# MEDICINA:

## Ciências da saúde e pesquisa interdisciplinar

3

*Benedito Rodrigues da Silva Neto*
*(Organizador)*

Atena Editora
Ano 2021

# MEDICINA:

## Ciências da saúde e pesquisa interdisciplinar

3

*Benedito Rodrigues da Silva Neto*
*(Organizador)*

Atena Editora
Ano 2021

**Editora chefe**
Profª Drª Antonella Carvalho de Oliveira
**Assistentes editoriais**
Natalia Oliveira
Flávia Roberta Barão
**Bibliotecária**
Janaina Ramos
**Projeto gráfico**
Natália Sandrini de Azevedo
Camila Alves de Cremo
Luiza Alves Batista
Maria Alice Pinheiro
**Imagens da capa**
iStock
**Edição de arte**
Luiza Alves Batista
**Revisão**
Os autores



Atena Editora
Ano 2021

**Ciências Agrárias e Multidisciplinar**



Atena Editora
Ano 2021

Atena Editora
Ano 2021

Atena Editora
Ano 2021

Ciências Biológicas e da Saúde

# Medicina: ciências da saúde e pesquisa interdisciplinar 3


**Diagramação:** Camila Alves de Cremo
**Correção:** Flávia Roberta Barão
**Indexação:** Gabriel Motomu Teshima
**Revisão:** Os autores
**Organizador:** Benedito Rodrigues da Silva Neto

**Dados Internacionais de Catalogação na Publicação (CIP)**

M489 Medicina: ciências da saúde e pesquisa interdisciplinar 3 / Organizador Benedito Rodrigues da Silva Neto. – Ponta Grossa - PR: Atena, 2021.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
ISBN 978-65-5983-468-6
DOI: https://doi.org/10.22533/at.ed.686210809

1. Medicina. 2. Saúde. I. Silva Neto, Benedito Rodrigues da (Organizador). II. Título.

CDD 610

**Elaborado por Bibliotecária Janaina Ramos – CRB-8/9166**

**Atena Editora**
Ponta Grossa – Paraná – Brasil
Telefone: +55 (42) 3323-5493
www.atenaeditora.com.br
contato@atenaeditora.com.br

Atena Editora
Ano 2021

## DECLARAÇÃO DOS AUTORES

Os autores desta obra: 1. Atestam não possuir qualquer interesse comercial que constitua um conflito de interesses em relação ao artigo científico publicado; 2. Declaram que participaram ativamente da construção dos respectivos manuscritos, preferencialmente na: a) Concepção do estudo, e/ou aquisição de dados, e/ou análise e interpretação de dados; b) Elaboração do artigo ou revisão com vistas a tornar o material intelectualmente relevante; c) Aprovação final do manuscrito para submissão.; 3. Certificam que os artigos científicos publicados estão completamente isentos de dados e/ou resultados fraudulentos; 4. Confirmam a citação e a referência correta de todos os dados e de interpretações de dados de outras pesquisas; 5. Reconhecem terem informado todas as fontes de financiamento recebidas para a consecução da pesquisa; 6. Autorizam a edição da obra, que incluem os registros de ficha catalográfica, ISBN, DOI e demais indexadores, projeto visual e criação de capa, diagramação de miolo, assim como lançamento e divulgação da mesma conforme critérios da Atena Editora.



Atena Editora
Ano 2021

## DECLARAÇÃO DA EDITORA

A Atena Editora declara, para os devidos fins de direito, que: 1. A presente publicação constitui apenas transferência temporária dos direitos autorais, direito sobre a publicação, inclusive não constitui responsabilidade solidária na criação dos manuscritos publicados, nos termos previstos na Lei sobre direitos autorais (Lei 9610/98), no art. 184 do Código penal e no art. 927 do Código Civil; 2. Autoriza e incentiva os autores a assinarem contratos com repositórios institucionais, com fins exclusivos de divulgação da obra, desde que com o devido reconhecimento de autoria e edição e sem qualquer finalidade comercial; 3. Todos os e-book são *open access, desta forma* não os comercializa em seu site, sites parceiros, plataformas de *e-commerce,* ou qualquer outro meio virtual ou físico, portanto, está isenta de repasses de direitos autorais aos autores; 4. Todos os membros do conselho editorial são doutores e vinculados a instituições de ensino superior públicas, conforme recomendação da CAPES para obtenção do Qualis livro; 5. Não cede, comercializa ou autoriza a utilização dos nomes e e-mails dos autores, bem como nenhum outro dado dos mesmos, para qualquer finalidade que não o escopo da divulgação desta obra.



Atena Editora
Ano 2021

# APRESENTAÇÃO

A interdisciplinaridade é fruto da tradição grega, onde os programas de ensino recebiam nome de *enkúklios Paidéia* e com objetivo de trabalhar a formação da personalidade integral do indivíduo, acumulando e justapondo conhecimentos e articulação entre as disciplinas. A partir da década de 70 esse conceito se tornou muito enfático em todos os campos do conhecimento, inclusive nas ciências médicas.

Sabemos que a saúde apresenta-se como campo totalmente interdisciplinar e também com alta complexidade, já que requer conhecimentos e práticas de diferentes áreas tais como as ambientais, clínicas, epidemiológicas, comportamentais, sociais, culturais etc. Deste modo, o trabalho em equipe de saúde, de forma interdisciplinar, compreende ações planejadas em função das necessidades do grupo populacional a ser atendido não se limitando às definições exclusivistas de cada profissional.

Tendo em vista a importância deste conceito, a Atena Editora nas suas atribuições de agente propagador de informação científica apresenta a nova obra no campo das Ciências Médicas intitulada "Medicina: Ciências da Saúde e Pesquisa Interdisciplinar" em seis volumes, fomentando a forma interdisciplinar de se pensar na medicina e mais especificadamente nas ciências da saúde. É um fundamento extremamente relevante direcionarmos ao nosso leitor uma produção científica com conhecimento de causa do seu título proposto, portanto, esta obra compreende uma comunicação de dados desenvolvidos em seus campos e categorizados em volumes de forma que ampliem a visão interdisciplinar do leitor.

