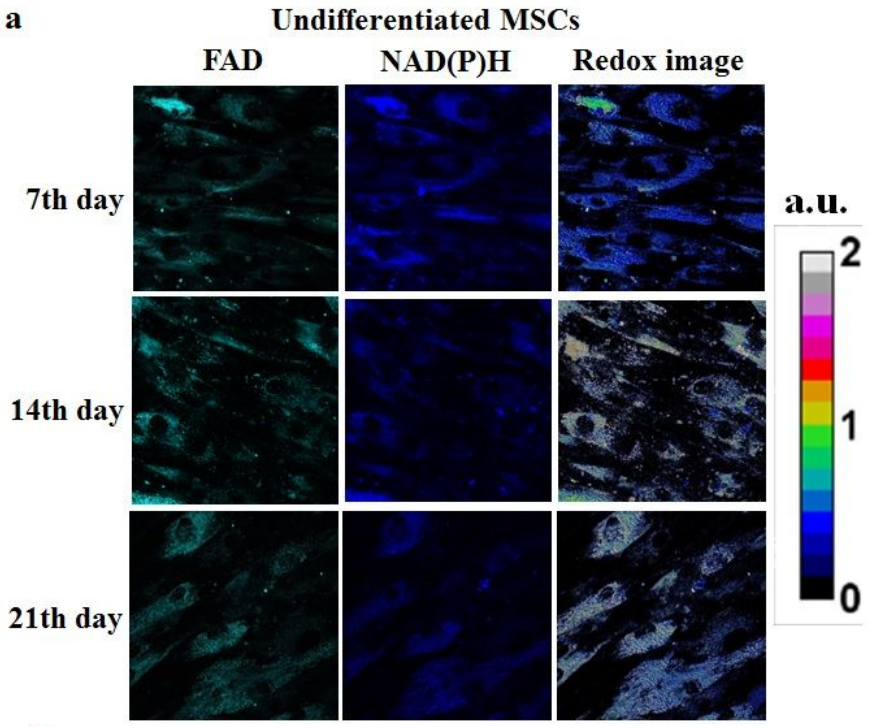
Metabolism

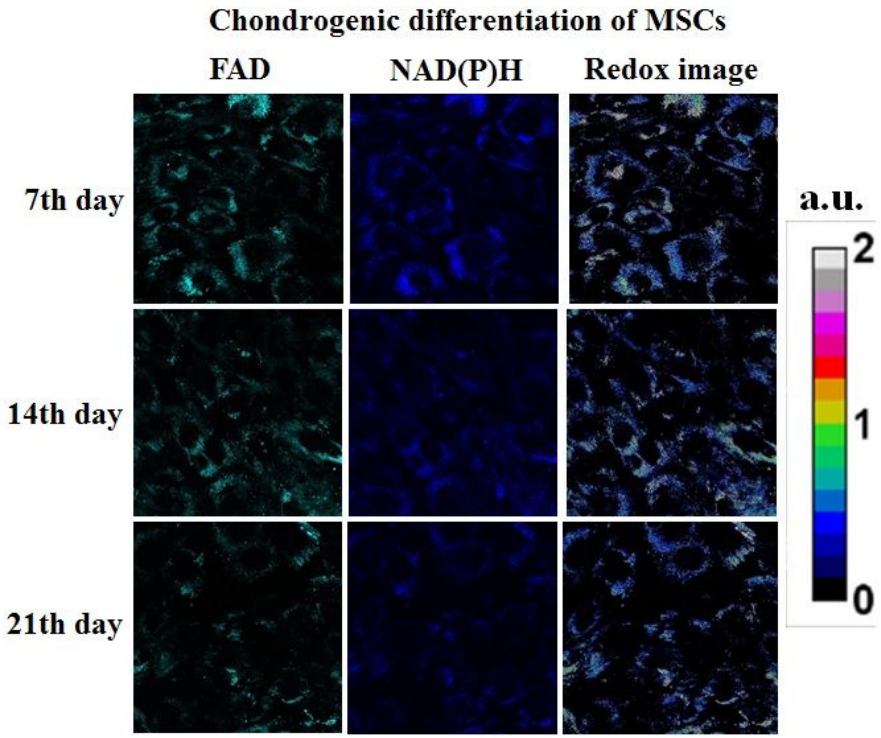


Mesenchymal Stem Cells



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

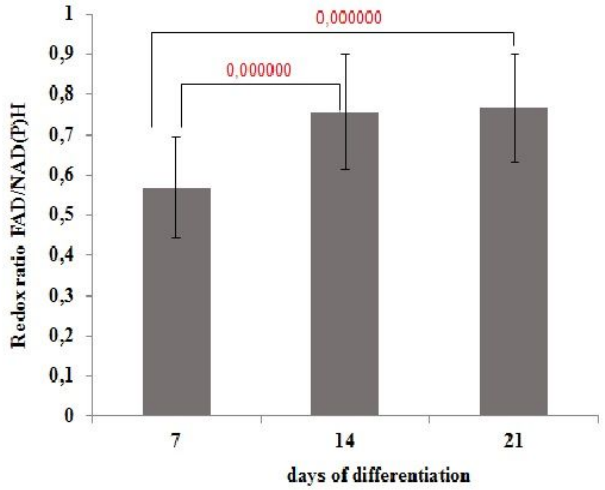


