Università degli Studi di Palermo
Dipartimento di Discipline Chirurgiche, Oncologiche e Stomatologiche
BIOPLAST
Laboratory of BIOlogy and regenerative medicine-PLASTic surgery
Direttore: Prof. Adriana Cordova
Responsabile scientifico: Prof. Francesco Moschella
Via del Vespro 129 – 90127 Palermo

ATTIVITÀ DI FORMAZIONE

- **Corso di Laurea magistrale II livello in Biotecnologie e Medicina Molecolare, Scuola di Medicina e Chirurgia, Dipartimento di Biomedicina, Neuroscienze e Diagnostica avanzata, Università degli Studi di Palermo:**

- 2017. Federica Grisafi, **Titolo della tesi:** *Analisi dei profili di espressione di microRNA in Sferoidi di Cellule Staminali Adipose (S-ASCs) e il loro ruolo sul mantenimento della staminalità e sul differenziamento mesenchimale.*
- 2017. Riccardo Gattuccio, **Titolo della tesi**: Analisi molecolare di una nuova popolazione di sferoidi di cellule staminali derivate da tessuto adiposo (S-ASCs) in condizioni di "in vitro long-term culture".
-2020. Marco Trapani, **Titolo della tesi**: *Nuova strategia di rigenerazione tissutale mediata da sferoidi di cellule staminali adipose.*
- 2020. Salvatore La Rosa, *In corso.*

- **Corso di Laurea Magistrale in Medicina e Chirurgia, Scuola di Medicina e Chirurgia, Dipartimento di Discipline Chirurgiche, Oncologiche e Stomatologiche, Sezione di Chirurgia Plastica e Ricostruttiva, Università degli Studi di Palermo:**

- 2018. Nicole Finocchiaro, **Titolo della tesi**: *Cellule staminali da tessuto adiposo: sedi di prelievo e tecniche di manipolazione.*
- 2020. Giorgio Pirrotta, **Titolo della tesi**: *Nuovi target molecolari nell'immunoterapia del melanoma.*

- **Scuola di specializzazione in Chirurgia Plastica, Ricostruttiva ed estetica, Scuola di Medicina e Chirurgia, Dipartimento di Discipline Chirurgiche, Oncologiche e Stomatologiche, Sezione di Chirurgia Plastica e Ricostruttiva, Università degli Studi di Palermo:**

- 2018. Luigi Montesano, **Titolo della tesi**: *Studio della capacità rigenerative degli Sferoidi di Cellule Staminali derivate da tessuto adiposo (SASCs) su un difetto della calvaria in un modello animale.*

- **Dottorato di ricerca in Oncologia e Chirurgia Sperimentali, Dipartimento di Discipline Chirurgiche Oncologiche e Stomatologiche, Università degli Studi di Palermo:**

- 2020. Federica Grisafi, **Titolo della tesi**: *3D in Suspension versus 2D in Adhesion: molecular profiles in stemness and mesenchymal differentiation of Spheroids from Adipose-derived Stem Cells*. Ciclo XXXIII

COLLABORAZIONI

-Oncologia medica, Dipartimento di Discipline Chirurgiche, Oncologiche e Stomatologiche Università degli Studi di Palermo;

-Central Laboratory of Advanced Diagnosis and Biomedical Research (CLADIBIOR), Dipartimento di Biomedicina Neuroscienze e Diagnostica avanzata, Università degli Studi di Palermo;

- Dipartimento di Ingegneria, Università degli Studi di Palermo;

- Istituto di BioFisica, Consiglio Nazionale delle Ricerche, Palermo;

- Advanced BioDesign, Parc Technologique de Lyon, France.

PUBBLICAZIONI BIENNIO 2018-2020

Impact Factor totale: 24,448

1. 2018. **Spheroids from adipose-derived stem cells exhibit an miRNA profile of highly undifferentiated cells.** Anna Barbara Di Stefano, Federica Grisafi, Marta Castiglia, Alessandro Perez, Luigi Montesano, Alessandro Gulino, Francesca Toia, Daniele Fanale, Antonio Russo, Francesco Moschella, Angelo A. Leto Barone, Adriana Cordova. J Cell Physiol. 2018 Nov;233(11):8778-8789. doi: 10.1002/jcp.26785. Epub 2018 May 24. PMID: 29797571 ***I.F.: 4.522*** ***Q1***

