An Acad Bras Cienc (2024) 96(Suppl. 1): e20240100 DOI 10.1590/0001-3765202420240100
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

HEALTH SCIENCES

Red propolis extract associated to platelet-rich plasma and stromal cells with focus in cell therapy and functional tissue regeneration

CHARLENE S.C. GARCIA, PAULO MIGUEL C. GARCIA, OTÁVIO B.A.F. SANTOS, DANIELA STEFFENS, SANDRO T. MARTINS, PATRICIA PRANKE, JANAÍNA S. CRESPO, JOÃO ANTONIO P. HENRIQUES & MARIANA ROESCH-ELY

Abstract: The use of platelet-rich plasma (PRP) and adipose-derived stromal cells (ADSC) have been investigated as a form of wound healing enhancement. The objective of this work was to evaluate the association of red propolis (RP) and PRP as inducers of ADSC for application in tissue regeneration. Adipose tissue post-collection and post-cryopreservation was isolated with type II collagenase, characterized by flow cytometry, and differentiated into osteogenic, chondrogenic and adipose cell. The viability of ADSC was evaluated when exposed to different concentrations of RP using the MTT and trypan blue assay. Acridine orange and ethidium bromide (AO/EB) was performed to evaluate cell death events. Horizontal migration methods were investigated in ADSC using autologous and homologous PRP associated with RP (PRP/RP). All assays were processed in triplicate. Flow cytometry and cellular differentiation showed that type II collagenase was effective for isolating ADSC post-collection and post-cryopreservation. RP extracts at concentrations of up to 50 $\mu g.mL^{-1}$ presented no cytotoxic effects. Association of PRP and RP at 25 and 50 $\mu g.ml^{-1}$ influenced ADSC migration, with total closure on the seventh day after exposition. The results here presented could stimulate proliferation of ADSC cells that may contribute directly or indirectly to the reconstructive process of tissue regeneration.

Key words: adipose derived stromal cells, cell therapy, platelet-rich plasma, red propolis, tissue regeneration.

INTRODUCTION

Therapy with adipose-derived stromal cells (ADSC) has shown promising results in dermatology, helping correct skin deformities caused by injury, disease and congenital deformity, like heart disease and musculoskeletal disorders (Lee et al. 2021, Shauly et al. 2022, Julian et al. 2023). The great advantage of ADSCs is that they are found in abundance in the human body, are easy to obtain with minimal morbidity for patients, generate almost no moral and political controversy regarding their use (Moak et al. 2021).

As support for the activation of dermal fibroblasts and remodelling of the extracellular matrix, cellular therapy with ADSC is an alternative that uses techniques to correct changes in the relief of the skin of the face and other regions of the body, through minimally invasive clinical procedures for rejuvenation (Suh et al. 2019). ADSC have paracrine activity and are capable of releasing bioactive molecules, such as cytokines, antioxidant factors, chemokines and growth factors, which contribute to the formation of tissue granulation, these processes

being considerably related to wound closure (Trzyna et al. 2021, Qin et al. 2023).

Another protocol used in cell therapy is platelet-rich plasma (PRP), that also promotes tissue regeneration through the minimally invasive treatment and can be obtained autologously (Samberg et al. 2019). PRP is rich in several growth factors and cellular metabolism, among which are the presence of insulin-like growth factor (IGF), platelet-derived growth factor α and β (PDGF-α and -β), transforming growth factor β1 (TGF-β1), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and interleukin-1 (IL-1), playing an important role in healing as they have anti-inflammatory and regenerative properties (Li et al. 2019).

Significant improvements are observed after PRP application for treatment of scars, soft tissue injuries, burns and healing wounds. It has been also reported that PRP in aesthetic procedures attenuates static and dynamic wrinkles (Menchisheva et al. 2021, Diab et al. 2022). The ability of fibroblasts to produce collagen is reduced over time due to the decrease in growth factors, showing a direct impact on the elasticity, sagging and skin aging (Xue et al. 2022). Thus, the application of PRP in association with ADSC can activate intracellular processes that stimulate proliferation, migration, cell survival, as well as the production of extracellular matrix proteins, favouring the formation of new collagen (Franck et al. 2019).

Studies in the area of biotechnology have associated tissue regeneration, cell therapy and natural products. Among them, red propolis (RP) has been widely studied in the last decade. The literature has pointed out interesting biological activities related to the hydroalcoholic extract of RP, such as antioxidant, antibacterial, antimicrobial, anti-inflammatory and antitumor activity (Frozza et al. 2013, Rufatto et al. 2018, Novais et al. 2021). RP has also been reported to increase cell proliferation, activate metabolites, and stimulate wound matrix remodelling (Picolotto et al. 2019). Some studies confirm the therapeutic efficacy of RP through quantitative and qualitative analyses of the expression of proteins such as type I and III collagen in the wound matrix, which favoured wound re-epithelialization (Conceição et al. 2022).

In view of the above statements and considering the benefits of its bioactive substances responsible for stimulating the reconstructive process of damaged tissues, red propolis (RP) extracts associated with adipose-derived stromal cells (ADSC) and platelet-rich plasma (PRP) are potential strategies for the treatment of skin regeneration, restoring impaired function. Therefore, this study aimed to isolate and culture adipose tissue cells and combine them with platelet-rich plasma gel-based vehicles and red propolis extracts and evaluate proliferation and migration aspects *in vitro* of cell culture.

MATERIALS AND METHODS

Sample collection

The present study was approved under the protocol number CAAE: 46199015.2.0000.534 - Health Ministry Platform, and Ethics Committee in Research of the University of Caxias do Sul under the number: 1.230.848. Prior informed consent for participation in the experiment was obtained from human subjects.

For fat liposuction surgeries, the patient was submitted to epidural anaesthesia, and the donor area where the liposuction was collected passed through strict asepsis and antisepsis. After the patient was under anaesthetic effect, a small incision was made with a scalpel blade where a liposuction cannula was inserted, coupled with pressure. When the procedure was finished, the incision was closed with suture. The collected

material was washed 3 (three) times with sterile 9% saline to remove the erythrocytes and broken fat cells, and placed in DMEM/F12 culture medium (Dulbecco's Modified Eagle's Medium/ Nutrient Mixture F-12 Ham - Sigma Aldrich, St. Louis, Missouri, USA), supplemented with 1% P/S (penicillin/streptomycin- Sigma Aldrich, St. Louis, Missouri, USA) and 10% FBS (Fetal bovine serum - Cultilab, Campinas- SP, Brazil). The adipose tissue collected was properly labelled, sealed, transported in an aseptic environment and immediately sent to the laboratory for processing.

Red propolis extracts

Red propolis was collected in Alagoas, located in North-eastern Brazil at geographic location of 9°41'59.7"S, 36°20'10.7"W. Altogether 12 samples were collected, one each month, during a year, to ensure that a representative sampling could be obtained and further tested. After collection, it was stored in a location away from light exposure and then frozen at -20°C until preparation of the extract. Initially, 50 g of the red propolis powder was mixed with 500 mL of 70% EtOH, stirring at room temperature for 24 h. After extraction, the mixture was filtered and the solvent evaporated (37 °C) with subsequent lyophilized at -80 ^{0}C. Final powder was maintained at -20 °C.

Platelet-rich plasma samples

The protocol of Vendramin et al. (2009) with modifications was used for the preparation of PRP samples. Initially, peripheral blood was centrifuged at 400 g for 10 minutes and plasma supernatant was collected and centrifuged to obtain platelet-rich plasma. For the autologous thrombin, 1 mL of the first centrifugation was used and 0.3 mL of 10% calcium gluconate was added with further heating in a water bath at 37°C for 15 minutes, until plasma gelatinize. Centrifugation was performed in 800 g for 10 minutes to obtain the thrombin. The platelet-rich plasma gel was prepared by adding autologous thrombin to the platelet-rich plasma in a proportion of 1:5. After mixing, the gel was formed in 1 min.