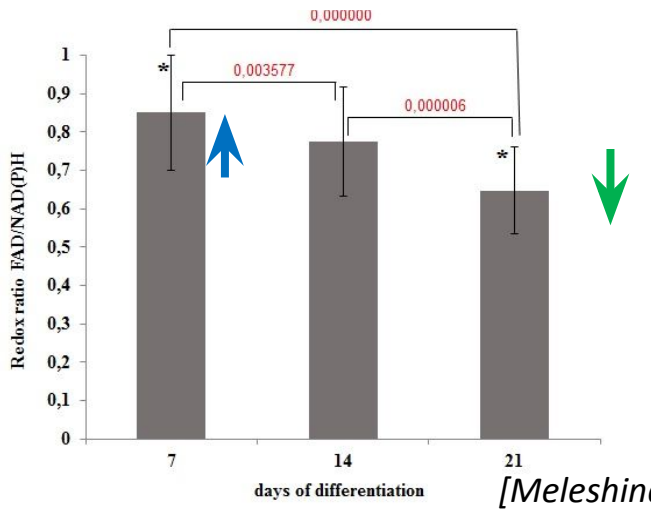


FAD:
excitation - 900 nm (5mW) ,
detection - 500-550 nm

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

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



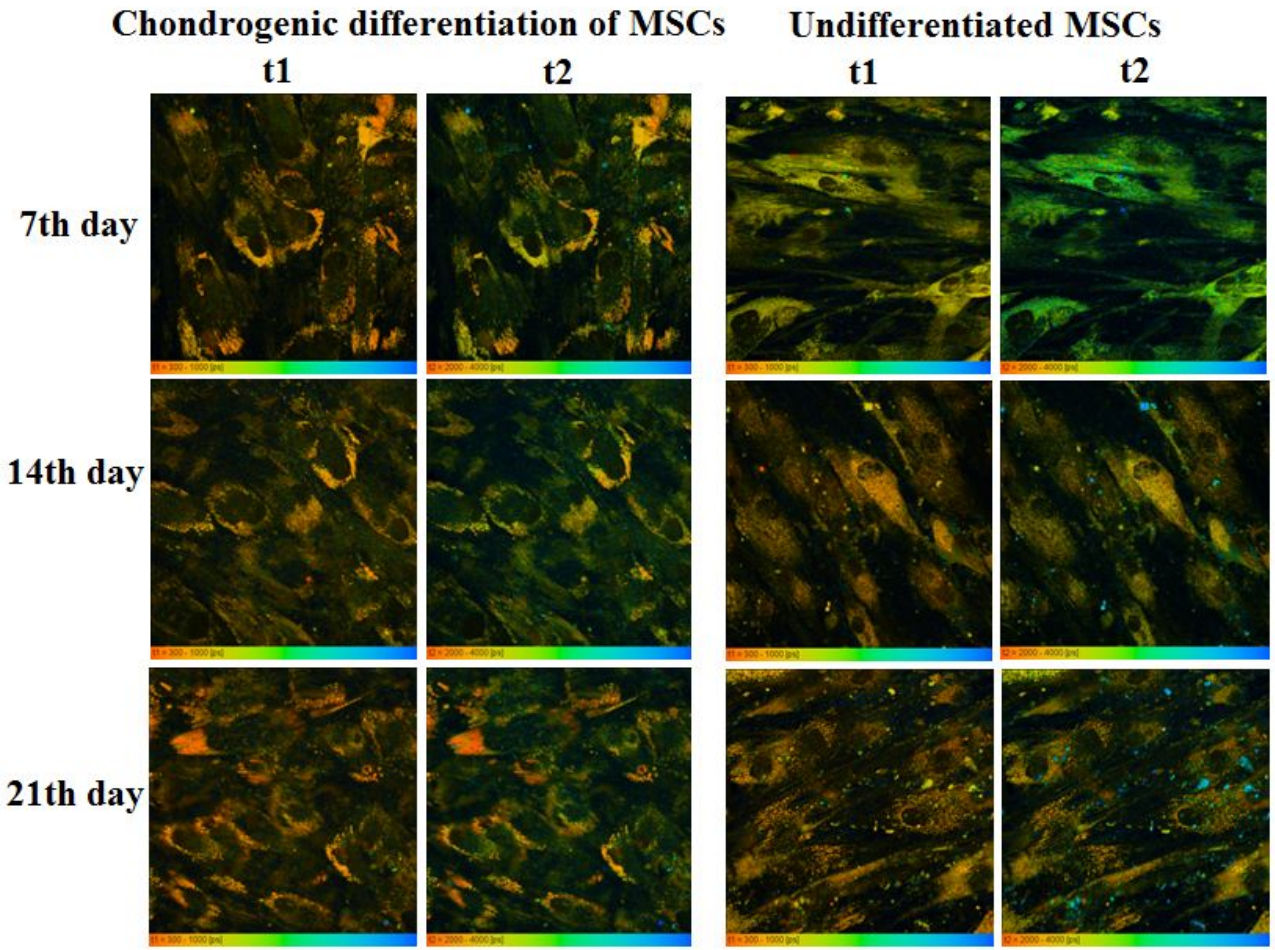


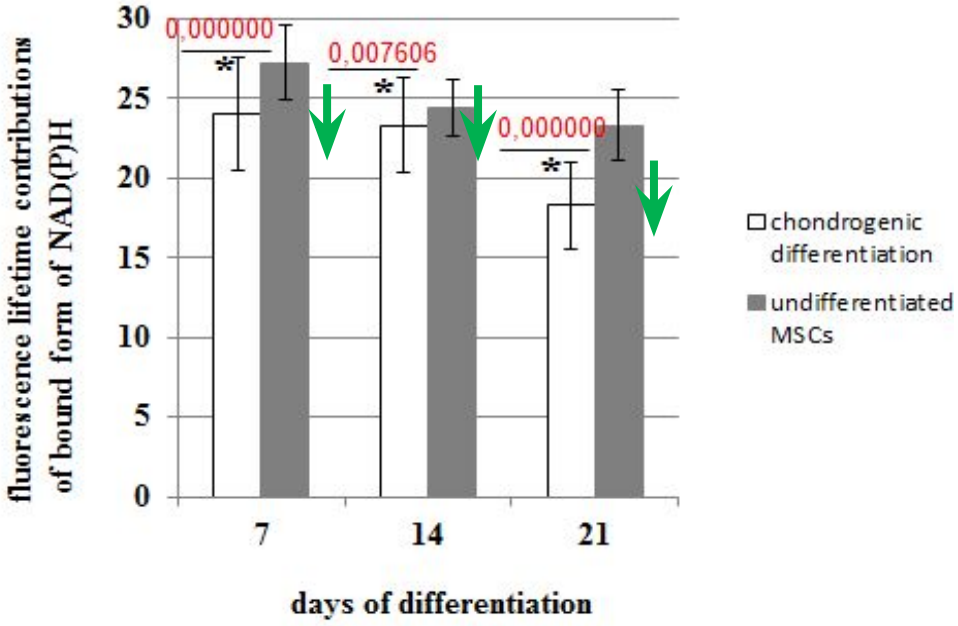
Redox ratio → OxPhos

Redox ratio → Glycolysis

[Meleshina et al. Stem Cell Research & Therapy (2017) 8:15]