2. 2018. **Adipose tissue, angiogenesis and angio-MIR under physiological and pathological conditions.** Di Stefano AB, Massihnia D, Grisafi F, Castiglia M, Toia F, Montesano L, Russo A, Moschella F, Cordova A. Eur J Cell Biol. 2019 Jun;98(2-4):53-64. doi: 10.1016/j.ejcb.2018.11.005. Epub 2018 Nov 30. PMID: 30527802 ***I.F.: 3.024*** ***Q2***

3. 2019. **Immunomodulation in Vascularized Composite Allotransplantation: what role for Adipose-derived stem cells?** Marco Pappalardo, Luigi Montesano, Francesca Toia, Antonio Russo, Sara Di Lorenzo, Francesco Dieli, Francesco Moschella, Angelo A. Leto Barone, Serena Meraviglia, Anna Barbara Di Stefano. Ann Plast Surg. 2019 Feb;82(2):245-251. doi: 10.1097/SAP.0000000000001763.PMID: 30628936 ***I.F.: 1.448*** ***Q2***

4. 2019. **Γδ T Cell-Based Immunotherapy in Melanoma: State of the Art.** F. Toia , A. B. Di Stefano, S.Meraviglia, E. Lo Presti, R. Pirrello, G. Rinaldi, F. Fulfaro, F. Dieli, and A. Cordova. J Oncol. 2019 May 23;2019:9014607. doi: 10.1155/2019/9014607. eCollection 2019.PMID: 31239842 ***I.F.: 2.60*** ***Q2***

5. 2020 **MicroRNAs in solid organ and vascularized composite allotransplantation: Potential biomarkers for diagnosis and therapeutic use.** Anna Barbara Di Stefano, Marco Pappalardo, Francesco Moschella, Adriana Cordova, Francesca Toia. Transplant Rev (Orlando). 2020 Oct;34(4):100566. doi: 10.1016/j.trre.2020.100566. Epub 2020 Jul 8. PMID: 32682704 ***I.F.: 3.26*** ***Q1***

6. 2020 **Human spheroids from adipose-derived stem cells (S-ASCs) induce calvarial bone production in a xenogeneic rabbit model.** Anna Barbara Di Stefano, Luigi Montesano, Beatrice Belmonte, Alessandro Gulino, Cesare Gagliardo, Ada Maria Florena, Giuseppa Bilello, Francesco Moschella, Adriana Cordova, Angelo A. Leto Barone, Francesca Toia. Ann Plast Surg. 2020 Dec 15; Publish Ahead of Print. doi: 10.1097/SAP.0000000000002579. Online ahead of print.PMID: 33346554 ***I.F.: 1.448*** ***Q2***

7. 2020 **In-situ gelling xyloglucan formulations as 3D artificial niche for adipose stem cell spheroids.** F. Toia A.B. Di Stefano, E. Muscolino, M.A. Sabatino, D. Giacomazza, F. Moschella, A. Cordova, C. Dispenza. Int J Biol Macromol. 2020 Oct 23:S0141-8130(20)34806-6. doi: 10.1016/j.ijbiomac.2020.10.158. Online ahead of print. PMID: 33470202 ***I.F.: 5.162*** ***Q1***

8. 2021 **Cell quality evaluation with gene expression analysis of spheroids (3D) and adherent (2D) adipose stem cells.** Anna Barbara Di Stefano, Federica Grisafi, Mileidys Perez-Alea, Marta Castiglia, Marta Di Simone, Serena Meraviglia, Adriana Cordova, Francesco Moschella, Francesca Toia. Gene. 2021 Feb 5;768:145269. doi: 10.1016/j.gene.2020.145269. Epub 2020 Oct 24. PMID: 33148459 ***I.F.: 2.984*** ***Q1***

Received: 30 November 2017 | Accepted: 30 April 2018

DOI: 10.1002/jcp.26785

ORIGINAL RESEARCH ARTICLE

WILEY Journal of Cellular Physiology

Spheroids from adipose-derived stem cells exhibit an miRNA profile of highly undifferentiated cells

A. Barbara Di Stefano PhD[1,2] | Federica Grisafi MSc[1,2] | Marta Castiglia PhD[2] | Alessandro Perez PhD[2] | Luigi Montesano MD[1] | Alessandro Gulino PhD[3] | Francesca Toia MD[1] | Daniele Fanale PhD[2] | Antonio Russo MD[2] | Francesco Moschella MD[1] | Angelo A. Leto Barone MD[4] | Adriana Cordova MD[1]