Cell culture assays

Isolation of stromal mesenchymal cells

Isolation of cells was based on the protocol described by Zuk et al. (2002) with modifications. The adipose tissue was consecutively washed with 1× PBS and submitted to the enzymatic process of complete tissue digestion with 1 mg.mL^{-1} of collagenase II (Sigma Aldrich, St. Louis, Missouri, USA) in 1× PBS for 30 minutes in a water bath, with shaking every 5 minutes. After, the adipose tissue was centrifuged for 10 minutes at 1400 rpm, the supernatant was removed and Stromal Vascular Fraction (SVF) was isolated. DMEM/F12 supplemented with 10% FBS and 1% P/S was added to the inactivation of the enzymatic digestion process and centrifugation was performed at 1000 rpm for 10 minutes. The pellet was resuspended in DMEM/ F12 supplemented and seeded in culture bottle and kept in a humidified incubator at 37°C with 5% CO_2.

Characterization of stromal cells derived from adipose tissue by flow cytometry

The identification of mesenchymal stromal cells was performed according to the methodology described by Dominici et al. (2006) with modifications. After the third passage, trypsin was added to cells and samples were further characterized by flow cytometry, as standard protocols using mesenchymal stromal cell models. Thus, the characterization occurred by the determination of positive specific cell surface antigens: CD29, CD44, CD73, CD90, CD105; and negative: CD14, CD34, CD45, CD 184, HLADR,

STRO1 on FACSAria III flow cytometer (Becton Dickison Immunocytometry Systems, San Jose, USA). Isotypes for all fluorochromes were used to confirm specific staining. Initially, 5 µL of each antibody was added to a 100 µL aliquot. The samples were incubated with the antibodies at 4 °C in the dark for 30 minutes. After this time, washes were performed with 1mL of PBS 1× and centrifuged at 1600 rpm for 5 minutes. Subsequently, the supernatant was discarded leaving 450 µL final volume. Finally, 7AAD was added to each tube to confirm the analysis of just live cells.

Osteogenic, chondrogenic and adipogenic differentiation

To confirm the isolation of ADCS, differentiation to other tissues was carried out, following standard protocols for mesenchymal cells. First, cells were detached with trypsin in the fifth passage, counted and plated at 1×10^4 cells. mL^{-1} in DMEM culture medium with 10% FBS and 1% P/S, in 6-well plate. After five days, the culture medium was replaced by the osteogenic and chondrogenic differentiation inducing medium (StemPro Kit, Gibco, USA) for 21 days, and adipogenic differentiation inducing medium (5 µM Indomethacin, 1 µM Rosiglitazone, 1.74 µM Insulin, 1 µM Dexamethasone and 1 mM 3-Isobutyl-1-methylxanthine) for 14 days, with medium changes every 48-72 hours. For the evaluation of the differentiation process, the monolayers were fixed in 4% paraformaldehyde for 30 minutes, washed twice with distilled water and covered with staining Alizarin Red S (Sigma-Aldrich, USA), Alcian Blue (Sigma-Aldrich, USA) for 5 minutes to osteogenic and chondrogenic differentiation, were washed twice with distilled water and analysed under reversed light microscopy. Adipogenic differentiation showed accumulation of lipid droplets in its cytoplasm.

Cell viability assays

Indirect cytotoxic activity by MTT

Cytotoxicity was assessed by the MTT indirect method, as described in the standards ISO 10993-05 (2009) and ISO 10993-12 (2012). This method is based on the reduction of MTT (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) by the mitochondrial dehydrogenase to form formazan crystals (Denizot & Lang 1986). ADSC cells were seeded in 96- well plate at a density of 8×10^4 $cells.mL^{-1}$ in 100 µL of DMEM culture medium supplemented with 10% FBS and 1% P/S. After 70-80% confluence, ADSC cells were treated with 25, 50, 100 and 200 $\mu g.mL^{-1}$ RP extract, incubated at 37°C in 5% CO_2 for 1, 2, 3 and 7 days. For the negative control, DMEM medium (10% SFB and 1% P/S) was used. For the negative control, DMEM medium (10% SFB and 1% P/S) was used. For the positive control, an agent that causes cell death was used, DMEM medium (10% SFB and 1% P/S) with 5% DMSO (dimethyl sulfoxide). The medium was removed and 1 $mg.mL^{-1}$ of MTT in SFB-free medium and P/S was added to the wells. The plates were incubated at 37°C for 2 h in humidified atmosphere with 5% CO_2. Subsequently, the MTT solution was removed and the formazan crystals were dissolved in 100 µL DMSO. Spectrophotometric reading at 570 nm was performed on a microplate reader (M2e, Molecular Devices, USA) and the results were expressed as percentage of viability. The absorbance of the negative control represented 100% viability and the values of the treated cells were calculated as a percentage of the control. The same conditions mentioned above were used for the evaluation of the ADSC cells exposed to PRP, thrombin to the platelet-rich plasma in a proportion of 1:5, autologous and homologous, and exposed to red propolis

extract at a concentration of 25, 50, 100 and 200 $\mu g.mL^{-1}$ associated to PRP (PRP/RP)

Cell viability analysis with Trypan Blue

The method of exclusion of Trypan blue is based on the fact that the non-viable cells allow the incorporation of the dye (Salvadori 2003). Thus, ADSC cells were initially seeded (8×10^4 $cells.mL^{-1}$) in 24-well plate. After 70-80% confluence, the cells were treated with different concentrations of RP extracts (25, 50, 100 and 200 $\mu g.mL^{-1}$) for 1, 2, 3 and 7 days, at 37°C in humidified atmosphere with 5% CO_2. Treatment was withdrawn and the cells washed twice with PBS and exposed to trypsin. 20 µL of cells with Trypan blue solution were prepared (1:1 v/v) and 10 µL of the prepared solution was pipetted and the cells were analysed on a Neubauer chamber. The cells were counted in the inverted microscope taking into account: $Cells.mL^{-1}$ = total counted × dilution factor × 10^4 and Cell viability (%) = total viable cells (not stained)/total cells (stained and not stained) × 100.

Dual staining through acridine orange and ethidium bromide

This assay is used to discriminate live from dead cells on the basis of membrane integrity, also by nuclear alterations and formation of apoptotic bodies that are characteristic of apoptosis (Kasibhatla et al. 2006). Thus, the ADSCs were initially seeded (8×10^4 $cells.mL^{-1}$) in 24-well plate and incubated at 37°C in humidified atmosphere with 5% CO_2. After 70-80% confluence, cells were treated with different concentrations of RP extracts (25, 50, 100 and 200 $\mu g.mL^{-1}$) for 1, 2, 3 and 7 days and washed twice with PBS prior to trypsin incubation. Twenty microliters of cells were placed on a glass slide with 2 µL of acridine orange (100 $\mu g.mL^{-1}$) and 2 µL of ethidium bromide (100 $\mu g.mL^{-1}$). Cells were observed in the fluorescence microscope, with 20× magnification. Two hundred cells were counted in each treatment condition. The analysis of images obtained by fluorescence with AO/EB staining were analysed through the ImageJ software to determine the number of normal cells and the ones induced to apoptosis and necrosis mechanisms. The analysis of the images obtained by fluorescence with AO/EB staining contributed to qualify and quantify mechanisms of cell death, such as apoptosis. Cellular staining patterns were analysed using ImageJ software to determine induced cell mechanisms of apoptosis and necrosis in contrast to normal cells. The AO/EB colouring method generates collared images, which were initially treated by channels. The intensities of the green and red channels below the threshold were not quantified and are represented in black.

Scratch-healing assay

The effect of platelet-rich plasma (PRP) and platelet-rich plasma with red propolis (PRP/RP) on migratory activity was evaluated using the scratch-healing assay (Glaß et al. 2012). ADSC cells ($8x10^4$ $cells.mL^{-1}$) were seeded in 24-well plate and incubated at 37°C in humidified atmosphere with 5% CO_2. After confluence of 90%, a linear scratch using a 200 µL tip was performed by configuring a free area of cells in the cell monolayer. Thereafter, the culture medium was replaced for the removal of suspension cells. The adherent cells were exposed to the PRP with different concentrations of RP extracts (25, 50, 100 and 200 $\mu g.mL^{-1}$) for 1, 2, 3 and 7 days, negative control DMEM (10% of SFB and 1% P/S) and positive control (DMSO 5%). After, cells were photographed using an image acquisition system coupled to the inverted microscope. Photomicrographs were analysed using ImageJ software (0.1 cm calibration) to determine the area occupied by cells after treatment. The

closure of the free area of cells characterized the cell Migration Index that was obtained by the following calculation:
Migration Index (%) = $\left[\frac{A0-At}{A0}\right] \times 100$, where A0 refers to the original area and AT to the area at time of scratch (1, 2, 3 e 7 days).

