Isolation and cultivation of adipose tissue-derived mesenchymal stromal cells

Commonly used acronym: *AT-MSC*

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Organisation

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Specific Research Group or Service In Vitro Toxicology and Dermato-Cosmetology

Country Belgium

Geographical Area Brussels Region

SCOPE OF THE METHOD

The Method relates to	Animal health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	adipose tissue-derived mesenchymal stromal cells

DESCRIPTION

Method keywords

adipose tissue

Stem cells

mesenchymal stromal cells

isolation

cultivation

Scientific area keywords

stem cell culture

stem cell isolation

mesenchymal stromal cells

Method description

Approximately 125 g of processed adipose tissue is incubated for 90 minutes at 37°C in dissociation medium (1:1) consisting of 1% (v/v) bovine serum albumin and 1 mg/mL collagenase A in phosphate buffered saline (PBS). After two filtration steps, the filtrate is carefully brought on top of 15 mL of Histopaque®-1077. Upon centrifugation for 20 minutes at 1000 g (4°C), the top layer is removed and the AT-MSC are collected in 50 mL PBS/BSA (1%). This procedure is carried out separately on two pieces of adipose tissue. Typically 5 - 20 x 10E7 viable cells are obtained per 250 g of processed adipose tissue. The isolated AT-MSC are then (sub)cultured as a monolayer in AT-MSC growth medium for 2 weeks, consisting of Dulbecco’s Modified Eagle Medium supplemented with 10% (v/v) foetal bovine serum (FBS), 50 µg/mL streptomycin sulphate, 7.33 IU/mL benzyl penicillin and 2.5 µg/mL fungizone. Cell cultures are incubated at 37°C in a 5% (v/v) CO2 humidified atmosphere and passaged at subconfluency using TrypLE® express. Growth media is changed every 3 days.

Lab equipment

Biosafety cabinet level 2;

Cell incubator;

Centrifuge.

Method status

History of use

Internally validated

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

Robust isolation method for adipose tissue-derived mesenchymal stromal cells.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

De Kock J, Najar M, Bolleyn J, Al Battah F, Rodrigues RM, Buyl K, Raicevic G, Govaere O, Branson S, Meganathan K, Gaspar JA, Roskams T, Sachinidis A, Lagneaux L, Vanhaecke T, Rogiers V. (2012) Mesoderm-derived stem cells: the link between the transcriptome and their differentiation potential. Stem Cells Dev. 21(18):3309-23

Najar M, Rodrigues RM, Buyl K, Branson S, Vanhaecke T, Lagneaux L, Rogiers V, De Kock J. (2014) Proliferative and phenotypical characteristics of human adipose tissue-derived stem cells: comparison of Ficoll gradient centrifugation and red blood cell lysis buffer treatment purification methods. Cytotherapy. 16(9):1220-8