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

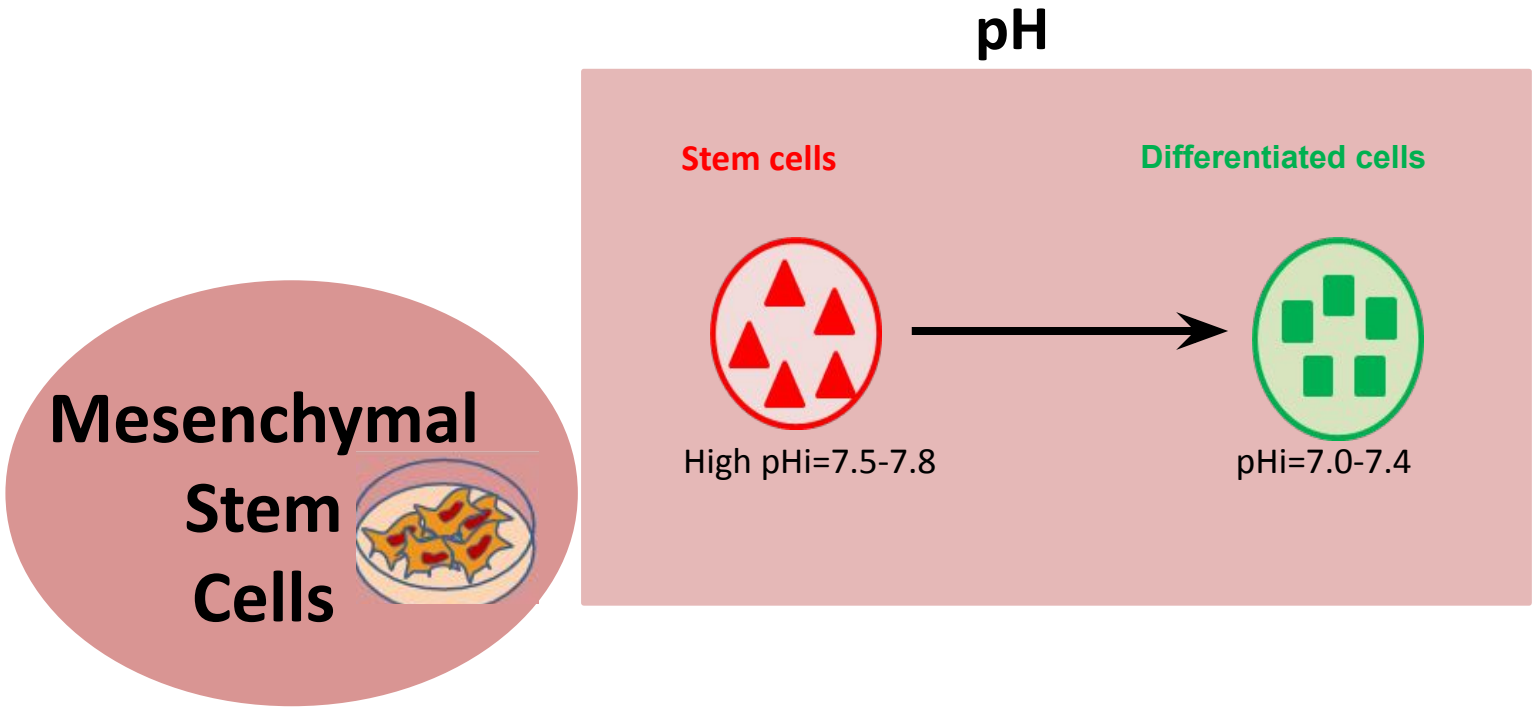




Pseudocolor-coded FLIM images of the free (t1) and protein-bound (t2) forms of NAD(P)H.
For NAD(P)H: excitation - 750 nm, detection - 455–500 nm. Field of view 213*213µm (512*512 pixels)

[Meleshina et al. Stem Cell Research & Therapy (2017) 8:15]

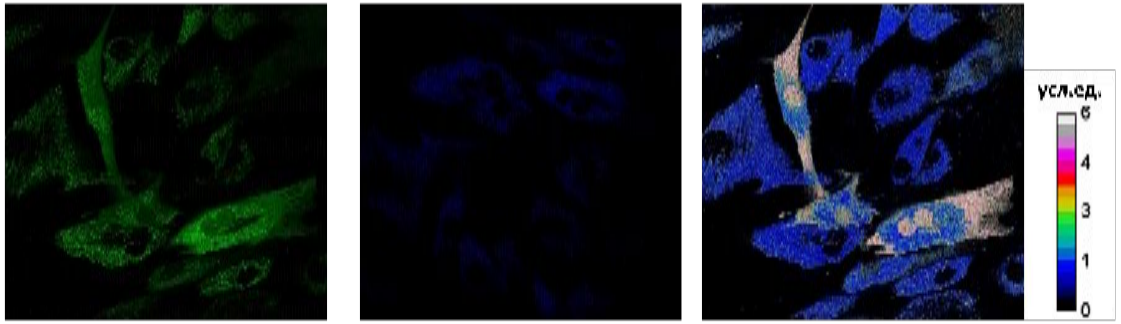
Functional-structural changes of MSCs during differentiation



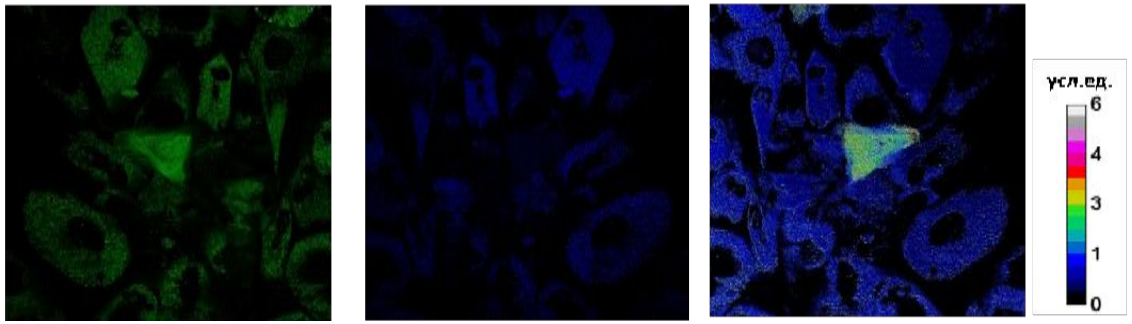
Intracellular pH analysis in MSCs during differentiation by fluorescence microscopy and SypHer–2

Fluorescence intensity I_{488} | Fluorescence intensity I_{405} | Redox ratio

Undifferentiated MSCs

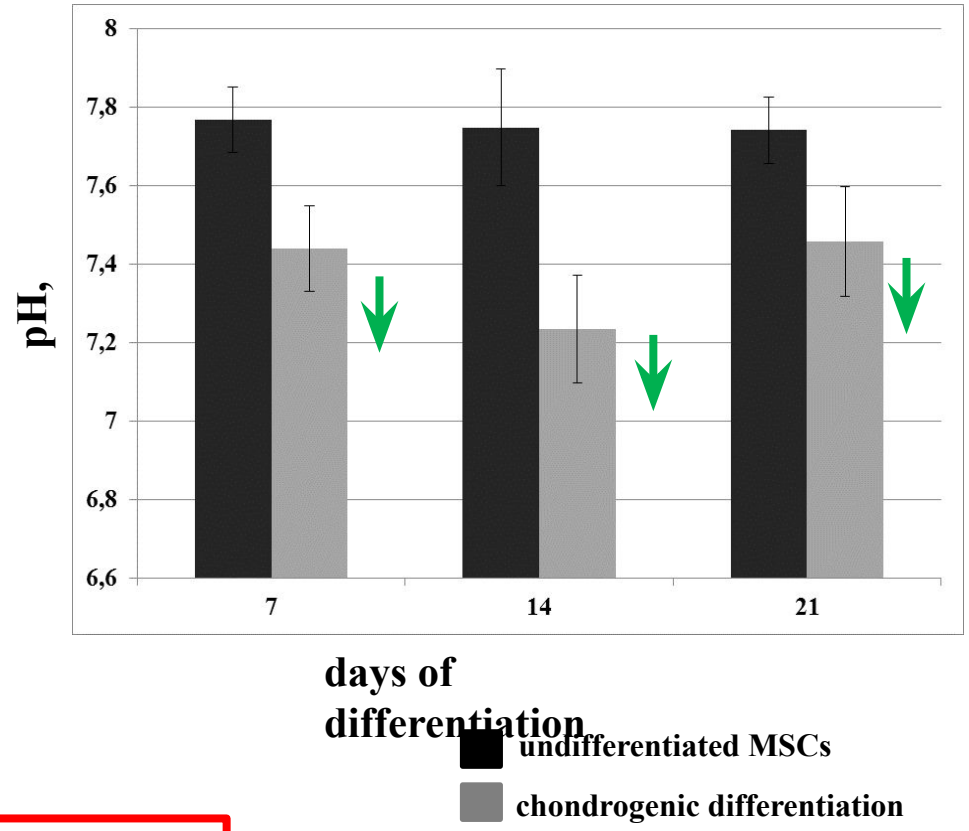


Chondrogenic differentiation of MSCs



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

$$pH = I488/I405+1,0219/0,2688$$

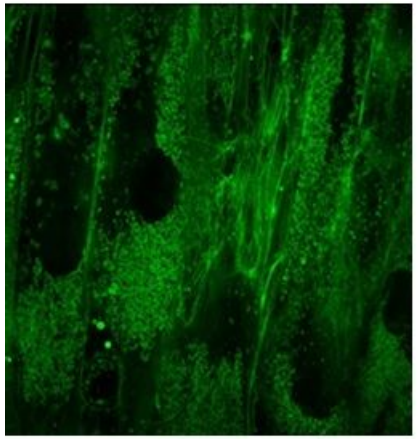


bias to acidic pH values

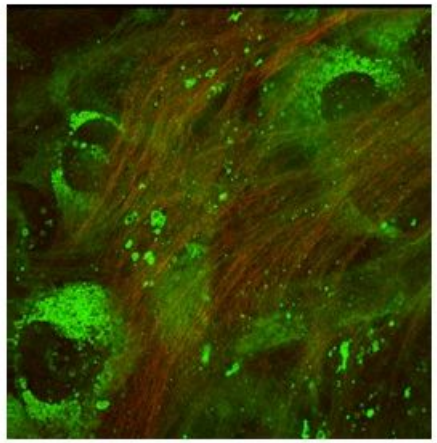
[*unpublished data*]

Analysis of collagen formation during chondrogenic differentiation using SHG

Undifferentiated MSCs



Chondrogenic differentiation of MSCs



Hematoxylin
staining

Alcian blue staining
on **acidic polysaccharides**

green –
cell autofluorescence

red- collagen fiber

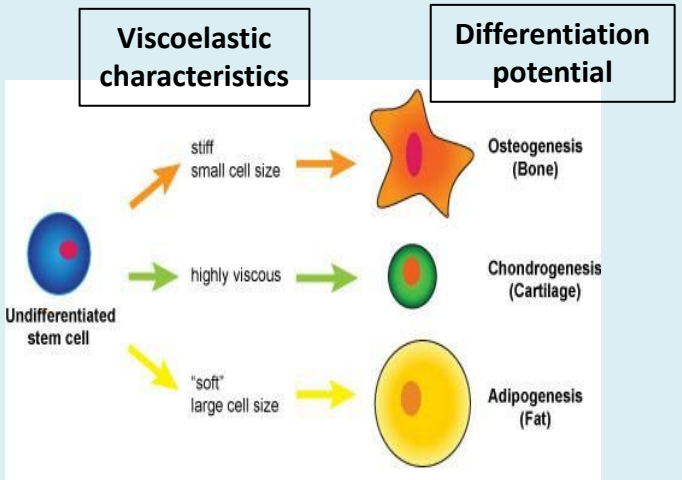
SHG of collagen was excited at wavelength of 750 nm and detected in the range 373-387 nm
the image size is 130×130 μm (512 × 512 pixels)