Finalmente reforçamos que a divulgação científica é fundamental para romper com as limitações ainda existentes em nosso país, assim, mais uma vez parabenizamos a estrutura da Atena Editora por oferecer uma plataforma consolidada e confiável para estes pesquisadores divulguem seus resultados.

Desejo a todos uma proveitosa leitura!

Benedito Rodrigues da Silva Neto

## SUMÁRIO

# CAPÍTULO 8

## CHARACTERIZATION OF NEURAL PRECURSORS OBTAINED FROM HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

*Data de aceite: 01/09/2021*
*Data de submissão: 04/06/2021*

**Nathalia Barth de Oliveira**
Advanced Therapy and Cellular Biotechnology in Regenerative Medicine Department, Pelé Pequeno Príncipe Institute, Child and Adolescent Health Research and Pequeno Príncipe Faculties
Curitiba – Paraná – Brazil
http://lattes.cnpq.br/8866080887373071

**Ana Carolina Irioda**
Advanced Therapy and Cellular Biotechnology in Regenerative Medicine Department, Pelé Pequeno Príncipe Institute, Child and Adolescent Health Research and Pequeno Príncipe Faculties
Curitiba – Paraná – Brazil
http://lattes.cnpq.br/2869392384748012

**Priscila Elias Ferreira Stricker**
Advanced Therapy and Cellular Biotechnology in Regenerative Medicine Department, Pelé Pequeno Príncipe Institute, Child and Adolescent Health Research and Pequeno Príncipe Faculties
Curitiba – Paraná – Brazil
http://lattes.cnpq.br/7617706302599916

**Bassam Felipe Mogharbel**
Advanced Therapy and Cellular Biotechnology in Regenerative Medicine Department, Pelé Pequeno Príncipe Institute, Child and Adolescent Health Research and Pequeno Príncipe Faculties
Curitiba – Paraná – Brazil
http://lattes.cnpq.br/5953351155176699

**Nádia Nascimento da Rosa**
Advanced Therapy and Cellular Biotechnology in Regenerative Medicine Department, Pelé Pequeno Príncipe Institute, Child and Adolescent Health Research and Pequeno Príncipe Faculties
Curitiba – Paraná – Brazil
http://lattes.cnpq.br/5518492186320543

**Katherine Athayde Teixeira de Carvalho**
Advanced Therapy and Cellular Biotechnology in Regenerative Medicine Department, Pelé Pequeno Príncipe Institute, Child and Adolescent Health Research and Pequeno Príncipe Faculties
Curitiba – Paraná – Brazil
http://lattes.cnpq.br/6520738424498523

**ABSTRACT:** Mesenchymal stem cells have been the focus of several studies, as they can be isolated from any vascularized tissue and can differentiate into mesodermal and non-mesodermal lineages. Adipose tissue is considered a good source for these cells, as it has a high yield in isolation and a greater capacity to form colonies. Regarding their differentiation capacity, mesenchymal stem cells from adipose tissue can differentiate into several *in vitro* lineages, such as adipogenic, chondrogenic, osteogenic, and neurogenic. During neurogenic differentiation, neurospheres, composed of neural precursors, are formed. These cells can be a potential alternative in treating neurodegenerative diseases such as Alzheimer's and Parkinson's. However, for the safe and effective use of these cells in regenerative medicine, immunophenotypic and

genotypic characterization is necessary and xenofree conditions. Therefore, this study aimed to characterize neural precursors derived from adipose tissue mesenchymal stem cells. For that, cells from the adipose tissue were isolated and cultured, expanded, and submitted to trilineage differentiation. The characterization of undifferentiated cells was carried out through flow cytometry which indicated that the isolated cells had characteristics of mesenchymal stem cells. The differentiation in neural precursors occurred through the formation of neurospheres after seeding the cells over a natural functional biopolymer matrix, called NFBX. Cells subjected to differentiation were positive for expression of Nestin and ß tubulin-III proteins in immunocytochemistry. Also, they expressed NEFM and TUBB3 genes in RT-PCR, indicating the characteristic neural phenotype of these differentiated cells and the epigenetic determination of this matrix in the differentiation of mesenchymal stem cells to neurospheres.
**KEYWORDS:** mesenchymal stem cells, adipose tissue, neurospheres, neural precursor, biopolymer.

## CARACTERIZAÇÃO DE PRECURSORAS NEURONAIS DERIVADAS DE CÉLULAS-TRONCO MESENQUIMAIS DO TECIDO ADIPOSO

**RESUMO:** As células-tronco mesenquimais têm sido o foco de diversos estudos na atualidade, pois podem ser isoladas a partir de qualquer tecido vascularizado e são capazes de se diferenciar em linhagens mesodermais e não mesodermais. O tecido adiposo é considerado uma boa fonte para obtenção dessas células, pois apresenta um alto rendimento no isolamento e uma maior capacidade de formar colônias. Com relação à sua capacidade de diferenciação, as células-tronco mesenquimais do tecido adiposo podem se diferenciar em diversas linhagens *in vitro* como, adipogênica, condrogênica, osteogênica e neurogênica. Durante a diferenciação neurogênica ocorre a formação de neuroesferas, que são compostas por precursoras neuronais, as quais são uma potencial alternativa no tratamento de doenças neurodegenerativas como o Alzheimer e o Parkinson. Contudo, para a utilização segura e eficaz dessas células na medicina regenerativa é necessária uma caracterização imunofenotípica e genotípica, assim como ser um processo livres de produtos de origem animal. Sendo assim, o objetivo desse estudo foi caracterizar as precursoras neuronais derivadas das células-tronco mesenquimais do tecido adiposo. Para isso, foram isoladas células do tecido adiposo que foram cultivadas, expandidas e, em seguida, submetidas à diferenciação *trilineage*. A caracterização das células indiferenciadas foi realizada por meio da citometria de fluxo que indicou que as células isoladas apresentaram características de células-tronco mesenquimais. A diferenciação em precursoras neuronais se deu por meio da formação de neuroesferas após a semeadura das células sobre uma matriz funcional de biopolímero natural, denominada NFBX. As células submetidas à diferenciação foram positivas para expressão das proteínas Nestina e ß tubulina-III na imunocitoquímica. Além disso, expressaram os genes NEFM e TUBB3 em RT-PCR, indicando o fenótipo neuronal característico dessas células diferenciadas e a determinação epigenética dessa matriz na diferenciação de células-tronco mesenquimais em neuroesferas.
**PALAVRAS-CHAVE:** células-tronco mesenquimais, tecido adiposo, neuroesferas, precursoras neuronais, biopolímero.