Statistical analysis

All assays were performed with three independent replicates. Statistical significance was assessed using t-test and One-way Analysis of Variance (ANOVA) with the multiple mean comparison test (Tukey) to evaluate statistical differences in the case of normal distribution. Significance was accepted for *p* less than 0.05 using the Statistical Package for the Social Sciences (SPSS, version 19.0) for Windows.

RESULTS

Cell culture assay

Isolation of stromal cells derived from adipose tissue

In most studies, stromal cell isolation is performed with type I collagenase, however, in this work, type II collagenase was used in the isolation of stromal cells, and 10 mL of liposuction was obtained 1×10^6 cells.mL^{-1}. As required by isolation protocols, after 7 days incubation the cells presented fibroblastic morphology.

With the intention of storing the lipoaspirate for later use, 10 mL of the liposuction was frozen at low temperatures (-80ºC) with FBS (1:2 v/v) and 20% DMSO. After 18 months of freezing, thawing and enzymatic digestion with type II collagenase were performed. It was possible to obtain 1×10^5 cells.mL^{-1}, and after 7 days of incubation the cells also showed fibroblastic morphology.

Characterization of stromal cells derived from adipose tissue and differentiation

The characterization by flow cytometry analysis shows the effectiveness of stromal cell isolation. This assay showed that there were no significant differences in surface markers profile among the three donor samples, for both, post-collection and post-freezing digestion with type II collagenase. In the third passage, the cell populations were homogeneous in the expression of the positive markers (Supplementary Material – Table SI).

To confirm ADSC isolation, differentiation to other tissues must be performed. The ADSC post-collection and post-freezing digestion with type II collagenase presented capacity for differentiation in osteogenic, chondrogenic and adipogenic. As mentioned, no differences were observed between post-collection and post-freezing results. Thus, when the ADSC culture was evaluated in a medium that induces osteogenic differentiation for 21 days, it was observed calcium-matrix deposits identified in red by Alizarin Red S staining. Rounded cells surrounded by a glycosaminoglycan matrix stained in blue by Alcian Blue dye were observed in the inducing medium of chondrogenic differentiation for 21 days. The ADSC cultured in inducing medium of adipogenic differentiation for 14 days presented morphological changes, rounded-like and with accumulation of lipid droplets in its cytoplasm.

Cell viability assay

Viability cells by MTT and Trypan Blue

ADSC cell viability showed similar effects of red propolis (RP) through MTT (*Figure 1a*), when compared trypan blue test (*Figure 1b*). All results shown, were compared to the negative control (DMEM, 10% of SFB and 1% P/S), which was normalized to 100% cell viability. The concentration of 25 µg.ml^{-1} of RP in MTT test

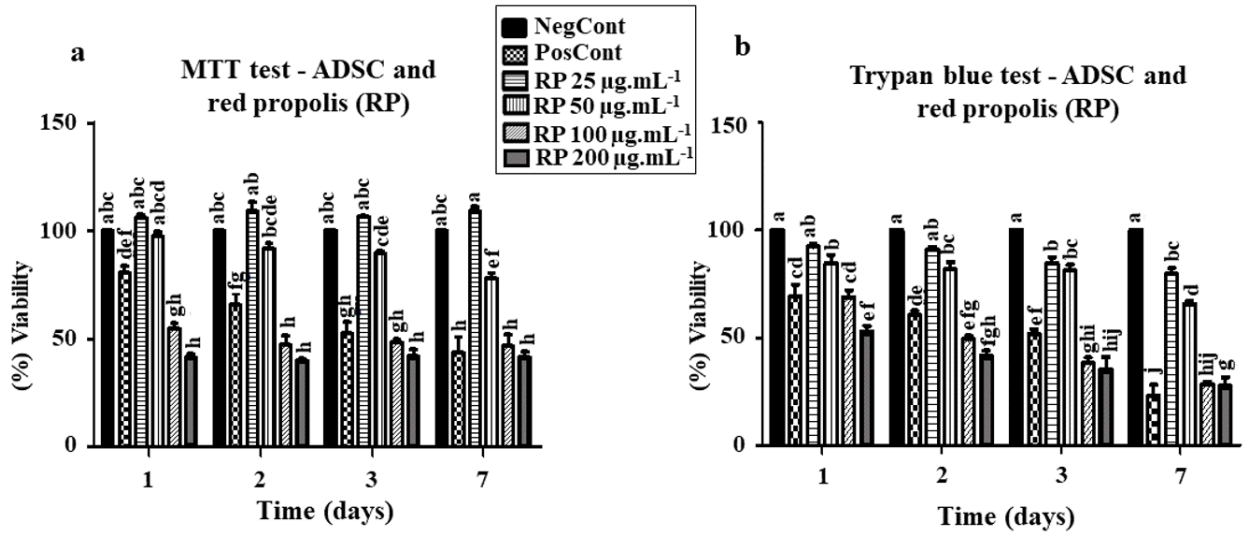

Figure 1. Cytotoxic effect obtained by performing an indirect test according to ISO 10993-5-2009-2 of the extracts of RP (µg.ml^{-1}) in comparison to NegCont (negative control - DMEM medium, 10% SFB and 1% P/S) and PosCont (positive control – DMEM medium, 10% SFB and 1% P/S and DMSO 5%) on the viability of ADSC cells treated for 1, 2, 3 and 7 days (a). Cytotoxic effect obtained by the trypan blue assay of RP extracts (µg.ml^{-1}) compared to NegCont (negative control - DMEM medium, 10% SFB and 1% P/S) and PosCont (positive control – DMEM medium, 10% SFB and 1% P/S and DMSO 5%) on the viability of treated ADSC cells for 1, 2, 3 and 7 days (b). All assays were performed with three independent replicates. * Letters correspond to the statistically significant differences, using the ANOVA-Tukey test ($p \leq 0.05$).

showed an increasing proliferative activity, with highest viability index at day 7 (109.27%). Thus, due to similarity of results, we will only report IC_{50} values for the MTT assay (*Table I*).

Once established RP concentrations, the platelet-rich plasma gel autologous and homologous associated with red propolis (PRP/RP) in contact with ADSC was evaluated. Therefore, differences in viability are shown according to *Figures 2a and 2b*.

Autologous PRP showed increasing proliferative activity with its highest viability index on the third day (125.30%) and small decrease on the seventh day (114.76%), same was observed for the homologous PRP with the lowest proliferative proportions, on the second day (104.9%) and on the seventh day (101.8%). The PRP with 25 and 50 µg.ml^{-1} of RP (PRP/RP), no demonstrate cytotoxic activity for ADSC, however, there was no increase in cell proliferation, as observed when we used only autologous PRP.

Dual staining with Acridine orange and ethidium bromide (AO / EB)

RP concentration was evaluated through normal cells with green fluorescence (OA) in contrast to dead cells with red fluorescence (EB). The analysis of the results obtained by the treatment of ImageJ software is presented in Table II.

At the concentration of 100 µg.ml^{-1}, an increase apoptosis/necrosis proportions of events were observed, until the second day, in which 71.47% of cells showed green fluorescence indicating viability. However, on the seventh day of exposure no viable cells could be counted, 10.7% of them yield yellow-orange fluorescence indicative of apoptosis and the majority, 89.3%, red fluorescence corresponding to necrotic events. The concentration of 200 µg.ml^{-1} showed a low viability. Thus, was possible to observe

Table I. IC_{50} **values – 50% inhibitory concentration obtained in the MTT assay for ADSC cell after RP exposure 1, 2, 3 and 7 days of treatment. All assays were performed with three independent replicates.**

IC_{50}				
Time	**1 day**	**2 days**	**3 days**	**7 days**
Concentration of RP µg.mL^{-1}	119.93±2.45	95.22±1.56	90.75±2.77	73.56±1.32

Results presented as mean ± SD.

