Moscow Institute of Physics and Technology
Research Institute of Transplantology and Artificial Organs

Isolation and differentiation adult human stem cells from adipose tissue as one of the stages for hybrid organs construction

Ponomareva A.S.

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General Goal

- To develop a technology to isolate MSC from human adipose tissue
- To prove MSC differentiation ability into adipogenic and myogenic lineages

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Sources of Stem Cells

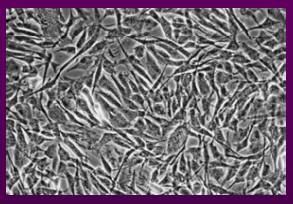
- Embryo
- Bone marrow
- Cord blood
- Adipose tissue*
- And etc. (peripheral blood, epithelia of the skin, hear follicle, synovial fluid, dental pulp, mucous membrane)

*Zuk P.A., Zhu M., Mizuno H., Huang J., Futrell W., Katz A.J., Benhaim P., Lorenz P., Hedrik M.H., Multilineage cells from human adipose tissue: implications for cell-based therapies, Tissue engineering, 2001, 7, pp. 211-228.

Adipose-derived stem cells

Fibroblast-like population of cells:

- Express multiple CD marker antigens similar to those observed on MSC from bone marrow and cord blood
- Can be maintained *in vitro* for extended periods with stable population doubling and low levels of senescence
- Differentiate *in vitro* into multiple lineages



Mizuno H., Hyakusoku H., Mesengenic potential and future clinical perspective of human processed lipoaspirate cells, Nippon Med. Sch., 2003, 70(4), pp. 300-306.

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The source of adipose tissue

- Stem cells were isolated from 4 samples of subcutaneous fat from 4 donors of Institute Transplantology and Artificial Organs
- 4 samples of operation material from 10 to 40 g

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Main protocol of isolation Stem Cells from adipose tissue

- Extensive washing with PBS, mincing, digestion with collagenase
- Inactivation of enzyme with control cell culture medium, centrifugation
- Removing of supernatant, pellet filtration through a 40-70 μm filters

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Culturing of adipose-derived cells

Maintenance in culture:

- 9 passages
- 12 days one passage
- 1000 cells/cm
- at 37°C and 5% CO2
- in noninductive control medium

Colony formations investigation:

- 2 weeks
- 400-12.5 cells/cm2
- at 37°C and 5% CO2
- in noninductive control medium

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Flow cytometry

- Adipose-derived cells were examined for expression of monoclonal antibodies specific to CD10, CD13, CD29, CD44, CD59, CD105, CD34, CD45, CD71, CD73, CD90, HLA-ABC, CD133
- Flow cytometry was performed on a FACScan argon laser cytometer (Bekton Dickinson, USA) with program FACScan Reseach

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Differentiation of adipose-derived cells

Induction of differentiation:

Adipogenic medium = MesenCalt + adipogenic supplements

Myogenic medium = control medium + dexamethasone + hydrocortisone + 10% HS

Assessment of differentiation:

Adipogenic: staining with Oil Red O (indicator of intracellular lipid accumulation)

Myogenic: estimation of antibodies expression: Anti-human MyoD1, Anti-Myogenin, Anti-human Smooth Muscle Myosin Heavy Chain

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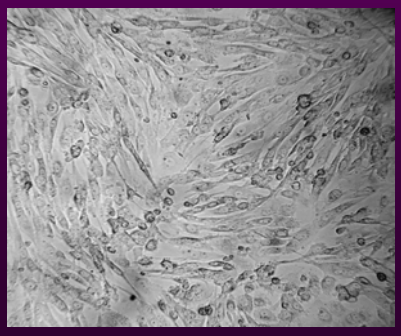
Cell yield and primary population

- The main yield of cells was 70 000-300 000 cells per gram of tissue
- Heterogeneous population of cells



Primary population of human adipose-derived cells.
Inverted-stage microscope.
Initial increase x100.