## 1 | INTRODUCTION

Currently, mesenchymal stem cells (MSCs) are in focus, as they can be isolated from any vascularized tissue and have high plasticity with the ability to originate mesodermal and non-mesodermal tissues. In addition, they secrete pro and anti-inflammatory cytokines and growth factors that provide modulation of the inflammatory response and tissue repair, contributing to the body's homeostasis (BACAKOVA et al., 2018; BATEMAN et al., 2018; BROWN et al., 2019; HAN et al., 2019).

Adipose tissue is derived from the embryonic mesoderm and has a diverse population of cells, including MSCs, capable of differentiating into osteoblasts, chondroblasts, cardiac myocytes, smooth muscle cells, and neural cells. Therefore, mesenchymal stem cells from adipose tissue (ASCs) are considered great candidates for regenerative medicine due to their wide differentiation capacity and their role in homeostasis and tissue repair throughout the life of adult organisms (MAZZEO; SANTOS, 2018; MIANA; GONZÁLEZ, 2018; SI et al., 2019).

The neurodifferentiation capacity of ASCs has made them promising for studies aimed at the treatment of neurodegenerative diseases. During neurogenic differentiation, cells form small spheres, called neurospheres, composed of neural precursors (NP), a mixed population of stem cells, and neural progenitors (LEE; LOUIS; REYNOLDS, 2015; MENDES FILHO et al., 2018; ZHANG et al., 2012). However, using these NPs in clinical treatments, it is necessary to expand the knowledge about the proteins and genes that these cells express. Consequently, basic studies such as the characterization of NPs derived from ASCs are needed, as they may assist future clinical projects enabling the safe and effective use of these cells.

## 2 | METHOD

The project used samples of adipose tissue, taken from healthy patients, over 18 years old, who signed the free and informed consent form, after approved by the ethics committee of Pequeno Príncipe Faculties (CEP / FPP) under the number: 3,049,033 on 11/30/2018.

### 2.1 Isolation, cultivation and expansion of ASCs

The samples were washed extensively with phosphate-buffered saline (PBS) containing 1% penicillin and streptomycin (P / S), were enzymatically digested with 0.075% type I collagenase at 37ºC under continuous agitation for 30 minutes. After incubation, collagenase type I activity was inactivated by adding an equal volume of Dulbecco/F12 modified Eagle (DMEM/F12), containing 10% fetal bovine serum (SFB) and 1% P/S (standard culture medium). Subsequently, the samples were centrifuged at 600g for 10 minutes, and filtered through a 100 $\mu$M mesh. Cells were plated ($10^5$ cells/cm$^2$) in 75 cm$^2$

culture flasks with standard culture medium and incubated at 37ºC and 5% $CO^2$ (adapted from BUNNELL et al., 2008; IRIODA et al., 2016; ZUK et al., 2002).

When the adherent cells reached confluence, they were detached by trypsin / EDTA (0.25%) (Sigma, St. Louis, MO - USA) for 5 minutes. The released cells were collected and replated for subculturing in culture flasks with the same culture medium. This process repeated until the cells reached passage 4 (P4) for analysis.

## 2.2 Trilineage differentiation

The cells were seeded in 24-well culture plates ($10^4$ cells per well). After reaching 80% confluence, the plates were treated with a specific differentiation medium. Cells were fixed with 4% paraformaldehyde (Sigma-Aldrich®, USA) for 20 minutes and then performed the stains.

Adipogenic differentiation was induced in standard culture medium supplemented with 0.5 µM dexamethasone (Sigma-Aldrich®, USA), 0.5 mM isobutyl-methylxanthine (Sigma-Aldrich®, USA), and 50 µM indomethacin (Sigma-Aldrich ®, USA). Cultivation with differentiation medium was maintained for 14 days and the medium was changed twice a week. The accumulation of lipid vesicles was detected by staining Oil Red O cells (Sigma-Aldrich®, USA) (BUNNELL et al., 2008; IRIODA et al., 2016).

Osteogenic differentiation was induced with standard culture medium supplemented with 1 nM dexamethasone (Sigma-Aldrich®, USA), 2 mM β-glycerol-phosphate (Sigma-Aldrich®, USA), and 50 $\mu$M ascorbate-2-phosphate (Sigma-Aldrich®, USA). The cells were cultured in this medium for 35 days. Mineralization was evaluated by staining the cells with 40 mM Alizarin Red (adapted from BUNNELL et al., 2008; IRIODA et al., 2016).

For chondrogenic differentiation, the cells were resuspended at a concentration of $1.6X10^7$ cells / mL, then 5 $\mu$L of the cell suspension was transferred to the center of the well of a 24-well plate and kept for two hours at 37 ° C. After incubation, the differentiation medium was added according to the manufacturer's specifications (StemPro® Chondrogenesis Differentiation Kit - GIBCOTM Life Technologies, USA). This medium was changed twice a week for a period of 14 days, and the production of proteoglycans was stained with the Alcian Blue in acidic pH (IRIODA et al., 2016; ZUK et al., 2001).