[1]Department of Surgical, Oncological and Oral Sciences, Section of Plastic and Reconstructive Surgery, University of Palermo, Palermo, Italy

[2]Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, Palermo, Italy

[3]Department of Health Science, Human Pathology Section, Tumor Immunology Unit, University of Palermo, Palermo, Italy

[4]Department of Plastic and Reconstructive Surgery, Johns Hopkins School of Medicine, Baltimore, MD

Correspondence
Antonio Russo, MD, Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, via del Vespro 129, 90127 Palermo, Italy
Email: antonio.russo@usa.net

Funding information
Italian Ministry of Health, Grant/Award Number: GR-2010-2321017

Two-dimensional (2D) cell cultures have been extensively used to investigate stem cell biology, but new insights show that the 2D model may not properly represent the potential of the tissue of origin. Conversely, three-dimensional cultures exhibit protein expression patterns and intercellular junctions that are more representative of their in vivo condition. Multiclonal cells that grow in suspension are defined as "spheroids," and we have previously demonstrated that spheroids from adipose-derived stem cells (S-ASCs) displayed enhanced regenerative capability. With the current study, we further characterized S-ASCs to further understand the molecular mechanisms underlying their stemness properties. Recent studies have shown that microRNAs (miRNAs) are involved in many cellular mechanisms, including stemness maintenance and proliferation, and adipose stem cell differentiation. Most studies have been conducted to identify a specific miRNA profile on adherent adipose stem cells, although little is still known about S-ASCs. In this study, we investigate for the first time the miRNA expression pattern in S-ASCs compared to that of ASCs, demonstrating that cell lines cultured in suspension show a typical miRNA expression profile that is closer to the one reported in induced pluripotent stem cells. Moreover, we have analyzed miRNAs that are specifically involved in two distinct moments of each differentiation, namely early and late stages of osteogenic, adipogenic, and chondrogenic lineages during long-term in vitro culture. The data reported in the current study suggest that S-ASCs have superior stemness features than the ASCs and they represent the true upstream stem cell fraction present in adipose tissue, relegating their adherent counterparts.

KEYWORDS
adipose stem cells, long-term culture, mesenchymal differentiation, miR-142-3p, miRNAs

European Journal of Cell Biology 98 (2019) 53–64

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb

Review

Adipose tissue, angiogenesis and angio-MIR under physiological and pathological conditions

Anna Barbara Di Stefano[a,b,*,1], Daniela Massihnia[b,1], Federica Grisafi[a,b], Marta Castiglia[b], Francesca Toia[a], Luigi Montesano[a], Antonio Russo[b], Francesco Moschella[a], Adriana Cordova[a]

[a] Department of Surgical, Oncological and Oral Sciences, Section of Plastic and Reconstructive Surgery, University of Palermo, 90127 Palermo, Italy
[b] Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, University of Palermo, 90127 Palermo, Italy

ARTICLE INFO

Keywords:
Adipose stem cells
Endothelial cells
Angiogenesis
miRNAs

ABSTRACT

Angiogenesis is a crucial process for the maintenance of normal tissue physiology and it is involved in tissue remodeling and regeneration. This process is essential for adipose tissue maintenance. The adipose tissue is composed by different cell types including stromal vascular cells as well as adipose stem cells (ASCs). In particular, ASCs are multipotent somatic stem cells that are able to differentiate and secrete several growth factors; they are recently emerging as a new cell reservoir for novel therapies and strategies in many diseases. Several studies suggest that ASCs have peculiar properties and participate in different disease-related processes such as angiogenesis. Furthermore, pathological expansion of adipose tissue brings to hypoxia, a major condition of unhealthy angiogenesis.

Recent evidences have shown that microRNAs (miRNAs) play a crucial role also on ASCs as they take part in stemness maintenance, proliferation, and differentiation. It has been suggested that some miRNAs (MIR126, MIR31, MIR221 MIR222, MIR17-92 cluster, MIR30, MIR100 and MIR486) are directly involved in the angiogenic process by controlling multiple genes involved in this pathway. With the present review, we aim at providing an updated summary of the importance of adipose tissue under physiological and pathological conditions and of its relationship with neovascularization process. In particular, we report an overview of the most important miRNAs involved in angiogenesis focusing on ASCs. Hopefully the data presented will bring benefit in developing new therapeutic strategies.

Immunomodulation in Vascularized Composite Allotransplantation

What Is the Role for Adipose-Derived Stem Cells?