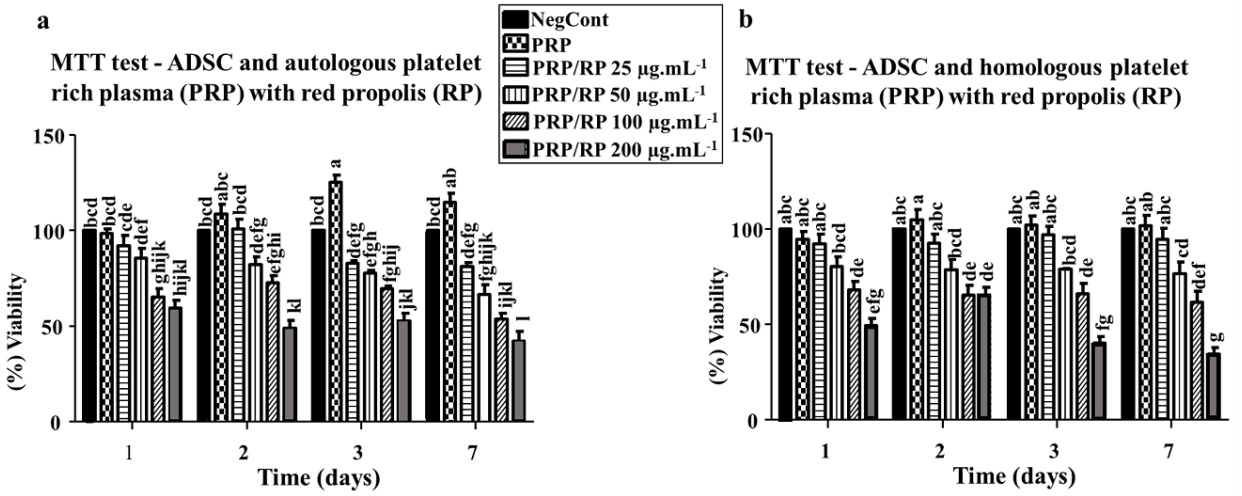

Figure 2. Cytotoxic effect obtained by performing MTT indirect test according to ISO 10993-5-2009-2 on PRP/RP. (a) Autologous platelet-rich plasma with red propolis (PRP/RP) (25-200 µg.ml^{-1}), and (b) Homologous platelet-rich plasma with red propolis (PRP/RP) (25-200 µg.ml^{-1}). PRP: platelet-rich plasma; NegCont: negative control (DMEM medium, 10% SFB and 1% P/S) on ADSC viability treated for 1, 2, 3 and 7 days. All assays were performed with three independent replicates. * Letters correspond to the statistically significant differences, using the ANOVA-Tukey test (p ≤ 0.05).

that concentrations of 25 and 50 µg.ml^{-1}of RP were those that caused less necrosis in the cells.

Scratch-healing assay

Previous results from the viability tests using concentrations of 25, 50 and 100 µg.ml^{-1} of RP maintained cell viability above 70%. The objective of the study was to evaluate the association of platelet-rich plasma in autologous gel associated with red propolis (PRP/RP) in contact with ADSC, thus, compared PRP/RP to the negative control (DMEM, 10% of SFB and 1% P/S) and PRP, as demonstrated in the MTT test (*Figure 2a*).

To determine the influence of platelet-rich plasma (PRP) and the association of platelet-rich plasma and red propolis (PRP/RP) on the ADSC migration profile, cells were treated for 1, 2, 3 and 7 days. The migration of ADSC in the presence of PRP and PRP/RP at 1, 2, 3 and 7 days was compared with the negative control of the respective day. After 1 day, when comparing free area of ADSC cells in the cell monolayer treated with PRP/RP 100 µg.ml^{-1} with the respective negative control from day 1, no ADSC migration was observed, therefore, the results in bars on the graph not shown.

On day 2, when we compared the free area of ADSC cells in the cell monolayer treated with PRP/RP 25, 50 and 100 µg.ml^{-1} with the negative control on day 2, not observed ADSC migration, results in bars on the graph not shown. The same occurred for day 3, however on day 3, PRP covered the entire the free area of ADSC

Table II. Percentages of cellular events obtained by the Image J plugin for viable, apoptosis and necrotic cells after 1, 2, 3 and 7 days of exposure to different concentrations of RP. All assays were performed with three independent replicates.

		25 µg	50 µg	100 µg	200 µg
		1 day			
Cell count (%)	Viable	96.03±0.90	93.99±1.28	86.42±1.53	7.00±1.89
	Apoptosis	3.76±0.84	6.01±1.28	5.57±0.54	2.95±0.54
	Necrosis	0.21±0.07	0.00±0.00	8.01±1.92	90.05±2.26
		2 days			
	Viable	97.59±0.63	97.25±0.58	81.84±0.98	1.47±0.66
	Apoptosis	2.41±0.63	2.75±0.58	4.84±0.73	3.52±1.53
	Necrosis	0.00±0.00	0.00±0.00	13.32±1.66	95.01±0.89
		3 days			
	Viable	98.37±0.50	94.79±1.40	46.53±2.64	0.00±0.00
	Apoptosis	1.63±0.50	2.80±0.50	4.61±0.30	10.08±1.43
	Necrosis	0.00±0.00	2.41±0.70	48.86±2.43	89.92±1.43
		7 days			
	Viable	98.00±0.57	76.52±1.02	0.00±0.00	0.00±0.00
	Apoptosis	1.88±0.55	7.59±0.53	9.44±0.91	10.10±0.15
	Necrosis	0.12±0.02	15.89±0.83	90.56±0.91	89.90±0.15

Results presented as mean ± SD.

cells in the cell monolayer. On day 7, when we compared the migration area with the negative control on day 7, we observed ADSC free area in the cell monolayer was complete closure for PRP and PRP/RP 25 and 50 µg.ml^{-1} (Figure 3). The results demonstrated that RP extracts associated with PRP were capable of stimulating proliferation of ADSC cells, that may contribute directly or indirectly in the cell therapy and tissue regeneration (Figure 4).

DISCUSSION

Patients with lost areas of tissue often require treatment with cell therapy, and consequently, the performance of several procedures to collect cells. With the aim of carrying out a single collection to obtain cells that can be used as needed and minimizing the exposure of patients to the invasive procedures, enzymatic digestion was also carried out with post-freezing adipose tissue. The protocol using enzymatic digestion with type II collagenase was adequate for the isolation of ADSC post-freezing (18 months), in which adherent cells were able to differentiate into other tissues (Feng et al. 2018, Laschke et al. 2018). This is a mandatory requirement to confirm whether the protocol used for ADSC isolation was effective (Yuanzheng et al. 2015). However, related to the yield of cell number after enzymatic digestion, we observed a reduction (1×10^5 cells.mL^{-1}).

Autologous ADSC treatment can increase collagen levels, reduce fibrosis, stimulate angiogenesis, providing better cell proliferation (Surowiecka & Strużyna 2022, Wang et al. 2023). ADSC can be used individually or combined with other substances to stimulate dermal fibroblasts