[Meleshina et al. Stem Cell Research & Therapy (2017) 8:15]

Functional-structural changes of MSCs during differentiation

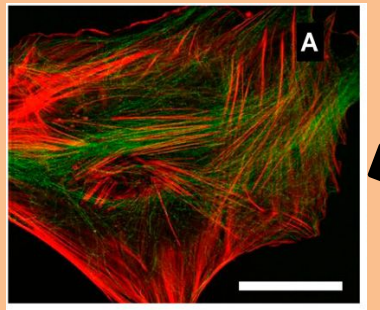
Mesenchymal Stem Cells

Viscosity



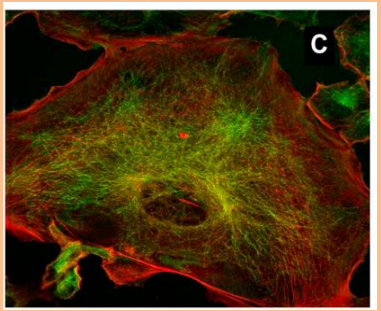
Cytoskeleton

Stem cells



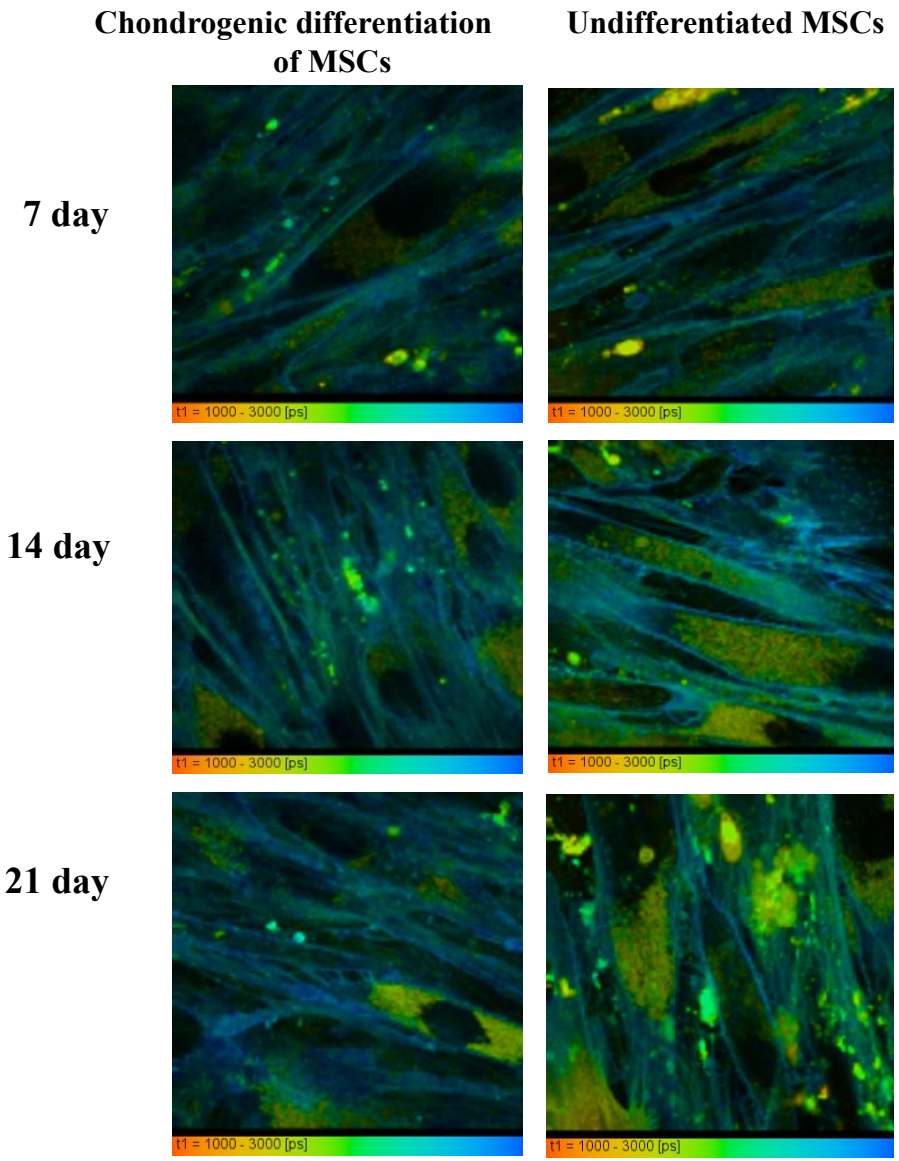
fibroblast-like, spindle shape,
long, thin stress fibers