Marco Pappalardo, MD, Luigi Montesano, MD,* Francesca Toia, MD, PhD,* Antonio Russo, MD, PhD,† Sara Di Lorenzo, MD, PhD,* Francesco Dieli, MD, PhD,‡§ Francesco Moschella, MD,* Angelo A. Leto Barone, MD,*|| Serena Meraviglia, MD,‡§ and Anna Barbara Di Stefano, PhD*†*

Abstract: Hand and face transplants are becoming increasingly common, recording progressively more penile, uterus, abdominal wall, and allotransplantation cases reported worldwide. Despite current protocols allow long-term survival of the allografts, the ultimate goal of donor-specific tolerance has not been achieved yet. In fact, the harmful adverse effects related to the lifelong administration of immunosuppressive agents are the main drawbacks for vascularized composite allotransplantations. Research is very active in investigating alternative methods to induce greater tolerance while minimizing toxicity. Adipose-derived stem cells (ASCs) represent promising cell therapies for immunomodulation in preclinical and clinical settings. Their clinical appeal is due to their easy harvest in large quantities through a noninvasive and well-accepted approach; they may well promote donor-specific tolerance and potentially reduce immunosuppression. Several experimental studies exist, but lacking review articles reporting current evidence. This work proposes a literature review on the immunomodulatory role of ASCs in vascularized composite allotransplantations. In vitro and in vivo evidence will be summarized. The role that cell passaging and upstream progenitors—the so-called spheroid ASCs—may play in modulating the immune response will also be discussed. Finally, this article will summarize current knowledge on biodistribution, migration, and homing of injected stem cells. This review may well provide useful information for preclinical and clinical studies, aiming at a breakthrough for donor-specific tolerance.

Key Words: adipose-derived stem cells, cell-based therapies, face transplantation, hand transplantation, vascularized composite allotransplantations

(*Ann Plast Surg* 2019;82: 245–251)

Since their first description, more than 100 human hand and 37 face vascularized composite allotransplantations (VCAs) have been reported in the past 18 years,[1–4] proving not only their technical feasibility, but also the superior outcomes compared with conventional reconstruction in selected patients. Nonetheless, VCA is not routinely performed because of the condition of lifelong immunosuppression and toxicity profiles.[2] The major complications resulting from immunosuppressive therapies include increased propensity to develop infections, organ toxicity (ie, nephrotoxicity), and malignancy.[5]

Furthermore, unlike solid organ transplantation, VCAs are life enhancing rather than lifesaving, leading to many ethical and medical issues involving individual surgeons as well as professional societies.[1]

The use of novel cell-based therapies that combine the benefits of immunoregulation with neuroregeneration proves to have a great potential in improving functional outcomes and the quality of life in VCA patients.[6]

It is known that mesenchymal stem cells (MSCs) are "immune privileged" because of their low expression of human leukocyte antigen and costimulatory molecules. Furthermore, they exert potent immunosuppressive actions.[7] These desirable properties make MSCs a new therapeutic option; MSCs have emerged as a promising cell-based therapy for immunomodulation and are currently being examined in preclinical and clinical settings as therapeutic solutions for autoimmune disorders or transplant rejection.[8–12]

To date, bone marrow–derived MSCs (BM-MSCs) are considered the standard cell type used in the fields of stem cell biology and clinical application. However, adipose-derived stem cells (ASCs), abundant in the human body, could be harvested with minimal invasiveness and are becoming increasingly widespread in treating various diseases.[13] Many studies have aimed to investigate the phenotypic and intrinsic biological differences between traditional adherent and nonadherent ASCs.[14,15] The recent isolation of a nonadherent ASC population paved the way to the possibility that this niche could include cells at a less advanced stage of differentiation and therefore more upstream in the stem cell hierarchy. Despite scientists have began using spheroids from ASCs (S-ASCs) in a number of laboratories worldwide, their identity as a separate stem cell niche with different phenotype, biological behavior, and differentiation ability has never been fully defined.

The following summarizes current literature available on in vitro and in vivo studies concerning the immunomodulatory effects of ASCs and discusses the potential role that the 3-dimensional (3D) niche and upstream progenitors have in modulating the immune system. Finally, we summarize current knowledge available on biodistribution, migration, and homing of injected stem cells.

Immunomodulatory Role of ASCs

The so-called ASCs are a variety of MSCs extracted from adipose tissue and also defined as fibroblast-like, adherent, and multipotent cells. Indeed, ASCs are able to differentiate along multiple lineages including adipocytes, osteoblasts, and chondrocytes, as well as other cell types including hepatocytes, myocytes, and neuronal-like cells.[16,17] The successful isolation of multipotent ASCs achieved in the past decade and their ability to preferentially home to damaged tissue make ASCs a promising tool in regenerative medicine.[17–19] Indeed, ASCs can be engineered and used as biological vehicles for vector-based genes to convey therapeutic molecules in vivo.[20,21] Furthermore,

Received February 15, 2018, and accepted for publication, after revision October 17, 2018.

From the *Plastic and Reconstructive Surgery and †Medical Oncology, Department of Surgical, Oncological and Oral Sciences; ‡Central Laboratory of Advanced Diagnosis and Biomedical Research (CLADIBIOR); and §Department of Biopathology and Biomedical Methodologies, University of Palermo, Palermo, Italy; and ||Plastic and Reconstructive Surgery, Johns Hopkins Medicine, Department of Plastic and Reconstructive Surgery, Baltimore, MD.

Author Contributions: Special topic conceived by M.P., F.T, S.M., and A.B.D.S. Manuscript drafted by M.P., A.A.L.B., L.M., S.D.L., F.T., S.M., and A.B.D.S. Manuscript revision carried out by A.R., F.D., F.M., and A.A.L.B.

Conflicts of interest and sources of funding: none declared.

Reprints: Francesca Toia, MD, PhD, Plastic and Reconstructive Surgery, Department of Surgical, Oncological and Oral Sciences, University of Palermo, Via del Vespro, 129, 90127, Palermo, Italy. E-mail: francescatoia@gmail.com.

ISSN: 0148-7043/19/8202–0245
DOI: 10.1097/SAP.0000000000001763

Hindawi
Journal of Oncology
Volume 2019, Article ID 9014607, 8 pages
https://doi.org/10.1155/2019/9014607

Review Article

Γδ T Cell-Based Immunotherapy in Melanoma: State of the Art

F. Toia,[1] A. B. Di Stefano,[1] S. Meraviglia,[2] E. Lo Presti,[2] R. Pirrello,[1] G. Rinaldi,[3] F. Fulfaro,[3] F. Dieli,[2] and A. Cordova[1]

[1]*Department of Surgical, Oncological and Oral Sciences, Plastic and Reconstructive Surgery Section, University of Palermo, 90127, Italy*
[2]*Department of Biopathology, Central Laboratory of Advanced Diagnosis and Biomedical Research (CLADIBIOR), University of Palermo, 90127, Italy*
[3]*Department of Surgical, Oncological and Oral Sciences, Oncology Section, University of Palermo, 90127, Italy*

Correspondence should be addressed to A. B. Di Stefano; annabarbara.distefano@gmail.com

Received 28 December 2018; Accepted 2 May 2019; Published 23 May 2019

Guest Editor: Aditya B. Pant

Metastatic melanoma is still associated with a poor prognosis, and there is increasing interest in immunotherapy alone or in combination with other adjuvant therapies. Γδ T lymphocytes play a pivot role in the immune response against cancer, but while γδ-based immunotherapy is already a clinical reality for several solid tumors, data on melanoma are still limited and fragmented. This systematic review presents preclinical and clinical evidence for a role of γδ T lymphocytes in immunotherapeutic strategies for advanced melanoma and discusses research state of the art and future perspectives. Current strategies focus on in vivo stimulation, and ex vivo adoptive therapy and vaccination; results are promising, but further studies are needed to better investigate the interactions in tumoral microenvironment and to improve clinical efficacy of immunotherapeutic protocols.

Transplantation Reviews 34 (2020) 100566

Contents lists available at ScienceDirect

Transplantation Reviews

journal homepage: www.elsevier.com/locate/trre

Review article

MicroRNAs in solid organ and vascularized composite allotransplantation: Potential biomarkers for diagnosis and therapeutic use

Anna Barbara Di Stefano, PhD [a,1,*], Marco Pappalardo, MD, PhD [a,1], Francesco Moschella, MD [a], Adriana Cordova, MD [a,b,c], Francesca Toia, MD,PhD [a,b,c]