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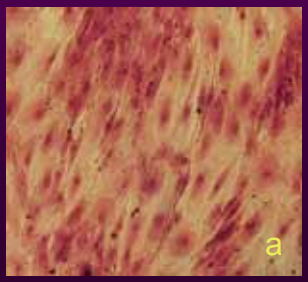
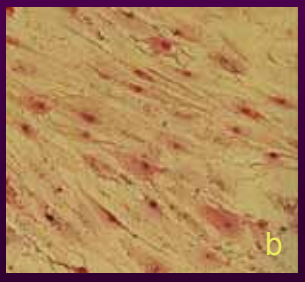
Adipose-derived cells culture

- The number of cells was increased in 2.5 – 3 times for one passage
- Culture of cells was morphologically changing when the number of passage was increasing





Culture morphology: a – II passage, b – V passage. Giemsa stain. *Inverted-stage microscope. Initial increase x400.*

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Colony formation

- Colony formations were observed after 2 weeks of culturing

Colony formation. Crystal violet stain. *Inverted-stage microscope. Initial increase x100.*

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Phenotypic characterization of adipose-derived cells: CD marker profile

- Adipose-derived cells were positive for the cell-surface markers CD10, CD13, CD29, CD44, CD59, CD73, CD90, CD105, HLA-ABC.
- And negative for the cell-surface markers CD34, CD45, CD71, CD133.
- The absence of expression of CD 34, CD 45, CD 133 signifies that hemopoetic and endotelial cells don't present in adipose-derived cells population

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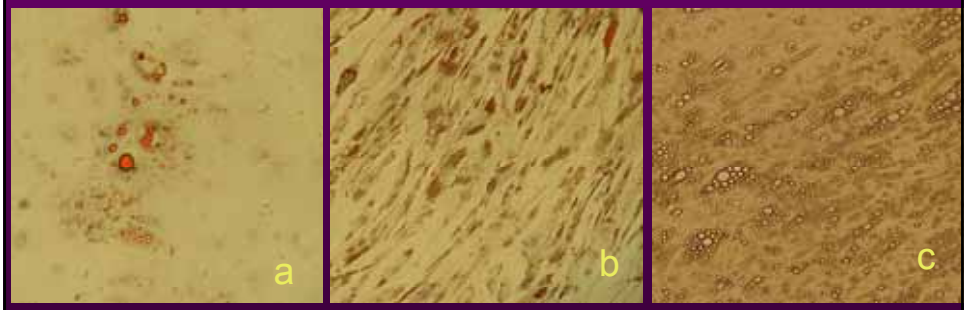
Adipose-derived cells under adipogenic differentiation *in vitro*

(3, 14 days of culturing, 2 passages)



a – 3^{d} day incubation in adipogenic medium, b,c – 14^{ht} day incubation in adipogenic medium. a, b –Oil Red O stain, hematocsilin stain, c –no stain.
Inverted-stage microscope. Initial increase x100.

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Adipose-derived cells under myogenic differentiation

(number of cells, %%)

weeks / proteins	2	3	5	6
Negative control IgG1, %	0.22	0.19	1.06	1.53
MyoD1, %	16.57	5.03	10.11	7.38
Myogenin , %	n.t.	0.51	0.53	0.33
Smooth Muscle Myosin Heavy Chain, %	n.t.	n.t.	4.49	39.93

n.t. – not tested

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Conclusions

- Original technology of isolation of fibroblast-like cells population from human adipose tissue is developed.
- Marker profile of adipose-derived human cells is similar to profile of Mesenhimal Human Stem Cells.
- An absence of hemopoetic and endotelial cells in obtained cells population have been shown.

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Conclusions

- An ability of MSC from human adipose tissue to differentiate in adipogenic lineage was observed.
- Primary data confirm an ability of MSC from human adipose tissue to differentiate in myogenic lineage.

Thank you for your attention

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