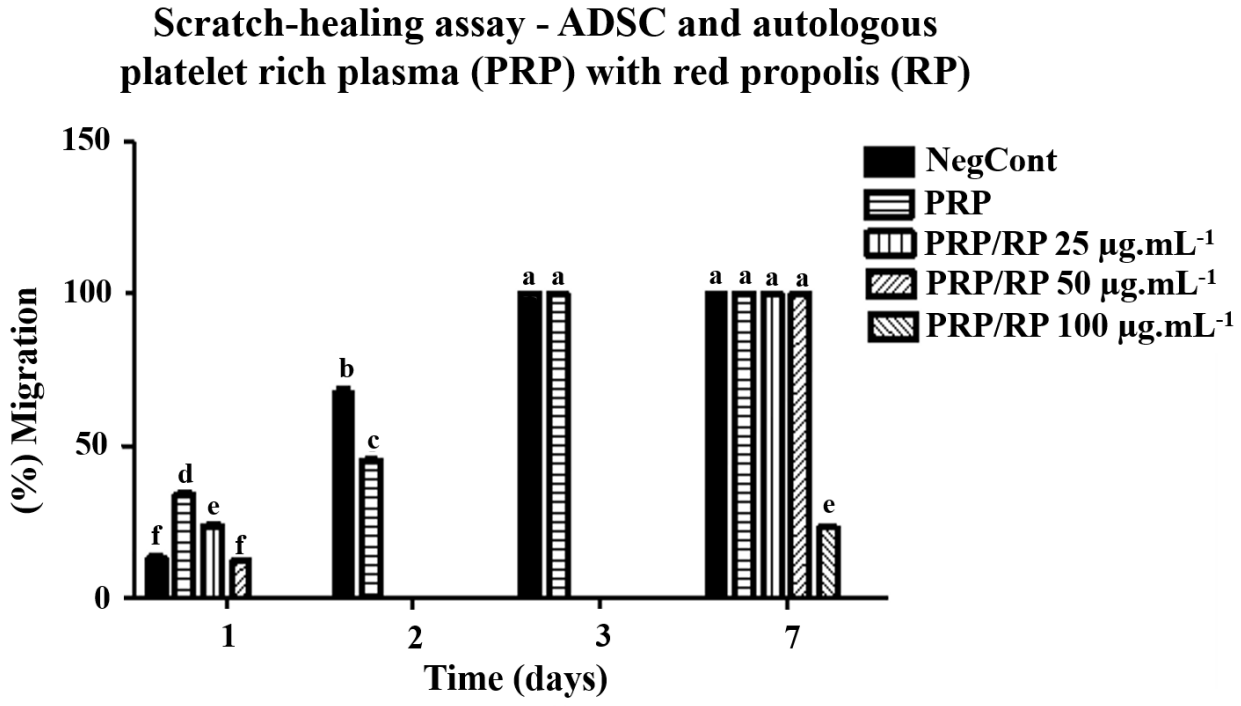

Figure 3. Scratch-healing assay using ADSC and autologous platelet rich plasma (PRP) with red propolis (RP). Effect of autologous PRP/RP on the ADSC migration in relation to the time 0 (NegCont – negative control – DMEM medium, 10% SFB and 1% P/S) and after 7 days for the red propolis (RP) and PRP/RP (autologous platelet rich plasma with red propolis concentrations). All assays were performed with three independent replicates. * Letters correspond to the statistically significant differences, using the ANOVA-Tukey test ($p \leq 0.05$).

and promote skin regeneration (Zhou et al. 2019, Liu et al. 2022). In addition to the use of ADSC for the treatment of wounds, we can mention their use in aesthetic protocols. The increasing age of the population leads to the search for products and methodologies that are not immunogenic and that prevent aging, attenuating expression marks (Glass & Ferretti 2019).

Our research group has investigated the chemical composition of red propolis from the northeast of Brazil and tested diferent applications such as antioxidant properties, antibacterial and antitumor activities (Frozza et al. 2013, Frozza et al. 2017, Rufatto et al. 2018, Picolotto et al. 2019). We have observed that red propolis extracts could also be combined for application in occlusive dressings with natural latex rubber (Garcia et al. 2021). A variety of compounds were found, among them are phenolic ones, including flavonoids, that have been considered the main biologically active constituents of red propolis, together with the cinnamic acid derivatives, esters, and some terpenes, which contribute to favourable biological activities such as antioxidant, antibacterial and regeneration process (Rufatto et al. 2018, dos Santos et al. 2019).

Total extract of RP is relatively safe to be used in most of the models tested, and in the present study, is also showed that can applied associated with ADSC and PRP for revitalization of tissues. Here, two approaches were used to evaluate the cytotoxic activity of RP: the first involving mitochondrial function variation - MTT assay (Denizot & Lang 1986), and a second

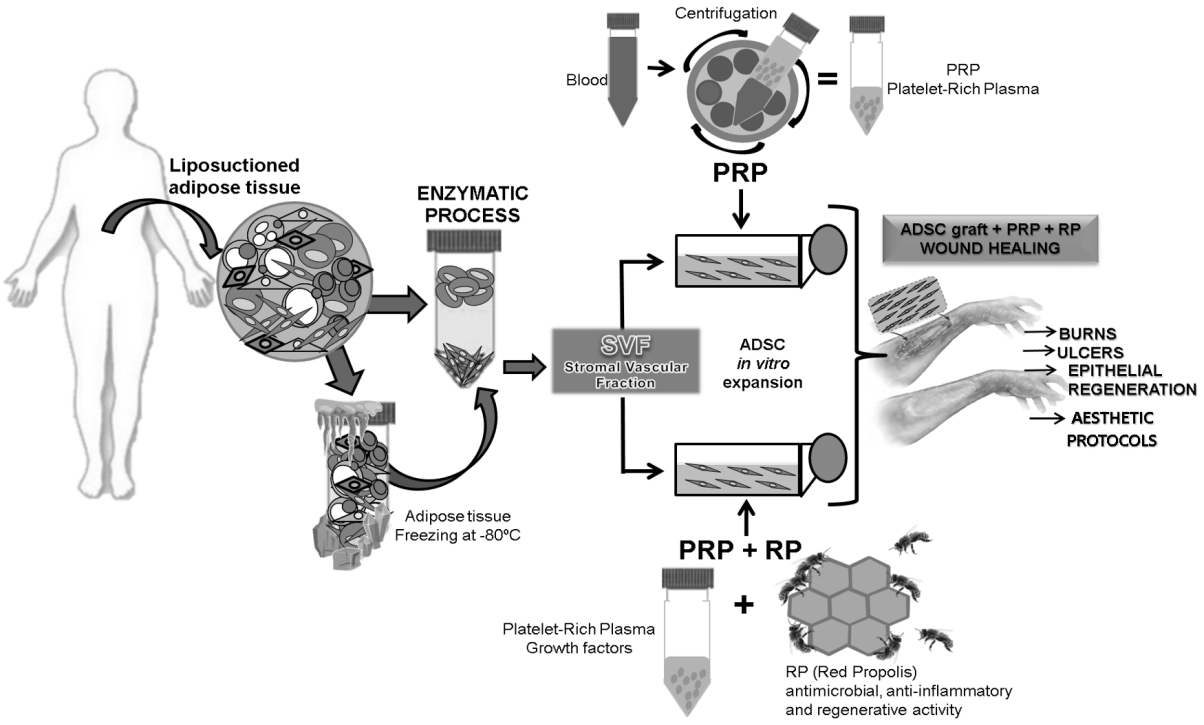

Figure 4. Representative scheme of treatment with association of platelet-rich plasma with red propolis extract (PRP/RP) and adipose-derived stromal cells (ADSC) for functional tissue regeneration.

involving the integrity of the plasma membrane - Trypan blue (Salvadori 2003).

Cellular viability of the trypan blue test showed a decrease in comparison to MTT test, but this event was observed for all conditions and was in accordance with the biological cell viability response seen for MTT. Initially, the trypan blue technique was used, as a rapid estimate of viability in mammalian cells, making it an efficient tool in toxicological assays to evaluate complex compounds (Gajski et al. 2012). Also, it is known that MTT staining interacts with the redox cell signalling in mitochondria, reflecting in different patterns of cell metabolism related to viability conditions, therefore both tests were performed.

MTT assay showed IC_{50} values that progressively decreased from day 1 to day 7, with lower values of 73.56±1.32 $\mu g.ml^{-1}$ after one week incubation. Lopez et al. (2015) exposed RP extracts to human keratinocytes (HaCat) and demonstrated cytotoxic concentration of RP above 50 $\mu g.ml^{-1}$. This same concentration using canine mesenchymal stromal cells showed that the extracts of RP at 50 $\mu g.ml^{-1}$ showed no cytotoxic activity (Bernardino et al. 2018). In general, ADSC presented greater resistance to exposure of different concentrations of red propolis extract relative to secondary keratinocytes and canine mesenchymal stromal cells. Apparently, red propolis can be involved in preventing and stabilizing pro-inflammatory immune response, which also contribute to wound healing (Magnavacca et al. 2022).

ADSC cells presented viability greater than 70% after exposition to RP, PRP and PRP/RP at concentrations of 25 and 50 $\mu g.ml^{-1}$ in the period of 1, 2, 3 and 7 days. The results for the autologous and homologous PRP/RP at concentrations of 100 and 200 $\mu g.ml^{-1}$, reduce cell viability greater than 30%. These results are in accordance with standards for medical devices (ISO

10993-5-2009-2). Cell proliferation was greater when ADSC were exposed to autologous PRP.

Although we observed similarity in the results when using autologous and homologous PRP in association with RP, it should be emphasized advantages in using autologous PRP, once absence of transmissible pathogens is expected, among other features, making its application safer and more effective for the patient (Li et al. 2019). In continuity, the growth factors present in the autologous PRP are not mutagenic and act stimulating the healing process in an effective way, being able to be used alone or in combination with stromal cells in the tissue regeneration (Ramaswamy Reddy et al. 2018).