[a] BIOPLAST-Laboratory of BIOlogy and Regenerative Medicine-PLASTic Surgery, Plastic and Reconstructive Surgery Section, Department of Surgical, Oncological and Oral Sciences, University of Palermo, 90127 Palermo, Italy
[b] Plastic and Reconstructive Surgery Section, Department of Surgical, Oncological and Oral Sciences, University of Palermo, 90127 Palermo, Italy
[c] Plastic and Reconstructive Unit, Department of Oncology, Azienda Ospedaliera Universitaria Policlinico "Paolo Giaccone", 90127 Palermo, Italy

ARTICLE INFO

Keywords:
Vascularized composite allotransplantations
Micro-RNAs
Biomarker
Immune rejection
Organ transplantation
Tolerance

ABSTRACT

Nowadays, solid organ transplantation (SOT) is an established treatment for patients with end-organ dysfunction, which dramatically improves the quality-of-life. Vascularized composite allotransplants (VCAs) including hand and face have been reported worldwide over the last 20 years. However, VCAs, differently to SOT, are life-enhancing instead of life-saving and are not routinely performed due to the risk of immune rejection and the adverse effects of immunosuppression.

Over the past decade, although considerable improvements in short-term outcomes after allotransplantation have been registered, these results have not been translated into major progress in long-term allograft acceptance and patient survival. Recently active researches in the field of biomarker discovery have been conducted to develop individualized therapies for allograft recipients.

MicroRNAs (miRNAs) are a small noncoding RNAs functioning as critical regulators of gene and protein expression by RNA interference. They have been connected in numerous biological processes and diseases. Due to their immunomodulatory functions, miRNAs have been amended as potential diagnostic and prognostic biomarker for the detection of rejection in allotransplantation. Due to their specific circulating expression profile, they could act as noninvasive predictive tools for rejection that may help clinicians in an early adjustment of the immunosuppression protocol during acute rejections episodes. Indeed, specific anti-sense oligonucleotides suppressing miRNAs expressed in rejection could reduce the rejection rate in allografts and decrease the use of immunosuppressants.

We present a literature review of the immunomodulatory properties and characteristics of miRNAs. We will summarize the current knowledge on miRNAs as potential biomarkers for allograft rejection and possible application in allotransplantation monitoring. Finally, we will discuss the advances in preclinical miRNA-based therapies for immunosuppression.

› Ann Plast Surg. 2020 Dec 15;Publish Ahead of Print. doi: 10.1097/SAP.0000000000002579.
Online ahead of print.

Human Spheroids from Adipose-Derived Stem Cells Induce Calvarial Bone Production in a Xenogeneic Rabbit Model

Anna Barbara Di Stefano [1], Luigi Montesano, Beatrice Belmonte, Alessandro Gulino, Cesare Gagliardo, Ada Maria Florena, Giuseppa Bilello, Francesco Moschella, Adriana Cordova, Angelo A Leto Barone, Francesca Toia

Affiliations + expand

PMID: 33346554 DOI: 10.1097/SAP.0000000000002579

Abstract

Calvarial defects can result from several causes. Tissue engineering hold the potential to restore native form and protective function. We have recently shown that stemness and differentiation ability of spheroids from adipose-derived stem cells (S-ASCs) promotes osteoblasts growth within Integra in a small vertebral lesion. In our study, we aimed to test osteogenic potential of S-ASCs in aiding regeneration of a calvarial defect. Groups containing Integra showed increased bone regeneration at the calvarial defect-Integra interface compared with the control group. In particular, S-ASC-derived osteoblasts group showed a superior calvarial remodeling than undifferentiated S-ASCs group. Clusters of ossification were observed in these both groups with enhanced microvasculature density and fibrosis. In conclusion, seeding of S-ASCs in dermal regeneration templates enhanced bone healing in a rabbit calvarial defect model. These findings could prompt the elective use of S-ASCs with enhanced multilineage differentiation potential for tissue engineering purposes.

International Journal of Biological Macromolecules 165 (2020) 2886–2899

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

In-situ gelling xyloglucan formulations as 3D artificial niche for adipose stem cell spheroids

F. Toia [a,b,1], A.B. Di Stefano [b,1], E. Muscolino [c], M.A. Sabatino [c], D. Giacomazza [d], F. Moschella [b], A. Cordova [a,b], C. Dispenza [c,d,*]

[a] Dipartimento di Discipline Chirurgiche, Oncologiche e Stomatologiche, Università degli Studi di Palermo, via del Vespro 129, 90127 Palermo, Italy
[b] BIOPLAST-Laboratory of BIOlogy and Regenerative Medicine-PLASTic Surgery, Dipartimento di Discipline Chirurgiche, Oncologiche e Stomatologiche, Università degli Studi di Palermo, via del Vespro 129, 90127 Palermo, Italy
[c] Dipartimento di Ingegneria, Università degli Studi di Palermo, Viale delle Scienze 6, 90128 Palermo, Italy
[d] Istituto di BioFisica, Consiglio Nazionale delle Ricerche, Via U. La Malfa 153, 90146 Palermo, Italy

ARTICLE INFO

Article history:
Received 25 June 2020
Received in revised form 16 October 2020
Accepted 20 October 2020
Available online 24 October 2020

Keywords:
Spheroids of adipose stem cells
Artificial niche
In-situ forming gel
Partially degalactosylated xyloglucan

ABSTRACT

Three-dimensional spheroidal cell aggregates of adipose stem cells (SASCs) are a distinct upstream population of stem cells present in adipose tissue, with enhanced regeneration properties *in vivo*. The preservation of the 3D structure of the cells, from extraction to administration, can be a promising strategy to ensure optimal conditions for cell viability and maintenance of stemness potential. With this aim, an artificial niche was created by incorporating the spheroids into an injectable, in-situ gelling solution of partially degalactosylated xyloglucan (dXG) and an ad hoc formulated culture medium for the preservation of stem cell spheroid features. The evolution of the mechanical properties and the morphological structure of this artificial niche was investigated by small amplitude rheological analysis and scanning electron microscopy, respectively. Comparatively, systems produced with the same polymer and the typical culture medium (DMEM) used for adipose stem cell (ASC) growth in adherent cell culture conditions were also characterised. Cell viability of both SASCs and ASCs incorporated inside the hydrogel or seeded on top of the hydrogel were investigated as well as the preservation of SASC stemness conditions when embedded in the hydrogel.

Gene 768 (2021) 145269

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Cell quality evaluation with gene expression analysis of spheroids (3D) and adherent (2D) adipose stem cells

Anna Barbara Di Stefano [a,*,1], Federica Grisafi [a,1], Mileidys Perez-Alea [b], Marta Castiglia [c], Marta Di Simone [d,e], Serena Meraviglia [d,e], Adriana Cordova [a,f,g], Francesco Moschella [a], Francesca Toia [a,f,g]

[a] BIOPLAST-Laboratory of BIOlogy and Regenerative Medicine-PLASTic Surgery, Plastic and Reconstructive Surgery, Department of Surgical, Oncological and Oral Sciences, University of Palermo, 90127 Palermo, Italy
[b] Advanced BioDesign, Parc Technologique de Lyon, Woodstock - Bâtiment Cedre 1, Saint Priest, France
[c] Medical Oncology, Department of Surgical, Oncological and Oral Sciences, University of Palermo, Palermo, Italy
[d] Central Laboratory of Advanced Diagnosis and Biomedical Research (CLADIBIOR), University of Palermo, Palermo, Italy
[e] Department of Biomedicine, Neurosciences and Advanced Diagnosis, University of Palermo, Palermo, Italy
[f] Plastic and Reconstructive Surgery, Department of Surgical, Oncological and Oral Sciences, University of Palermo, 90127 Palermo, Italy
[g] Plastic and Reconstructive Surgery, Department of Oncology, Azienda Ospedaliera Universitaria Policlinico "Paolo Giaccone", 90127 Palermo, Italy

ARTICLE INFO

Keywords:
Adipose stem cells
Spheroid
Aging
Shelterin complex
ALDH family
Telomere length

ABSTRACT

Adipose stem cells (ASCs) represent a reliable source of stem cells with a widely demonstrated potential in regenerative medicine and tissue engineering applications. New recent insights suggest that three-dimensional (3D) models may closely mimic the native tissue properties; spheroids from adipose derived stem cells (SASCs) exhibit enhanced regenerative abilities compared with those of 2D models. Stem cell therapy success is determined by "cell-quality"; for this reason, the involvement of stress signals and cellular aging need to be further investigated. Here, we performed a comparative analysis of genes connected with stemness, aging, telomeric length and oxidative stress, in 3D and 2D primary cultures. The expression levels of stemness-related markers and anti-aging Sirtuin1 were significantly up-regulated ($P < 0.001$) in SASCs-3D while gene expression of aging-related p16INK4a was increased in ASCs-2D ($P < 0.001$). The 3D and 2D cultures also had a different gene expression profile for genes related to telomere maintenance (Shelterin complex, RNA Binding proteins and DNA repair genes) ($P < 0.01$ and $P < 0.001$) and oxidative stress (aldehyde dehydrogenase class1 and 3) ($P < 0.05$, $P < 0.01$ and $P < 0.001$) and presented a striking large variation in their cellular redox state. Based on our findings, we propose a "cell quality" model of SASCs, highlighting a precise molecular expression of several genes involved with stemness (SOX2, POU5F1 and NANOG), anti-aging (SIRT1), oxidative stress (ALDH3) and telomeres maintenance.

RELAZIONI A CONVEGNI NAZIONALI E INTERNAZIONALI

1. **Human spheroids from adipose-derived stem cells (s-ascs) induces calvarial bone production in a xenogeneic rabbit model.** Di Stefano A. B., Montesano L., Maenza A., Belmonte B., Moschella P., Cassata G., Florena A.F., Russo A., Cordova A., Moschella F. and Leto Barone A.A. *Goim 2016*

2. **Analysis of gene expression profiles of microRNAs in Spheroids from Adipose-derived Stem Cells (S-ASCs) and their involvement in mesenchymal differentiation and stemness potential.** A. Barbara Di Stefano, Daniele Fanale, Luigi Montesano, Alessandro Perez, Michele A. Manahan, Justin M. Sacks, Gedge D. Rosson, Antonio Russo, Adriana Cordova, Francesco Moschella and Angelo A. Leto Barone. *IFATS, San Diego (CA) November 17-20 2016.*

3. **Involvement of microRNAs in the characterization of mesenchymal differentiation and stemness potential of Spheroids from Adipose-derived Stem Cells (S-ASCs).** A. Barbara Di Stefano, Daniele Fanale, Luigi Montesano, Alessandro Perez, Antonio Russo, Adriana Cordova, Angelo A. Leto Barone and Francesco Moschella. *Regenerative surgery, Roma 2-3 Dicembre 2016.*

4. **Molecular analysis of Spheroids from Adipose-derived Stem Cells (S-ASCs) during in vitro long-term culture.** A. Barbara Di Stefano, Federica Grisafi, Alessandro Perez, Marta Castiglia, Antonio Russo, Francesco Moschella, Angelo A. Leto Barone and Adriana Cordova. *Regenerative surgery, Roma 15-16 Dicembre 2017.*

5. **"Capacità rigenerative delle cellule staminali derivate da tessuto adiposo (SASCs) su matrice dermica (INTEGRA®): nostra esperienza su modelli animale". Focus su matrici dermiche: Indicazioni, Prospettive e limiti.** Luigi Montesano, Anna Barbara Di Stefano, Moschella Francesco, Adriana Cordova. *Pisa 13/04/2018.*

6. **“Capacità rigenerative ossee e vascolari degli sferoidi di cellule staminali derivati da tessuto adiposo”.** A. Barbara Di Stefano e F. Moschella *SIMCRI, Taormina 26/10/2018*.

7. **Hydrogel scaffolds blends to host Spheroids from human adipose stem cells.** A.B. Di Stefano, M.A. Sabatino, F. Grisafi, E. Muscolino, M. Perez Alea, F. Toia, C. Dispenza, A. Cordova, F. Moschella. *Regenerative surgery, Roma 21-22 Giugno 2019.*

8. **Studio delle proprietà immunomodulatorie degli sferoidi di cellule staminali adipose nei trapianti vascolari compositi.** A.B. Di Stefano. *SICPRE, 26-28 Settembre 2019*

9. **Emerging immunological checkpoints put the brakes on melanoma infiltrating γδ t cells** Marta Di Simone, Anna Barbara Di Stefano, Anna Maria Corsale, Luigi Montesano, Elena Lo Presti, Francesca Toia, Adriana Cordova, Francesco Dieli, Serena Meraviglia. *Siica 2021*

10. **K-carrageenan/PVA hydrogels as a bio-ink for cartilage reconstruction.** E. Muscolino, A.B. Di Stefano, F. Toia, M.A. Sabatino, F. Moschella, A. Cordova, D. Giacomazza C. Dispenza *AERC 2021*

11. **Injectable hydrogel formulations to host adipose stem cell spheroids for stemness maintenance and bone and cartilage regeneration.** E. Muscolino, A.B. Di Stefano, F. Toia, M.A. Sabatino, F. Moschella, A. Cordova, D. Giacomazza C. Dispenza. *AERC 2021*