## 2.3 Differentiation in neural precursors

For the differentiation of ASCs into NPs, the NFBX membrane was placed in 24-well plates, sterilized under UV light, and hydrated with a standard culture medium. After 24 hours the medium was removed from the wells and the cells were seeded on the membrane at a concentration of $1X10^4$ in 20 $\mu$l of standard medium and incubated at 37°C and 5% $CO^2$ for 20 minutes. After this period, 500 $\mu$l of the standard medium was added to each well. The medium was changed twice a week until the neurospheres were obtained. With the aid of a 1000 $\mu$L micropipette, the neurospheres were removed from the wells and transferred to 75

$cm^2$ bottles for expansion and 6- and 48-well plates for analysis.

### 2.4 Flow cytometry

Undifferentiated cells were analyzed for the expression of surface markers, using monoclonal antibodies against specific antigens conjugated to fluorochromes (CD13, CD34, CD45, CD73, CD90, CD105, HLA-DR, and HLA-ABC) and analyzed by flow cytometry.

After trypsinization, cells were resuspended in 1mL PBS with 5% human albumin (5% PBS / HA). Then, 200 $\mu$L of the suspension were distributed in cytometry tubes, the conjugated antibodies were added, the tubes were vortexed and incubated in the dark for 15 minutes. After incubation, 400 $\mu$L of 5% PBS / HA was added and the tubes were vortexed again, the supernatant was discarded and the cells were resuspended with 100 $\mu$L of 5% PBS / HA. 5 $\mu$L of 7-AAD (7-aminoactinomycin D) was added to the specific tubes and the cells were incubated for 5 minutes. After incubation, 400 $\mu$L of 5% PBS / HA were added to each tube, followed by analysis on the flow cytometer (FACS Canto II; Becton Dickinson, USA) (CARVALHO et al., 2008; IRIODA et al., 2016), 10,000 cells were analyzed and data analysis was performed using the Infinicyt™ software: Flow Cytometry Software 1.6.0 (Cytognos S.L., Spain).

### 2.5 Immunocytochemistry

Undifferentiated cells and neurospheres were characterized by the expression of Nestin and $\beta$III-tubulin proteins. After the neurosphere dissociation, cells were washed with PBS and fixed with 4% paraformaldehyde for 20 minutes. The cells were permeabilized with 0.1% Triton X-100 (Amresco®, USA) diluted in PBS with 1% human albumin (1% PBS/HA) for 30 minutes. After incubation, the wells were washed with PBS and the primary antibodies diluted in 1% PBS / HA were added, which were incubated overnight at 4 °C. After incubation, the solutions with the primary antibodies were discarded and the wells washed with PBS. The cells were incubated for 1 hour at room temperature with the secondary antibody diluted in 1% PBS / HA, in the absence of light. After incubation, the wells were washed and 300 $\mu$L of PBS was added with 1 $\mu$g / mL of Hoechst 33258 (Invitrogen®M - USA) to identify the nucleus (adapted from TRZASKA; RAMESHWAR, 2011). Immunocytochemical analyzes were performed using an inverted fluorescence microscope (Axio Vert. A1 – Zeiss, Oberkochen- Germany).

### 2.6 RT-PCR

The neurospheres were grown in 6-well plates with a standard culture medium changed twice a week. When the cells reached confluence, they were submitted to the RNA extraction protocol that was carried out following the instructions of the manufacturer of the kit "PureLink ™ RNA Mini Kit" (Invitrogen®M - USA). cDNA production was performed following the instructions of the "High-Capacity cDNA Reverse Transcription Kit" kit (Invitrogen®M

– USA). The primer' sequences (forward and reverse), molecular weight of the amplified material, and annealing temperature are described in TABLE 1.

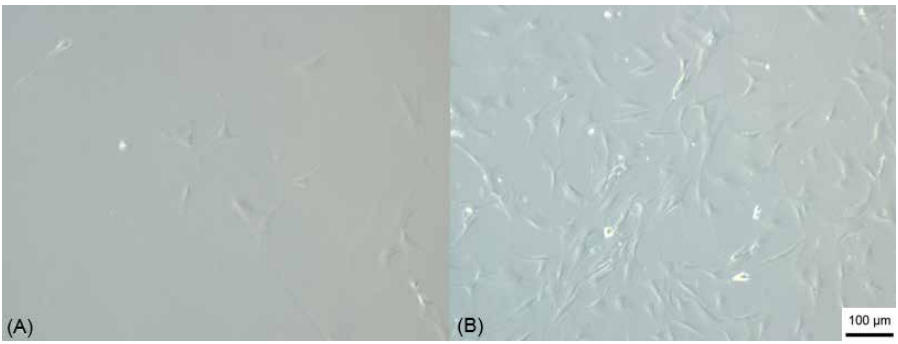
| GENE | PRIMERS | *AT (ºC) | AMPLIFIED SIZE (bp)** |
|---|---|---|---|
| *NEFM* | F: 5'ACATCGAGAGCGCCACAA3'<br>R: 5'GACGAGCCATTTCCCACTTTG3' | 60 | 98 |
| *ACTB* | F: 5'CTGGGACGACATGGAGAAAA3'<br>R:5'AAGGAAGGCTGGAAGAGTGC3' | 57 | 564 |
| *TUBB3* | F: 5'GGAGATCGTGCACATCCAGG3'<br>R: 5' CAGGCAGTCGCAGTTTTCAC' | 62 | 385 |

Note: Primers obtained from Sigma Aldrich®. * AT (ºC) = annealing temperature of the primer in degrees Celsius. ** (bp) = base pairs. Source: Author (2019).

TABLE 1 - PRIMERS USED IN THE RT-PCR REACTION.