When comparing the results obtained in the MTT assay using PRP associated with RP (PRP/RP) or RPP alone, it is clear that PRP alone increases cell viability. However, when we compare the results obtained in the MTT assay using only the RP extract concentration 25 $\mu g.ml^{-1}$ (RP 25 concentration 25 $\mu g.ml^{-1}$) with the association of PRP with RP concentration 25 $\mu g.ml^{-1}$ (PRP/RP 25 $\mu g.ml^{-1}$) we observed no difference in the cell viability at lower concentrations, although at 100 and 200 $\mu g.ml^{-1}$, cell viability was greater. These data may indicate that a mechanism of synergy may occur between the compounds, allowing greater cellular resistance at higher concentrations of RP when associated with PRP.

MTT assay showed that RP extract associated with PRP at concentrations of 100 and 200 $\mu g.ml^{-1}$ increased viability. According to ISO 10993-5 (2009), a material causes cytotoxic effects when it causes a reduction in viability above 30% after 24 hours exposition. Higher concentrations of RP, such as 100 and 200 µg.ml-1, in association or not with PRP, were considered cytotoxic for all exposure times tested in the present study. A decrease in cell viability was observed in both MTT tests, RP extract only and RP extract in association with PRP, however at concentrations up to 50 $\mu g.ml^{-1}$, cell viability remained above the cutoff point established by ISO 10993-5-2009- 2, therefore, being suitable for application.

Further tests were performed in order to evaluate apoptotic activity of RP in ADSC through the AO/EB staining analysis. Beside checking the integrity of the plasma membrane, AO/EB assay also covers nuclear alterations and formation of apoptotic bodies (Rodrigues et al. 2019). Viable ADSC cells percentages were observed until the second day at a concentration of 100 $\mu g.ml^{-1}$ (81.84%). The data corroborate with those previously showed by the MTT and trypan blue. Bernardino et al. (2018) showed similar results reported by flow cytometry using Annexin V/propidium iodide of RP extract against canine mesenchymal stromal cells, after the 3 days incubation at the concentration of 50 $\mu g.ml^{-1}$, with 98.5% of viable cells. In our study, apoptotic events were observed on the third day in the concentration of 100 $\mu g.ml^{-1}$ (46.53%) and at all days in the concentration of 200 $\mu g.ml^{-1}$.

Research shows promising results for the scratch-healing assay using only extract RP. According to Basílio (2018), red propolis extract showed an important role in the migration process, allowing the closure of the free area of cells, demonstrating that RP had an important role in the migration in the 3T3 cells (mouse embryonic fibroblasts). In this work, after establishing the concentrations of the RP extract in the MTT assay, with or without PRP, we proceeded with the scratch-healing assay in comparison to PRP.

The results showed that the presence of PRP favoured the proliferation profile scratch-healing assay. Qu et al. (2021) demonstrated that after PRP exposition, a proliferation marker (Ki67) was up-regulated, suggesting pro-proliferative effect. A decrease of the free cell area was observed for concentrations of 25, 50 and 100

$\mu g.mL^{-1}$ of PRP/RP, allowing the ADSC to migrate, with their total closure only at the seventh day of treatment. However, the concentration of 200 $\mu g.mL^{-1}$ of RP, even associated with PRP, showed deleterious effects on the stromal cells.

The PRP can be easily prepared, through the collection of whole blood with subsequent centrifugation, and, after the centrifugation process, a greater proportion of platelets can be obtained, with activation of their granules, which in turn release factors that stimulate tissue repair (Menchisheva et al. 2021).

Research has shown that with increasing age, skin stem cells decrease, as well as the ability of fibroblasts to produce collagen I and II is reduced due to a decrease in growth factors (Rorteau et al. 2022). Autologous PRP is well tolerated and can stimulate and accelerate the healing of complex wounds, especially in elderly patients (Tian et al. 2019). In this work, we suggest that the association of PRP with RP could increase collagen stimulation, in addition to presenting an important antibacterial potential attributed to RP extract, due to its ability to inhibit the proliferation of microorganisms, as demonstrated in other studies of our group (Rufatto et al. 2018, Picolotto et al. 2019).

The results demonstrated that red propolis extracts associated with platelet-rich plasma were capable of stimulating proliferation of ADSC cells that may contribute directly or indirectly to the reconstructive process of damaged tissues and tissue regeneration. However, new analyses must be carried out to confirm this hypothesis.

CONCLUSIONS

Although there have been advances in procedures for burns and other injuries involving skin loss, it is important to study therapies which are capable of aiding in the recovery of individuals with skin lesions. The *in vitro* association of stromal cells derived from adipose tissue, platelet-rich plasma and red propolis extracts at concentrations up to 50 $\mu g.ml^{-1}$ presents no cytotoxicity and may contribute to enhancing the wound healing process, decreasing the peripheral area of the wound and stimulating cell proliferation, thus providing proper cell growth with the aim of reliably reproducing the lost structure and function of the original skin.

This study showed the possibility of collecting adipose tissue, submitting these cells to cryopreservation at -80°C for several months. The use of this protocol on a large scale and in public health systems can be applied to minimize complications, treatment time and costs, thus ensuring the return of the individual to normal activities as soon as possible.

Therefore, autologous grafts using ADSCs, in combination with platelet-rich plasma and antibacterial compounds, like the red propolis, indicate potential for application in the treatment of skin wounds and tissue regeneration. This offers a promising perspective in reconstructive plastic surgery for the treatment and recovery of burn patients or individuals with other types of injuries requiring skin grafts, reducing pain and modulating parameters such as wettability in a wound, thus promoting neovascularization and wound re-epithelialization. However, it is essential to carry out more studies related to this association, to better understand its benefits.

Acknowledgments

We thank the Biotechnology Institute from the University of Caxias do Sul, the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Hospital Beneficente São Carlos from Farroupilha – Brazil, and students of Scientific Initiation Allana Thaís Cauduro and Gabriela Lorenzet. The authors declare no conflict of interest.

Author contributions

Author's name	Description of contributions
Charlene Silvestrin Celi Garcia	X, Y, A and B
Paulo Miguel Celi Garcia	X and Z
Otávio Branco Araújo de Farias Santos	X and Z
Daniela Steffens	Z and A
Sandro Tomaz Martins	A
Patricia Pranke	Z and A
Janaína da Silva Crespo	Z and A
João Antonio Pêgas Henriques	Y and A
Mariana Roesch-Ely	Y, Z and A

X performed the research study; Y designed the research study; Z contributed essential reagents or tools; A Analyzed the data; B wrote the paper. CSC Garcia, PMC Garcia, OBAF Santos, D Steffens, ST Martins, P Pranke, JS Crespo, JAP Henriques, M Roesch-Ely, all authors have read and approved the final manuscript.

REFERENCES

BASÍLIO JAD. 2018. Desenvolvimento e avaliação *in vitro* da atividade cicatrizante de membranas poliméricas incorporadas com própolis vermelha. Tese de Doutorado. http://www.repositorio.ufal.br/handle/riufal/3603.

BERNARDINO PN, BERSANO PRO, NETO JFL & SFORCIN JM. 2018. Positive effects of antitumor drugs in combination with propolis on canine osteosarcoma cells (spOS-2) and mesenchymal stromal cells. Biomed Pharmacother 104: 268-274. doi: 10.1016/j.biopha.2018.05.027.

CONCEIÇÃO M, GUSHIKEN LFS, ALDANA-MEJÍA JA, TANIMOTO MH, FERREIRA MVS, ALVES ACM, MIYASHITA MN, BASTOS JK, BESERRA FP & PELLIZZON CH. 2022. Histological, Immunohistochemical and Antioxidant Analysis of Skin Wound Healing Influenced by the Topical Application of Brazilian Red Propolis. Antioxidants (Basel) 4: 2188. doi: 10.3390/antiox11112188.

DENIZOT F & LANG R. 1986. Rapid colorimetric assay for cell growth and survival: modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods 89: 271-277. doi: 10.1016/0022-1759(86)90368-6.

DIAB HM, ELHOSSEINY R, BEDAIR NI & KHORKHED AH. 2022. Efficacy and safety of plasma gel versus platelet-rich plasma in periorbital rejuvenation: a comparative split-face clinical and Antera 3D camera stud. Arch Dermatol Res 314: 661-671. doi: 10.1007/s00403-021-02270-7.