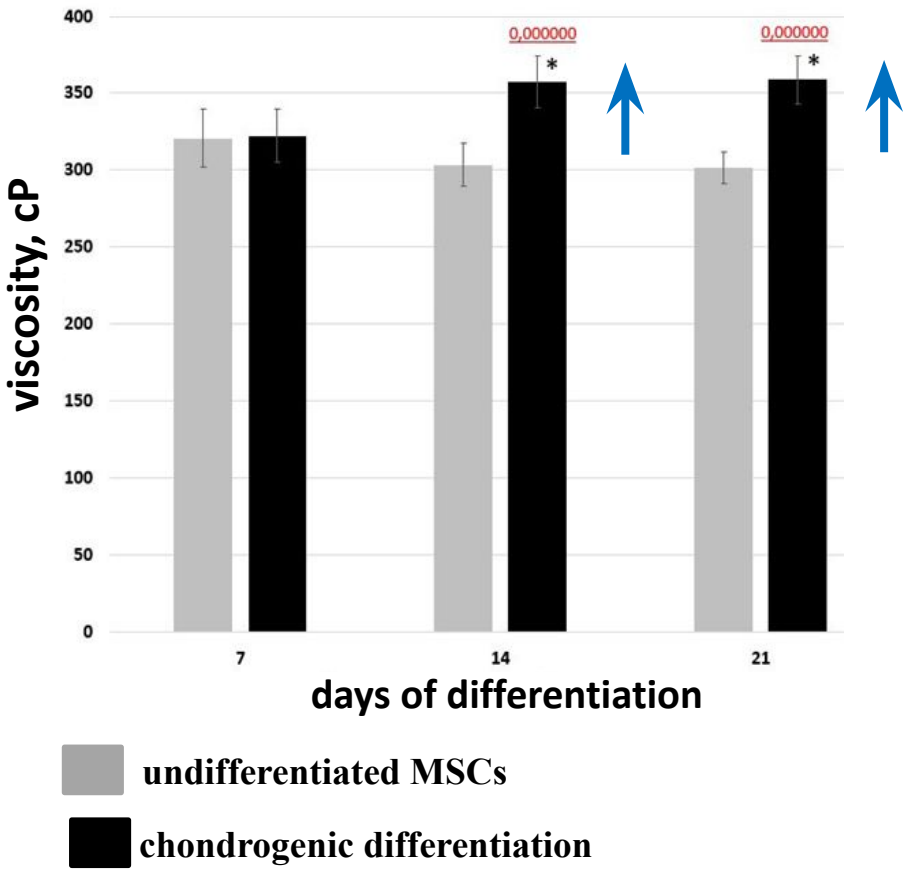
Differentiated cells



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

MSCs viscosity analysis during differentiation using FLIM and Bodipy 2





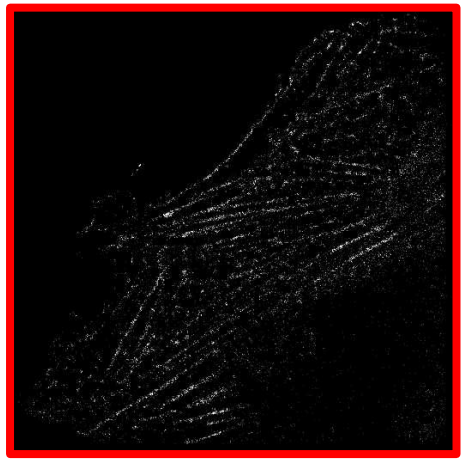
viscosity increase – cholesterol accumulation

ex of Bodipy 2 = 800 nm, detection range = 409-660 nm

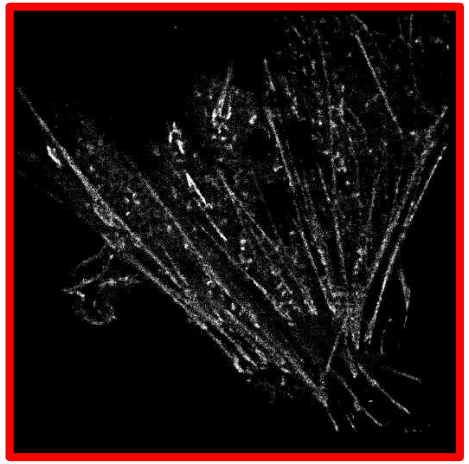
[*unpublished data*]

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

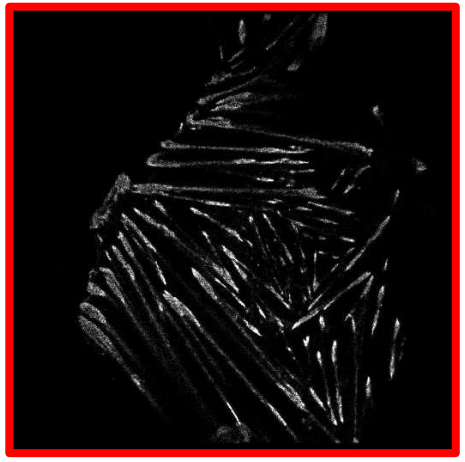
Undifferentiated MSCs



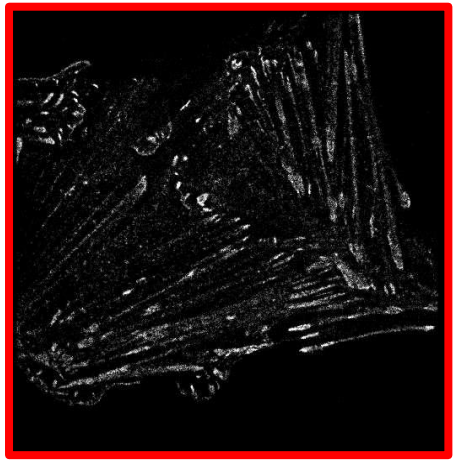
7 day

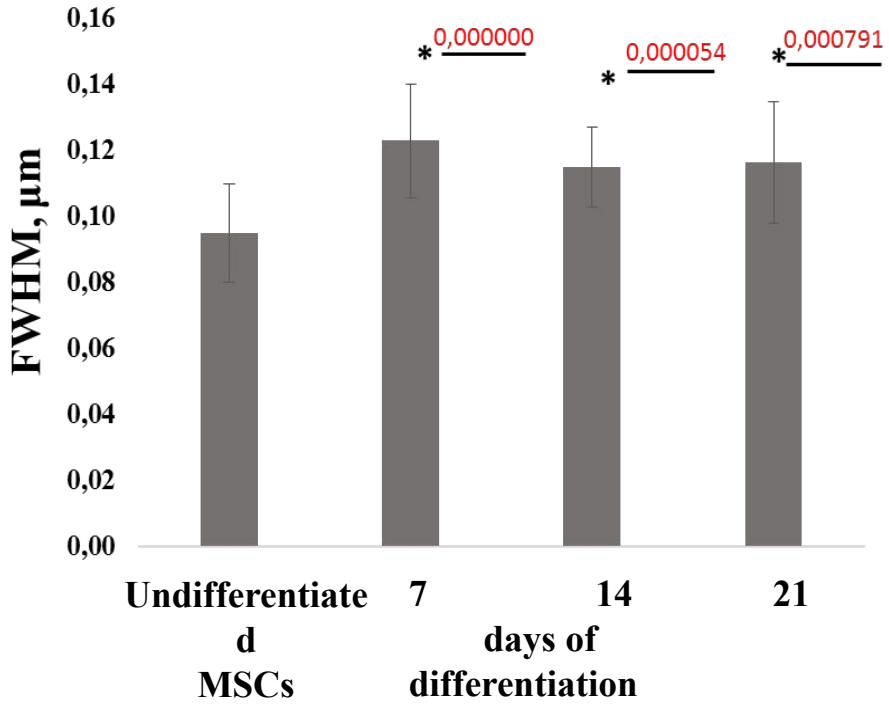


14 day



21 day





Increase of actin fibers thickness

ex of TagRFP = 555 nm, em=584 nm

[*unpublished data*]

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

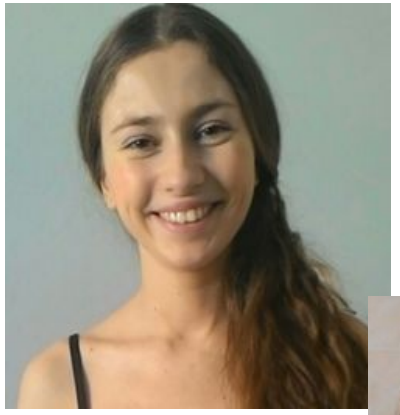
viscosity increase – cholesterol accumulation

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

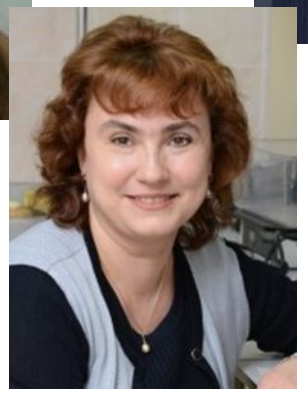
This work has been financially supported by Russian Science Foundation (grants No. 14-15-00536)



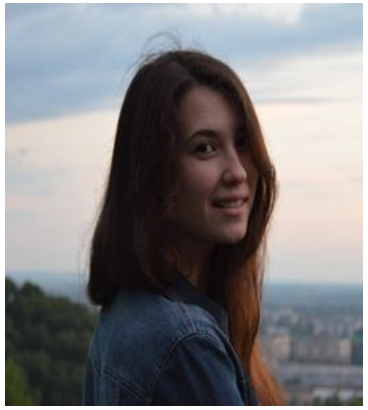
V.V. Dudenkova

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!