## 3 I RESULTS AND DISCUSSION

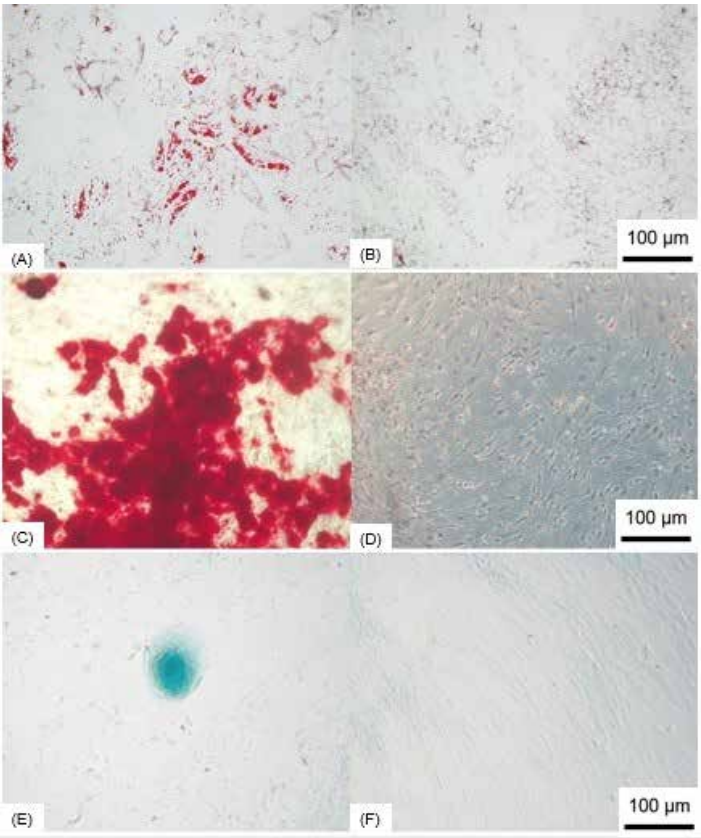
The 20 isolated samples were identified from A1 to A20 according to the order of collection. During the isolation process, the contaminating blood cells were removed after successive washes with PBS, the isolated cells adhered to the plastic and showed fibroblast morphology in approximately 3 days of culture, the cell confluence was reached after an average time of 10 days (FIGURE 1) (BORKOWSKA et al., 2015). In the study by Zuk et al. (2001), the confluence was reached in about 48 hours, but the cell concentration used in the plating is not reported, thus the difference in the confluence time may be related to the plating concentration, which in the present study was $10^5$ cells/cm$^2$ for each 75cm$^2$ bottle. Cells were grown until P4 for analysis.




(A) Cells 3 days after isolation showing fibroblast morphology. (B) Cells with 80% confluence. (Source: The Author, 2019. Image obtained by inversion optical microscopy, 100X - Axio Vert. A1 - Zeiss, Germany). Scale bar, 100 $\mu$m.

FIGURE 1 – ASCs CULTIVATION.

Eight samples were chosen at random to be submitted to *trilineage* differentiation. All samples were able to differentiate in the three strains, the adipocytes showed lipid vacuoles stained with Oil Red O (FIGURE 2a), in the osteoblasts it was possible to observe the mineralization through the staining with red alizarin (FIGURE 2c) and, the differentiation in chondroblasts was proven by staining proteoglycans with blue Alcian (FIGURE 2e).



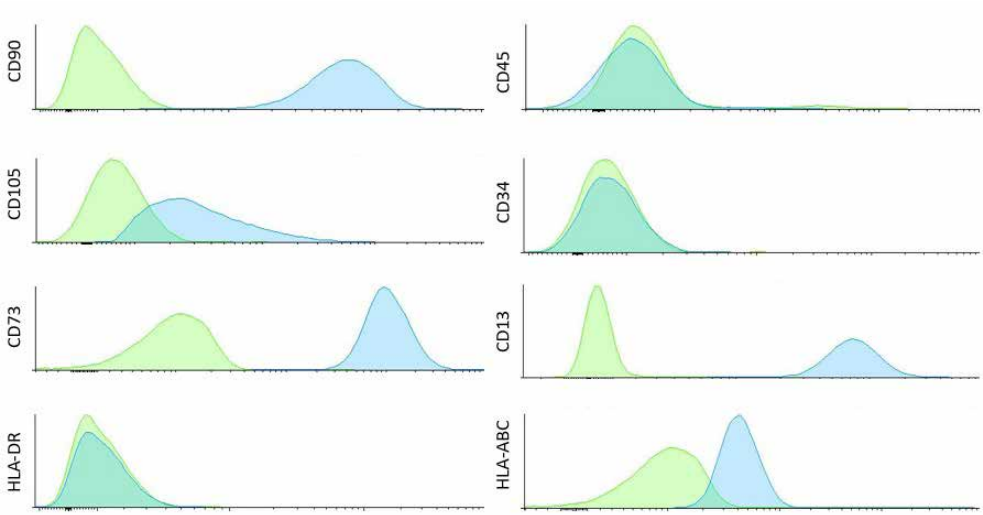

(A) ASCs subjected to adipogenic differentiation, staining the lipid vacuoles with Oil Red O. (B) Control of undifferentiated ASCs. (C) ASCs submitted to osteogenic differentiation showing mineralization stained by red alizarin. (D) Undifferentiated control. (E) ASCs differentiated in chondroblasts with the production of proteoglycans stained with Alcian blue. (F) Undifferentiated control. (Source: O Autor, 2019. Image obtained by inversion optical microscopy, 100X - Axio Vert. A1 - Zeiss, Germany). Scale bar, 100 $\mu$m.

FIGURE 2 - *TRILINEAGE* DIFFERENTIATION.

Flow cytometry was performed on 12 samples and the histograms of the sample A15 are shown in FIGURE 3. The results show that more than 80% of the cells showed positive expression of CD13, CD73, CD90, CD105, and HLA-ABC and were negative for CD45 and HLA-DR. All samples showed an expression of less than 20% of CD34 which may show positivity at the beginning of culture, however, after successive passages, this positivity

tends to disappear (BOURIN et al., 2013).

