





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 Neuron Cardiomyocyte Adipocyte Differentiated Stem Fibroblast cells iPS Cell **Stem Cell** High pHi=7.0pHi=7.5-7.8 7.4 Mesenchymal Glycolysis Oxidative Metabolism Glycolysis Stem **Cytoskeleton** Viscosity Cells **Stem cells** Differentiation Viscoelastic potential characteristics **Differentiated cells** Osteogenesis (Bone) mall cell size Chondrogenesis (Cartilage) Undifferentiat stem cel fibroblast-like, spindle shape, Adipogenesis long, thin stress fibers large cell size (Fat) cuboidal shape, crisscrossed pattern of actin cytoskeleton, thick stress fibers

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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 Fluorescence Lifetime Imaging Microscopy (FLIM) +exso/endogenous markers Stochastic Optical Reconstruction Microscopy (STORM) +fluorescence dyes/protein

Outline of the experiment

• MSCs – human mesenchymal stem cells bone marrow





 λ, nm^{540}

Outline of the experiment

• MSCs – human mesenchymal stem cells bone marrow

(Becker & Hickl GmbH)



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Functional-structural changes of MSCs during differentiation Metabolism





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





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*213 μ m (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





[unpublished data]

Analysis of collagen formation during chondrogenic differentiation using SHG





Chondrogenic differentiation of MSCs



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 $130 \times 130 \ \mu m$ (512 × 512 pixels)

green – cell autofluorescence

red- collagen fiber

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

Functional-structural changes of MSCs during differentiation



Viscosity



Mesenchymal Stem

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





[*unpublished data*]

0,000000

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

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



Undifferentiated MSCs



[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



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Thank you for your attention!