DOMINICI M, LE BLANC K, MUELLER I, SLAPER-CORTENBACH I, MARINI F, KRAUSE D, DEANS R, KEATING A, PROCKOP DJ & HORWITZ E. 2006. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8: 315-317. doi: 10.1080/14653240600855905.

DOS SANTOS DA, MUNARI FM, DA SILVA FROZZA CO, MOURA S, BARCELLOS T, HENRIQUES JAP & ROESCH-ELY M. 2019. Brazilian red propolis extracts: study of chemical composition by ESI-MS/MS (ESI+) and cytotoxic profiles against colon cancer cell lines. Biotech Res Innov 3: 120-130. doi: 10.1016/j.biori.2019.02.001.

FENG X, LIU L, YU B-Q, HUANG J-M, GU L-D & XU D-F. 2018. Effect of optimized collagenase digestion on isolated and cultured nucleus pulposus cells in degenerated intervertebral discs. Medicine (Baltimoree). 97: e12977. doi: 10.1097/MD.0000000000012977.

FRANCK CL, SENEGAGLIA AC, LEITE LMB, MOURA SAB, FRANCISCO NF, FILHO JMR. 2019. Influence of adiposo tissue deriverd stromal cells on the burn wound healind process. Stromal Cells Int 11: 2340725. doi: 10.1155/2019/2340725.

FROZZA CO ET AL. 2013. Chemical characterization, antioxidant and cytotoxic activities of Brazilian red propolis. Food Chem Toxicol 52: 137-142. doi: 10.1016/j.fct.2012.11.013.

FROZZA CODS ET AL. 2017. Antitumor activity of Brazilian red propolis fractions against Hep-2 cancer cell line. Biomed Pharmacother 91: 951-963. doi: 10.1016/j.biopha.2017.05.027.

GAJSKI G, OREŠČANIN V & GARAJ-VRHOVAC V. 2012. Chemical composition and genotoxicity assessment of sanitary landfill leachate from Rovinj, Croatia. Ecotoxicol Environ Saf 78: 253-259. doi: 10.1016/j.ecoenv.2011.11.032.

GLAß M, MÖLLER B, ZIRKEL A, WÄCHTER K, HÜTTELMAIER S & POSCH S. 2012. Cell migration analysis: Segmenting scratch assay images with level sets and support vector machines. Patt Recognit 45: 3154-3165. doi.org/10.1016/j.patcog.2012.03.001.

GARCIA CSC, MADDALOZZO AED, GARCIA PMC, FONTOURA CP, RODRIGUES MM, GIOVANELA M, HENRIQUES JAP, AGUZZOLI C, CRESPO JS & ROESCH-ELY M. 2021. Natural rubber films incorporated with red propolis and silver nanoparticles aimed for occlusive dressing application. 2021. Mat Res 24: e20200415. doi:10.1590/1980-5373-MR-2020-0415.

GLASS GE & FERRETTI P. 2019. Adipose-Derived Stem Cells in Aesthetic Surgery. Aesthet Surg J 14: 423-438. doi: 10.1093/asj/sjy160.

JULIAN K, GARG N, HIBINO N & JAIN R. 2023. Stromal Cells and Congenital Heart Disease: The Future Potential Clinical Therapy Beyond Current Treatment. Curr Cardiol Rev 19: 1-4. doi: 10.2174/1573403X18666220531093326.

KASIBHATLA S, AMARANTE-MENDES GP, FINUCANE D, BRUNNER T, BOSSY-WETZEL E & GREEN DR. 2006. Acridine orange/ethidium bromide (AO/EB) staining to detect apoptosis. CSH Protoc: pdb. prot4493. doi: 10.1101/pdb.prot4493.

LASCHKE MW, KARSCHNIA P, SCHEUER C, HEß A, METZGER W & MENGER MD. 2018. Effects of cryopreservation on adipose tissue-derived microvascular fragments. J Tissue Eng Regen Med. 12:1020-1030. doi: 10.1002/term.2591.

LEE S, CHAE DS, SONG BW, LIM S, KIM SW, KIM IK & HWANG KC. 2021. ADSC-Based Cell Therapies for Musculoskeletal Disorders: A Review of Recent Clinical Trials. Int J Mol Sci 30:10586. doi: 10.3390/ijms221910586.

LI T, MA Y, WANG M, WANG T, WEI J, REN R, HE M, WANG G, BOEY J, ARMSTRONG DG, DENG W & CHEN B. 2019. Platelet-rich plasma plays an antibacterial, anti-inflammatory and cell proliferation-promoting role in an in vitro model for diabetic infected wounds. Infect Drug Resist 29: 297-309. doi: 10.2147/IDR.S186651.

LIU Y, LIU Y, WU M, RUFEI Z, SHUN M, PEIFANG C, MINGXIAO H, HONGXU J, YAN Z & YONGLI B. 2022. Adipose-derived mesenchymal stromal cell-loaded β-chitin nanofiber hydrogel promote wound healing in rats. J Mater Sci Mater Med 20: 12. doi: 10.1007/s10856-021-06630-7.

LOPEZ BC, DE LOURENÇO C, ALVES D, MACHADO D, LANCELLOTTI M & SAWAYA A. 2015. Antimicrobial and cytotoxic activity of red propolis: an alert for its safe use. J Appl Microbiol 119: 677-687. doi: 10.1111/jam.12874.

MAGNAVACCA A, SANGIOVANNI E, GELMINI F, PIAZZA S, FUMAGALLI M, MARTINELLI G, POZZOLI C, ANGARANO M, BERETTA G & DELL'AGLI M. 2022. Brazilian propolis: a multifaceted natural product that modulates HIF-1 pathway–new perspectives for cutaneous wound healing and regeneration. Planta Med 88: 1518. doi: 10.1055/s-0042-1759203.

MENCHISHEVA Y, MIRZAKULOVA U, USUPOVA D, YERMUKHANOVA G & RYSBAYEVA Z. 2021. Platelet-rich plasma improves esthetic postoperative outcomes of maxillofacial surgical procedures. Acta Chir Plast Fall 63: 118-126. doi: 10.48095/ccachp2021118. PMID: 34814693.

MOAK TN, EBERSOLE TG, TANDON D & TENENBAUM M. 2021. Assessing Clinical Outcomes in Autologous Fat Grafting: A Current Literature Review. Aesthet Surg J 18:S50-S60. doi: 10.1093/asj/sjab148.

NOVAIS GB ET AL. 2021. Development of a New Formulation Based on In Situ Photopolymerized Polymer for the Treatment of Spinal Cord Injury. Polymers (Basel) 7: 4274. doi: 10.3390/polym13244274.

PICOLOTTO A, PERGHER D, PEREIRA GP, MACHADO KG, DA SILVA BARUD H, ROESCH-ELY M, GONZALEZ MH, TASSO L, FIGUEIREDO JG & MOURA S. 2019. Bacterial cellulose membrane associated with red propolis as phytomodulator: Improved healing effects in experimental models of diabetes mellitus. Biomed Pharmacother 112: 108640. doi: 10.1016/j.biopha.2019.

QIN Y, GE G, YANG P, WANG L, QIAO Y, PAN G, YANG H, BAI J, CUI W & GENG D. 2023. An Update on Adipose-Derived Stem Cells for Regenerative Medicine: Where Challenge Meets Opportunity. 2023. Adv Sci (Weinh) 10: e2207334. doi: 10.1002/advs.202207334.

QU Q ET AL. 2021. Platelet-rich plasma for androgenic alopecia: A randomized, placebo-controlled, double-blind study and combined mice model experiment. J Cosmet Dermatol 20: 3227-3235. doi: 10.1111/jocd.14089.

RAMASWAMY REDDY SH, REDDY R & BABU NC, ASHOK GN. 2018. Stem-cell therapy and platelet-rich plasma in regenerative medicines: A review on pros and cons of the technologies. J Oral Maxillofac Pathol 22: 367-374. doi: 10.4103/jomfp.JOMFP_93_18.