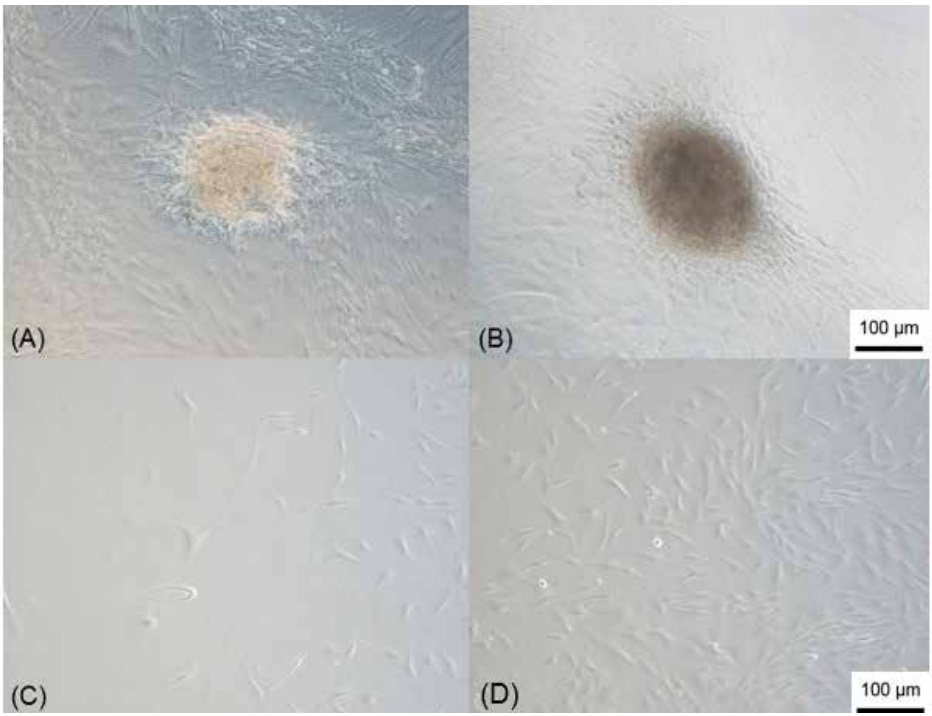
According to the International Society for Cell Therapy and the International Federation for Therapeutics and Adipose Science, ASCs must be adherent to plastic, capable of differentiating into adipocytes, osteoblasts, and chondroblasts, they must present a positive expression above 80% for CD13, CD73, CD90, and CD105, must be negative for CD45 (<2%) and, CD34 is considered an unstable marker that can be positive in up to 20% of cells (BOURIN et al., 2013).




Histograms for the analysis of flow cytometry of the sample A15. The blue peak represents positivity for CD90, CD105, CD73, CD13, and HLA-ABC and negativity for CD34, CD45, and HLA-DR markers; and in green, the isotypic control demarcating the area in which the samples are negative for a specific marker. Source: Author (2020).

FIGURE 3 – HISTOGRAMS.

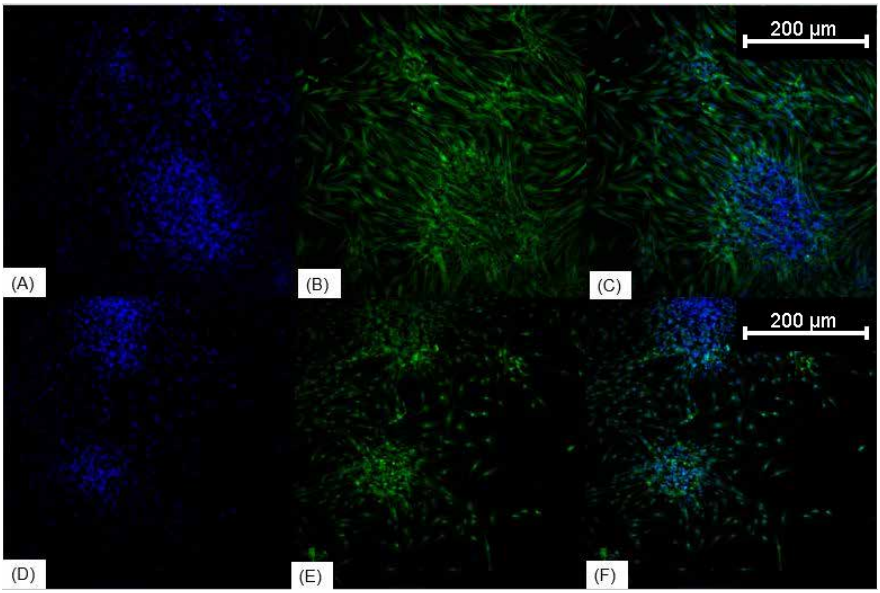
The production of neurospheres is crucial for the efficiency of neural differentiation, and most protocols use high-cost supplements or gene transfection (BORKOWSKA et al., 2015). In this study, the differentiation in NPs was through the formation of neurospheres that took place after 15-20 days of cultivation on the NFBX membrane (FIGURE 4). In the study by Zhang et al. (2012) neurospheres formation could be observed after approximately 7-9 days, but they used a neurobasal medium supplemented with epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and B27. The present study did not use any neurogenic growth factor or neurobasal medium, only the standard culture medium and the plating on the NFBX membrane. Therefore, the difference in the formation time of neurospheres may be related to the supplementation of the differentiation medium.




(A) Neurosphere formation process. (B) Neurosphere formed. (C) Expansion of NPs. (D) Expansion with 80% confluence. (Source: O Autor, 2019. Image obtained by inversion optical microscopy, 100X - Axio Vert. A1 - Zeiss, Germany). Scale bar, 100 $\mu$m.

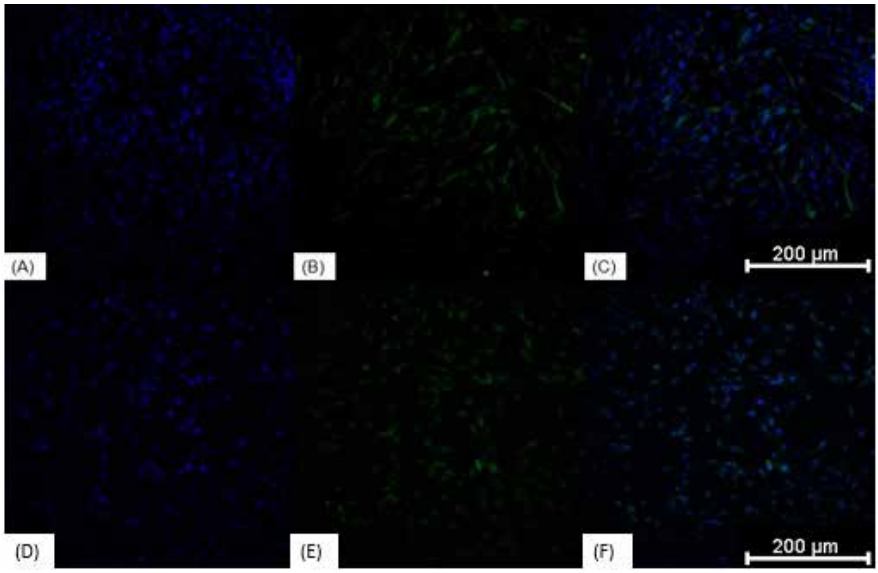
FIGURE 4 - NEUROPHERE FORMATION AND EXPANSION.

At the end of neurogenic induction, samples A3, A9, A13, and A19 were submitted to the immunocytochemistry protocol and showed positive marking for $\beta$III-tubulin and Nestin (FIGURE 5) as well as the undifferentiated cells (FIGURE 6). ASCs can express early and late neural markers, about 1-5% of the cells are positive for Nestin, however, this expression decreases as the number of passages increases. $\beta$III-tubulin is positive in about 90% of cells regardless of passage (FOUDAH et al., 2014).




(A) Neurospheres showing hoescht marking on the nucleus (blue). (B) Neurospheres with anti-βIII-tubulin antibody (FITC). (C) Images A and B overlapping. (D) Neurospheres showing hoescht marking on the nucleus (blue). (E) Neurospheres with anti-nestin antibody (FITC). (F) Images D and E overlapping (Source: O Autor, 2021. Image obtained by Fluorescence optical inversion microscope, 100X - Axio Vert. A1 - Zeiss, Germany). Scale bar, 200 $\mu$m.

FIGURE 5 – IMMUNOCYTOCHEMISTRY OF NEUROSPHERES.

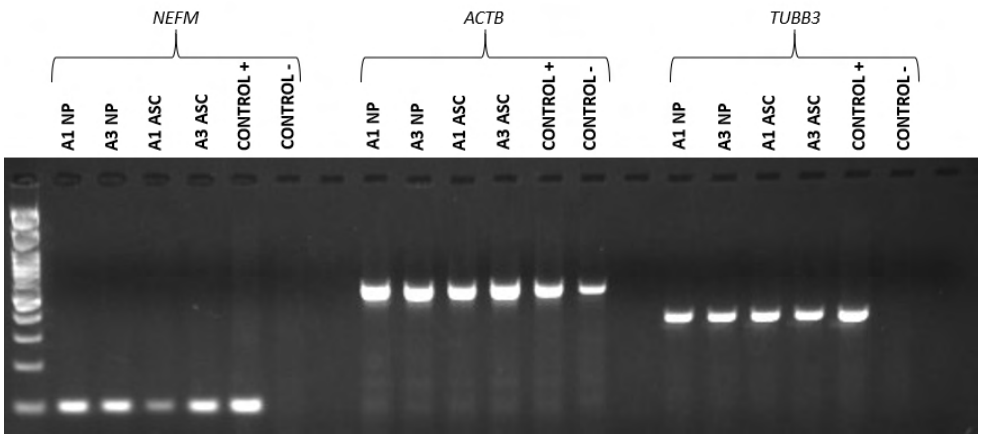



(A) ASCs showing Hoescht marking on the nucleus (blue). (B) ASC labeled with anti-βIII-tubulin antibody (FITC). (C) Images A and B overlapping. (D) ASCs with Hoescht marking on the nucleus (blue). (E) ASC labeled with anti-nestin antibody (FITC). (F) Images D and E overlapping (Source: O Autor, 2021. Image obtained by Fluorescence optical inversion microscope, 100X - Axio Vert. A1 - Zeiss, Germany). Scale bar, 200 $\mu$m.

FIGURE 6 - IMMUNICYTOCHEMISTRY OF ASCs.

RT-PCR analysis was performed for *NEFM*, *TUBB3*, and *ACTB* genes. The *NEFM* gene encodes a medium neurofilament protein that is commonly used as a biomarker of neural damage, and its expression is mostly concentrated in the brain and cerebellum (STROUS et al., 2007). The *TUBB3* gene encodes a protein expressed mainly in neurons that is involved in neurogenesis, orientation, and maintenance of axons, its expression is mainly concentrated in the fetal brain (ÖZTOP et al., 2019). The *ACTB* gene is a constitutive gene and was used as a control and validation of the technique.

ASCs express neural marker genes at the transcription level and, after neurogenic induction, this expression increases (ZHENG et al., 2017). In the present study, both the undifferentiated cells and the neurosphere-derived NPs expressed the three genes analyzed (FIGURE 7), but the quantification was not performed. The "ReNcell™ CX Human Neural Progenitor Cell Line" cell line was used as a positive control (Millipore Cat. No. SCC007) and human fibroblasts were used as a negative control.




RT-PCR for amplification of *NEFM* (98bp), *ACTB* (564bp) and *TUBB3* (385bp) genes in ASCs and NPs (Source: O Autor, 2021).

FIGURE 7 – RT-PCR ANALYSIS OF ASCs and NPs.

## 4 I CONCLUSION

This study demonstrated that ASCs could differentiate into cells with neural characteristics phenotype through cultivation on the NFBX membrane, without adding neurogenic growth factors in the culture medium or gene transfection. Future studies will be necessary to quantify the expression of neural markers both in ASCs and in NPs derived from the neurospheres. Furthermore, the study provides evidence on the ability of ASCs to respond to environmental stimuli and define the fate of cell differentiation as an epigenetic factor, like this used matrix.

## REFERENCES


BACAKOVA, L. et al. Stem cells: their source, potency and use in regenerative therapies with focus on adipose-derived stem cells - a review. **Biotechnology Advances**, v. 36, n. 4, p. 1111–1126, Aug. 2018.

BATEMAN, M. et al. Using Fat to Fight Disease: A Systematic Review of Non - Homologous Adipose - Derived Stromal / Stem Cell Therapies. **STEM CELLS**, v. 36, Feb 1 2018.

BORKOWSKA, P. et al. Differentiation of adult rat mesenchymal stem cells to GABAergic, dopaminergic and cholinergic neurons. **Pharmacological reports: PR**, v. 67, n. 2, p. 179–186, abr. 2015.

BOURIN, P. et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal / stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). **Cytotherapy**, v. 15, n. 6, p. 641–648, Jun. 2013.

BROWN, C. et al. Mesenchymal stem cells: Cell therapy and regeneration potential. **Journal of Tissue Engineering and Regenerative Medicine**, v. 13, n. 9, p. 1738–1755, set. 2019.

BUNNELL, B. A. et al. Adipose-derived stem cells: isolation, expansion and differentiation. **Methods (San Diego, Calif.)**, Vol. 45, n. 2, p. 115–120, Jun. 2008.

CARVALHO, K.A.T. et al. Angiogenesis without functional outcome after mononuclear stem cell transplant in a doxorubicin-induced dilated myocardiopathy murine model. **The International Journal of Artificial Organs**, v. 31, n. 5, p. 431–438, May 2008.

FOUDAH, D. et al. Expression of neural markers by undifferentiated mesenchymal-like stem cells from different sources. **Journal of Immunology Research**, vol. 2014, p. 987678, 2014.

HAN, Y. et al. Mesenchymal Stem Cells for Regenerative Medicine. **Cells**, v. 8, n. Aug 8, 13. 2019.

IRIODA, A. C. et al. Human Adipose-Derived Mesenchymal Stem Cells Cryopreservation and Thawing Decrease α4-Integrin Expression. **Stem Cells International**, v. 2016, p. e2562718, 15 fev. 2016.

LEE, V. M .; LOUIS, SHARON A .; REYNOLDS, BRENT A. The Central Nervous System. **Neural Stem Cells**, p. 6, abr. 2015.

MAZZEO, A .; SANTOS, E. J. C. Nanotechnology and multipotent adult progenitor cells in Reparative Medicine: therapeutic perspectives. **Einstein (São Paulo)**, v. Nov 16, 29 2018.

MENDES FILHO, D. et al. Therapy With Mesenchymal Stem Cells in Parkinson Disease: History and Perspectives. **The Neurologist**, v. 23, n. 4, p. 141–147, jul. 2018.

MIANA, V. V .; GONZÁLEZ, E. A. P. Adipose tissue stem cells in regenerative medicine. **ecancermedicalscience**, v. 12, 28 mar. 2018.

ÖZTOP, S. et al. Class III β-tubulin Expression in Colorectal Neoplasms Is a Potential Predictive Biomarker for Paclitaxel Response. **Anticancer Research**, v. 39, n. 2, p. 655–662, Feb. 2019.

SI, Z. et al. Adipose-derived stem cells: Sources, potency, and implications for regenerative therapies. **Biomedicine & Pharmacotherapy = Biomedicine & Pharmacotherapie**, v. 114, p. 108765, Jun. 2019.

STROUS, R. D. et al. Association of the dopamine receptor interacting protein gene, NEF3, with early response to antipsychotic medication. International **Journal of Neuropsychopharmacology**, v. 10, n. 3, p. 321–333, 1 Jun. 2007.

TRZASKA, K. A .; RAMESHWAR, P. Dopaminergic neural differentiation protocol for human mesenchymal stem cells. **Methods in Molecular Biology (Clifton, N.J.)**, v. 698, p. 295–303, 2011.

ZHANG, H.-T. et al. Neural differentiation ability of mesenchymal stromal cells from bone marrow and adipose tissue: a comparative study. **Cytotherapy**, v. 14, n. 10, p. 1203–1214, 1 set. 2012.

ZHENG, Y. et al. Comparison of the neural differentiation abilities of bone marrow - derived and adipose tissue - derived mesenchymal stem cells. **Molecular Medicine Reports**, v. 16, n. 4, p. 3877–3886, out. 2017.

ZUK, P.A. et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. **Tissue Engineering**, v. 7, n. 2, p. 211–228, abr. 2001.

ZUK, P. A. et al. Human adipose tissue is a source of multipotent stem cells. **Molecular Biology of the Cell**, vol. 13, n. 12, p. 4279–4295, ten. 2002.

## ÍNDICE REMISSIVO

# MEDICINA:

## *Ciências da saúde e pesquisa interdisciplinar*

3

www.atenaeditora.com.br
contato@atenaeditora.com.br
@atenaeditora
www.facebook.com/atenaeditora.com.br

Atena Editora
Ano 2021

# MEDICINA:

## Ciências da saúde e pesquisa interdisciplinar

3

www.atenaeditora.com.br
contato@atenaeditora.com.br
@atenaeditora
www.facebook.com/atenaeditora.com.br

Atena Editora
Ano 2021