RODRIGUES MM, FONTOURA CP, GARCIA CSC, MARTINS ST, HENRIQUES JAP, FIGUEROA CA, ROESCH-ELY M & AGUZZOLI C. 2019. Investigation of plasma treatment on UHMWPE surfaces: Impact on physicochemical properties, sterilization and fibroblastic adhesion. Mater Sci Eng C Mater Biol Appl 102: 264-275. doi: 10.1016/j.msec.2019.04.048.

RORTEAU J, CHEVALIER FP, BONNET S, BARTHÉLEMY T, LOPEZ-GAYDON A, MARTIN LS, BECHETOILLE N & LAMARTINE J. 2022. Maintenance of Chronological Aging Features in Culture of Normal Human Dermal Fibroblasts from Old Donors. Cells 11: 858. doi.org/10.3390/cells11050858.

RUFATTO LC, LUCHTENBERG P, GARCIA C, THOMASSIGNY C, BOUTTIER S, HENRIQUES JAP, ROESCH-ELY M, DUMAS F & MOURA S. 2018 Brazilian red propolis: Chemical composition and antibacterial activity determined using bioguided fractionation. Microbiol Res 214: 74-82. doi: 10.1016/j.micres.2018.05.003.

SALVADORI D. 2003. Teste do micronúcleo em células humanas in vitro. In: Ribeiro LR, Salvadori DMF & Marques EK (Eds), Mutagênese Ambiental. ULBRA, Canoas, p. 201-219.

SAMBERG M, STONE R 2ND, NATESAN S, KOWALCZEWSKI A, BECERRA S, WRICE N, CAP A & CHRISTY R. 2019. Platelet rich plasma hydrogels promote in vitro and in vivo angiogenic

potential of adipose-derived stem cells. Acta Biomater 15: 76-87. doi: 10.1016/j.actbio.2019.01.039.

SHAULY O, GOULD DJ & GHAVAMI A. 2022. Fat Grafting: Basic Science, Techniques, and Patient Management. Plast Reconstr Surg Glob Open 8: e3987. doi: 10.1097/GOX.0000000000003987.

STANDARDIZATION IOF. 2009. ISO 10993-5: Biological Evaluation of Medical Devices. Part 5: Tests For In Vitro Cytotoxicity. ISO Geneva, Switzerland. https://www.iso.org/standard/36406.html.

STANDARDIZATION IOF. 2012. ISO 10993-12: Biological Evaluation of Medical Devices: Sample Preparation and Reference Materials: Partie 12, Préparaton Des Échantillons Et Matériaux de Référence: ISO. https://www.iso.org/standard/36406.html.

SUH A, PHAM A, CRESS MJ, PINCELLI T, TERKONDA SP, BRUCE AJ, ZUBAIR AC, WOLFRAM J & SHAPIRO SA. 2019. Adipose-derived cellular and cell-derived regenerative therapies in dermatology and aesthetic rejuvenation. Ageing Res Rev 54: 100933. doi: 10.1016/j.arr.2019.100933.

SUROWIECKA A & STRUŻYNA J. 2022. Adipose-Derived Stromal Cells for Facial Rejuvenation. J Pers Med 16:117. doi: 10.3390/jpm12010117.

TIAN J, CHENG LH, CUI X, LEI XX, TANG JB & CHENG B. 2019. Application of standardized platelet-rich plasma in elderly patients with complex wounds. Wound Repair Regen 27: 268-276. doi: 10.1111/wrr.12702.

TRZYNA A & BANAŚ-ZĄBCZYK A. 2021. Adipose-Derived Stem Cells Secretome and Its Potential Application in "Stem Cell-Free Therapy". Biomolecules 11: 878. doi: 10.3390/biom11060878.

VENDRAMIN FS, FRANCO D & FRANCO TR. 2009. Método de obtenção do gel de plasma rico em plaquetas autólogo. Rev Bras Cir Plást 24: 212-218. http://www.rbcp.org.br/details/471/pt-BR/metodo-de-obtencao-do-gel-de-plasma-rico-em-plaquetas-autologo.

WANG L, LI T, MA X, LI Y, LI Z, LI Z, YU N, HUANG J, HAN Q & LONG X. 2023. Exosomes from human adipose-derived mesenchymal stem cells attenuate localized scleroderma fibrosis by the let-7a-5p/TGF-βR1/Smad axis. J Dermatol Sci 112: 31-38. doi: 10.1016/j.jdermsci.2023.09.001.

XUE M, ZHAO R, MARCH L & JACKSON C. 2022. Dermal fibroblast heterogeneity and its contribution to the skin repair and regeneration. Adv Wound Care 11: 87-107. doi.org/10.1089/wound.2020.1287.

YUANZHENG C, YAN G, TING L, YANJIE F, PENG W & NAN B. 2015. Enhancement of the repair of dog alveolar cleft by an autologous iliac bone, bone marrow-derived mesenchymal stromal cell, and platelet-rich fibrin mixture. Plast Reconstr Surg 135: 1405-1412. doi: 10.1097/PRS.0000000000001166.

ZHOU ZQ, CHEN Y, CHAI M, TAO R, LEI YH, JIA YQ, SHU J, REN J, LI G, WEI WX, HAN YD & HAN Y. 2019. Adipose extracellular matrix promotes skin wound healing by inducing the differentiation of adipose-derived stem cells into fibroblasts. Int J Mol Med 43: 890-900. doi: 10.3892/ijmm.2018.4006.

ZUK PA, ZHU M, ASHJIAN P, DE UGARTE DA, HUANG JI, MIZUNO H, ALFONSO ZC, FRASER JK, BENHAIM P & HEDRICK MH. 2002. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 13: 4279-4295. doi: 10.1091/mbc.e02-02-0105.

SUPPLEMENTARY MATERIAL

Table SI.

How to cite

GARCIA CSC, GARCIA PMC, SANTOS OBA, STEFFENS D, MARTINS ST, PRANKE P, CRESPO JS, HENRIQUES JAP & ROESCH-ELY M. 2024. Red propolis extract associated to platelet-rich plasma and stromal cells with focus in cell therapy and functional tissue regeneration. An Acad Bras Cienc 96: e20240100. DOI 10.1590/0001-3765202420240100.

Manuscript received on January 30, 2024;
accepted for publication on May 11, 2024

CHARLENE S.C. GARCIA[1]
https://orcid.org/0000-0003-3654-3659

PAULO MIGUEL C. GARCIA[1,2]
https://orcid.org/0009-0000-1428-7792

OTÁVIO B.A.F. SANTOS[3]
https://orcid.org/0009-0003-1883-5698

DANIELA STEFFENS[1]
https://orcid.org/0000-0002-6041-8260

SANDRO T. MARTINS[4]
https://orcid.org/0000-0001-8861-3837

PATRICIA PRANKE[5,6]
https://orcid.org/0000-0003-4698-6314

JANAÍNA S. CRESPO[4]
https://orcid.org/0000-0002-0935-242X

JOÃO ANTONIO P. HENRIQUES[1]
https://orcid.org/0000-0002-5298-932X

MARIANA ROESCH-ELY[1*]
https://orcid.org/0000-0003-3339-1901

[1]Universidade de Caxias do Sul, Institute of Biotechnology, Rua Francisco Getúlio Vargas 1130, Petrópolis, 95070-560 Caxias do Sul, RS, Brazil

[2]Brazilian Society of Plastic Surgery – SBCP, Rua Funchal 129, 2º andar, Vila Olímpia, 04551-060 São Paulo, SP, Brazil

[3]Brazilian Society of Anesthesiology – SBA, Rua Prof. Alfredo Gomes, 36, Botafogo, 22251-080 Rio de Janeiro, RJ, Brazil

[4]Universidade de Caxias do Sul, Area of Knowledge of Exact Sciences and Engineering, Rua Francisco Getúlio Vargas 1130, Petrópolis, 95070-560 Caxias do Sul, RS, Brazil

[5]Universidade Federal do Rio Grande do Sul, Hematology and Stem Cell Laboratory, Faculty of Pharmacy, Av. Ipiranga, 2752, Azenha, 90610-000 Porto Alegre, RS, Brazil

[6]Stem Cell Research Institute, R. dos Andradas, 1464 - Conj 133, Centro Histórico, 90050-170 Porto Alegre, RS, Brazil

Correspondence to: **Mariana Roesch-Ely**
E-mail: mrely@ucs.br

Author contributions

We declare that we participated sufficiently in the work to make public our responsibility for its content, as described in the contributions below: