Comparative Study of Blood-Derived Scaffolds for the Culture of Human Adipose Derived Stem Cells (ASCS) and Dermal Fibroblasts

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ABSTRACT. This study aimed to compare the performance of blood-derived scaffolds with a well-known and accepted scaffold, chitosan, in maintaining cell cultures of ASCs and fibroblasts for future wound healing applications. Cells were characterized, immunophenotyped and cultivated into the following scaffolds: 1) Chitosan (CH, control), 2) Platelet gel (PG), 3) Chitosan blended with platelet-derived growth factors (CHPG), and 4) Fibrin glue (FG). Parameters were evaluated: i) Maintenance of cell morphology ii) Cell proliferation and iii) Citotoxicity. Our results show that ASCs and fibroblasts presented similar proliferation behaviors, which were scaffold-dependent. Regarding cell density, there were more cells in PG, followed by CHPG, FG, and CH scaffolds, for both cell types. Moreover, apoptosis assays revealed that CH had the highest rates of early (4.7%) and late apoptosis (13.9%). The proposed scaffolds demonstrated significantly lower levels of apoptosis, at less than 10%. For all these reasons, our findings demonstrate that when compared to CH, both ASCs and fibroblasts may be grown more efficiently in all three proposed scaffolds. Furthermore, we can also conclude that PG and CHPG seems to be the better choices as biomaterials for the expansion of these cells, due to higher cell proliferation, and lower apoptosis levels. Finally, it is possible to conclude that a surplus from blood bank components may be used as scaffolds with bioactive properties, providing a suitable microenvironment for cells, which could then be employed to establish tissue banks for wound healing applications.

Key words: Scaffolds; ASCS; Fibroblasts; Blood bioactive scaffolds; Cell therapy

INTRODUCTION

Regenerative medicine consists of the repair, replacement, or construction of tissues and organs, always performed in vitro aiming to be used *in vivo*. This new branch of medicine unites different areas of knowledge such as tissue engineering, cell therapy, and scaffolding technology (Whitman; Green; Berry, 1997). Almost 25 years ago, back in 1993, Langer and Vacanti's group published an important review of the real possibilities of in vitro tissue design for organ repair. These authors were concerned that aging is a natural process, which highlights the needs for research into tissue engineering.

However, it is currently known that most differentiated cell types require a 3D environment to maintain their phenotypes, or to maintain their plasticity (when talking about stem cells). Scaffolds are important tools for tissue engineering, used to substitute all or part of a biological matrix (Whitman; Green; Berry, 1997), and can be composed of different materials, including metallic, synthetic, or natural polymers, such as purified collagen, polysaccharides, other extracellular matrix components, and decellularized tissues (Birchall; Macchiarini, 2008). Furthermore, scaffolds provide important characteristics to seeded cells that are not present in 2D monolayer cultures, such as mechanical resistance, cell migration, proliferation, differentiation, and cell-to-cell and cell-to-extracellular matrix interactions (Moroz et al, 2009; Moroz et al. 2013; Moroz and Deffune, 2014).

Chitosan, along with sodium alginate, is one of the most widely used scaffolds; composed of (1-4)-2-acetamido-2-deoxy-b-D-glucan (N-acetyl D-glucosamine) and (1-4)-2-amino-2-deoxyb-D-glucan (D-glucosamine) units, which are partially derived from the deacetylation of chitin polymers (Rinaudo, 2006). Chitosan is regarded as a well-established option for tissue engineering, and is therefore suitable to be used as a 3D control scaffold in these studies. This component is an abundant hydrophilic biopolymer polysaccharide found in crustacean shells and fungus, and it is biocompatible and bioabsorbable (Birchall and Macchiarini 2008; O´Brien, 2011). Another important characteristic of these compound is that it is able to assemble in a gel state in weak acid solutions such as acetic acid, which enables its pharmaceutical form to treat skin injures such as chronic wounds and burn injuries (Muzzarelli, Tanfani, Scarpini, 1980; Rinaudo, 2006). This characteristic allows for the insertion of cells prior to gelification, which promotes a well-balanced cell seeding concentration. This biopolymer also enhances the functions of inflammatory cells, thereby promoting granulation and remodeling of damaged tissue in large open wounds of animals, as seen in Ueno´s study (2001).

Bioactive scaffolds have been in the spotlight for some years now, and can be defined as those that actively interact with and/or modulate biological responses from cells embedded within the scaffold. This bioactive

effect is generally achieved due to the presence of growth factors, hormones, cytokines and/or other biological components such as fibronectin, which interacts with seeded cells via membrane receptors, ultimately modifying gene and protein expression (Moroz et al, 2009; Moroz et al. 2013). Blended biopolymers are one of these bioactive compounds, having emerged in the literature in 2006, and one of the best known is chitosan blended with collagens (Aranaz et al., 2009). Another good example is chitosan blended with hydrogel, which interacts with fibroblast growth factor 2 (FGF-2) and also provided good results regarding wound contraction, formation of granulation tissue and wound healing in a mouse model (Aranaz et al., 2009). Novel bioactive scaffolds may have different effects not observed in some of the already described scaffolds, which may provide a better environment for cells and may be usable directly in tissue engineering.

In turn, when talking about blood components that can be used as bioactive scaffolds, controversially, dozens of surplus blood bags are discarded every day as part of transfusion practices; however, these components could be used in the production of biological scaffolds to treat chronic wounds, among other wound types and could be employed to design novel blood-derived biomaterials, providing a wide research basis while directing this precious biological waste to a noble objective (Burnouf et al., 2009). These biomaterials could be obtained from different processes, which could be handled to encourage specific compositional and molecular characteristics. Different blood components such as packed red blood cells, platelet rich plasma, fresh frozen plasma, platelet concentrate and cryoprecipitate have undergone preliminary studies to develop blood-derived scaffolds, such as fibrin glue and platelet gel (Sierra 1993; Obara et al. 2005; Burnouf et al. 2009). As a further example, scaffolds blended with platelet concentrate can be a tremendous source of growth factors, specifically PDGF, VEGF, EGF, TGF-βandFP-4 (Green and Klink 1998).

Putting all this together, the aim of this study was to compare the performance of blood derived scaffolds with a well-known biopolymer scaffold in maintaining cell cultures from human adipose mesenchymal stem cells (ASCs) and human dermal fibroblasts, for future wound healing applications. To accomplish this, fibrin glue, platelet gel, and chitosan blended with platelet-derived growth factors were tested as scaffolds, comparing them with chitosan scaffold as controls.

MATERIALS AND METHODS

Cell harvesting and dissociation

Both cell types used in this study were harvested from 4 adult humans (23 to 42 years old) who underwent elective abdominoplasty procedures at the Department of Surgery and Orthopedics of Sao Paulo State University, School of Medicine, Botucatu-SP, Brazil. The patients agreed to donate tissue fragments by filling in a consent form, and all protocols were approved by the Governmental Ethics Committee on human biologic materials (Process number: OF.151/2013-CEP). Small tissue fragments (dermis and adipose tissue) were stored at 4°C (24hrs) in HEPES medium (Gibco, Grand Island, NY) containing 2% penicillin/streptomycin (Invitrogen, Carlsbad, CA) and 2% amphotericin B (Invitrogen, Carlsbad, CA) . These fragments were washed 3 times with sterile PBS(Gibco, Grand Island, NY) and the samples were submitted to the previously described enzymatic digestion protocol (Rodbell, 1964) to obtain dermal fibroblasts and ASCs, which were then seeded into cell culture and cultivated until the fourth passage. For a more detailed description of the harvesting methods, readers are directed to the previous report by Busser et al. (2015) on ASCs harvesting and (Janmaat et al., 2015) study on human dermal fibroblast harvesting.

Cell culture

ASCs and fibroblasts were seeded in tissue-treated culture flasks. ASCs were maintained with DMEM-F12 medium (Gibco, Grand Island, NY), while dermal fibroblasts were maintained with M199 medium (Gibco, Grand Island, NY). Both media were supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY) and 1% penicillin/streptomycin solution (Gibco BRL, Grand Island, NY). Cells were cultivated at 37ºC in a humidified atmosphere of 5% CO_2. A cell bank was established to provide the total number of cells required for all experiments.

Cell characterization

a) Immunophenotyping

ASCs and fibroblasts were detached from culture flasks using 0.25% trypsin/EDTA and diluted to 1×10^6 Isoton®. Cells were analyzed by FACSCalibur flow cytometer (BD, Franklin Lakes, NJ) using the following FITC antibodies: mouse anti-human CD90 (Thy1) cod328108; mouse anti-human CD45 cod304006; mouse anti-human CD105 cod323204; mouse anti-human Stro-1 cod340106; and PE antibodies: mouse anti-human and mouse Anti-human CD73 cod344004 (ecto-5`-nucleotidase); all purchased from Biolegend® (San Diego,

CA) and CD44 cod555749 purchased from BD® (BD, Franklin Lakes, NJ). Protocols were performed as previously described, with minor modifications (Calloni et al., 2013). Cells were briefly incubated with the antibodies, in the dark, following manufacturer instructions, for 45min. After this incubation period, cells were centrifuged 3 times in Isoton® at 1200rpm for 10min to wash away unbound antibodies. Cells were then fixed in a 4% formalin solution and analyzed by the flow cytometer. The cell gate population was chosen based on forward-scatter and side-scatter parameters in order to eliminate cell debris and to maintain a homogeneous cell distribution. A total of 1×10^5 cells were analyzed per marker/cell line. Finally, recommended software BD Cell Quest® was used to acquire and analyze sample data.

b) Tri-lineage differentiation

In order to confirm the mesenchymal stem cell phenotype, a tri-lineage differentiation potential assay (adipogenic, osteogenic and chondrogenic lineages) was conducted using a commercially available kit (Stem Pro®,Gibco, Grand Island, NY), according to manufacture instructions. After 14 and 21 days, the cells were stained with Oil Red O®, Alizarin Red S® and Alcian Blue (Sigma-Aldrich, St. Louis, MO) and analyzed in an inverted microscope (Zeiss Axiovert®).

c) Immunocytochemistry

For human dermal fibroblast characterization, cells were fixed in a 4% formaldehyde solution and endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide in methanol for 10 min. Fixed cells were washed 3 times in PBS and blocked for 1hrwith3% bovine serum albumin (BSA). The cells were then incubated overnight at 4ºC with primary antibodies: vimentin (ab92547 - Abcam®) and type I collagen (ab292 - Abcam®); washed with PBS again, and incubated for 2hrs at room temperature with anti-rabbit secondary antibody (ab6741- Abcam®), labeled with peroxidase. Chromogen color was developed by incubation with 3.3-diaminobenzidine tetrahydrochloride (Sigma-Aldrich, MO). Slides were then counterstained with haematoxylin (10s).

Blood-derived scaffold preparation

Scaffolds were developed using fibrin glue or platelet gel, employing classical techniques in a sterile environment, as described below:

a) Platelet gel scaffold (leucocyte-poor)

Initially, average platelet counts were determined in a pool of human platelet concentrates using a semi-automatic counter (Micros60, Horiba ABX®). After leucocyte removal, its counting was performed by manual counting using a Nageotte chamber. The concentration of growth factors such as PDGF, VEGF, EFG, TGF-β and FP-4were also measured using a commercially available colorimetric enzyme-linked immunosorbent assay (ELISA, Quantikine®; R&D Systems), in accordance with the protocol provided by the manufacturer. Finally, 45 µL of this platelet pool, 10 µL of human purified thrombin and 5 µL of calcium gluconate were gently pipetted on a cover slip at room temperature to allow gelatinization. Before complete gelatinization, 1×10^4 cells (suspended in a minimum quantity of DMEM-F12 or M199 media) were seeded to be sure that the cells would stay both on the surface and inside the scaffolds.

b) Fibrin glue scaffold

Fibrin glue scaffold was prepared with a 45 µL pool of fresh frozen human plasma, 10 µL of purified human thrombin and 5 µL of calcium gluconate. The gelatinization and cell seeding processes were conducted in the same conditions as the platelet gel scaffold, described above.

Chitosan scaffold preparation

Purified chitosan from shrimp shells (Sigma-Aldrich, St. Louis, MO) was diluted at 4% concentration in distilled water (Integral Water Purification System-Milli-Q™, MerkMillipore™) and acetic acid (Synth®) for 24 h in a shaker machine. This solution was kept at 4ºC for an additional 24h to complete stabilization, and 60 µL of chitosan gel was seeded on the cover slip of six well plates. A 0.5N NaOH solution was added to neutralize the acidity. The pH was measured to confirm that the microenvironment was neutral. One hour before complete polymerization, 1×10^4 cells (suspended in a minimum quantity of DMEM-F12 or M199) were added to the scaffolds.

The blended chitosan variant was made by adding the 1×10^4 cells suspended in 45 µL of platelet concentrated in cover slips with chitosan gel. All scaffolds were analyzed with an inverted microscope (Zeiss Axiovert®) to confirm their structure and integrity.

Cell proliferation assay

Cell number and viability was assessed using the classical Trypan blue staining method, according to routine procedures. ADSCs and fibroblasts (1×10^4 cells/ml), were seeded into the four scaffolds. After 7 days, living cells on each well were counted in a Neubauer chamber. The assay was performed in triplicates.

Citotoxicity assay

Apoptosis was assessed after 7 days of culture in a FACSCalibur Flow Cytometer (BD Biosciences) using an annexin V/iodide propidium kit (BD, Franklin Lakes, NJ). The percentage of cells in early apoptosis was quantified using the annexin V-FITC method, which detects the phosphatidyl serine externalized in the early phases of apoptosis (annexin-V+/7-AAD−); cells in late apoptosis were quantified based on the presence of the complex formed by annexin-V+ and 7-AAD+, and viable cells were also counted (annexin-V−/7-AAD−).

Statistical analysis

All the experiments were carried out in triplicate, and statistical analyses were performed using a parametric one-way ANOVA test, followed by Tukey's multiple comparisons test. Results were considered statistically significant when $p < 0.05$.

RESULTS

Cell culture and characterization

Adipose tissue and dermis cells were seeded and after 72 h of cultivation, adherent cells started to assemble into fibroblasts colony-forming units (CFU-Fs) or clusters as seen in Figure 1A. Both cells presented classic fibroblastoid or spindle like morphology represented in Figures 1B and 1C. Flow cytometry results showed that: i) ASCs exhibited high expression of classically described positive cell surface markers CD90 (95.6%), CD44 (97.46%), CD105 (54.92%), Stro-1 (54.45%), and CD73 (96.03%), some expression of CD45 (36%), which is a negative cell surface marker (Figure 1D); ii) fibroblasts expressed high rates of positive cell surface markers to CD90 (97.55%) and CD44 (95.94%); and medium rates of negative cell surface markers CD34 (41.62%) and CD45 (22.72%) (Figure 1E).
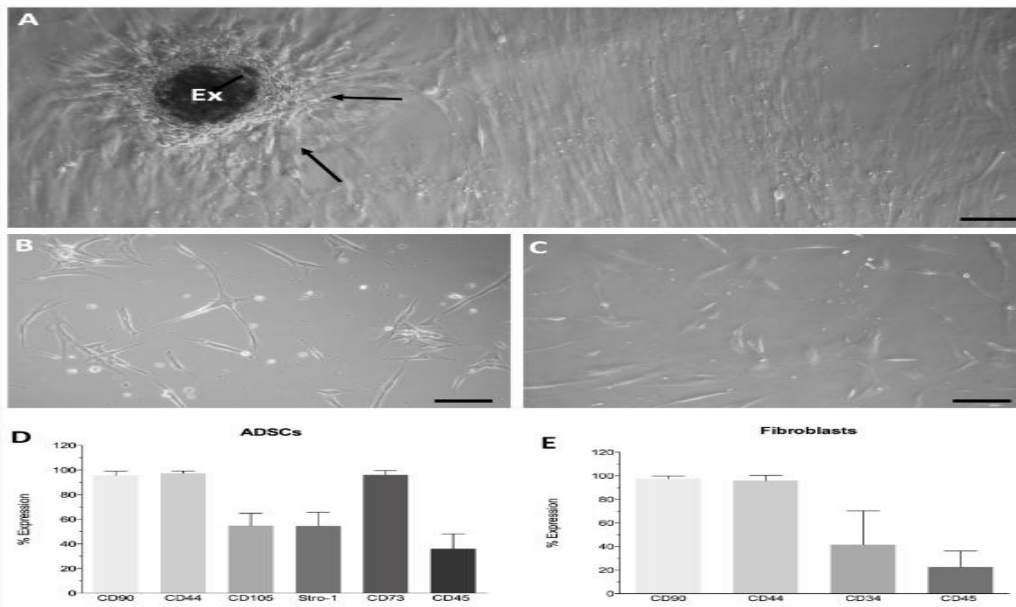

Figure 1. Colony-forming units (CFU-Fs) or clusters represented in Figure 1A. Both cells Fibroblastoid or spindle like morphology represented in Figures 1B and 1C. Expression of cell surface markers of ASCs and fibroblasts: (A) CD90(+), CD44(+), CD105(+), Stro-1(+), CD73(+), and CD45(-) for ASCs; and (B) CD90(+), CD44(+), CD34(-), and CD45(-) for fibroblasts.

In the tri-lineage differentiation analysis, ASCs showed positive staining for adipogenic (Figure 2B), osteogenic (Figure 2C) and chondrogenic (Figure 2D) lineages. Figure 2A shows the negative control stained with H/E.
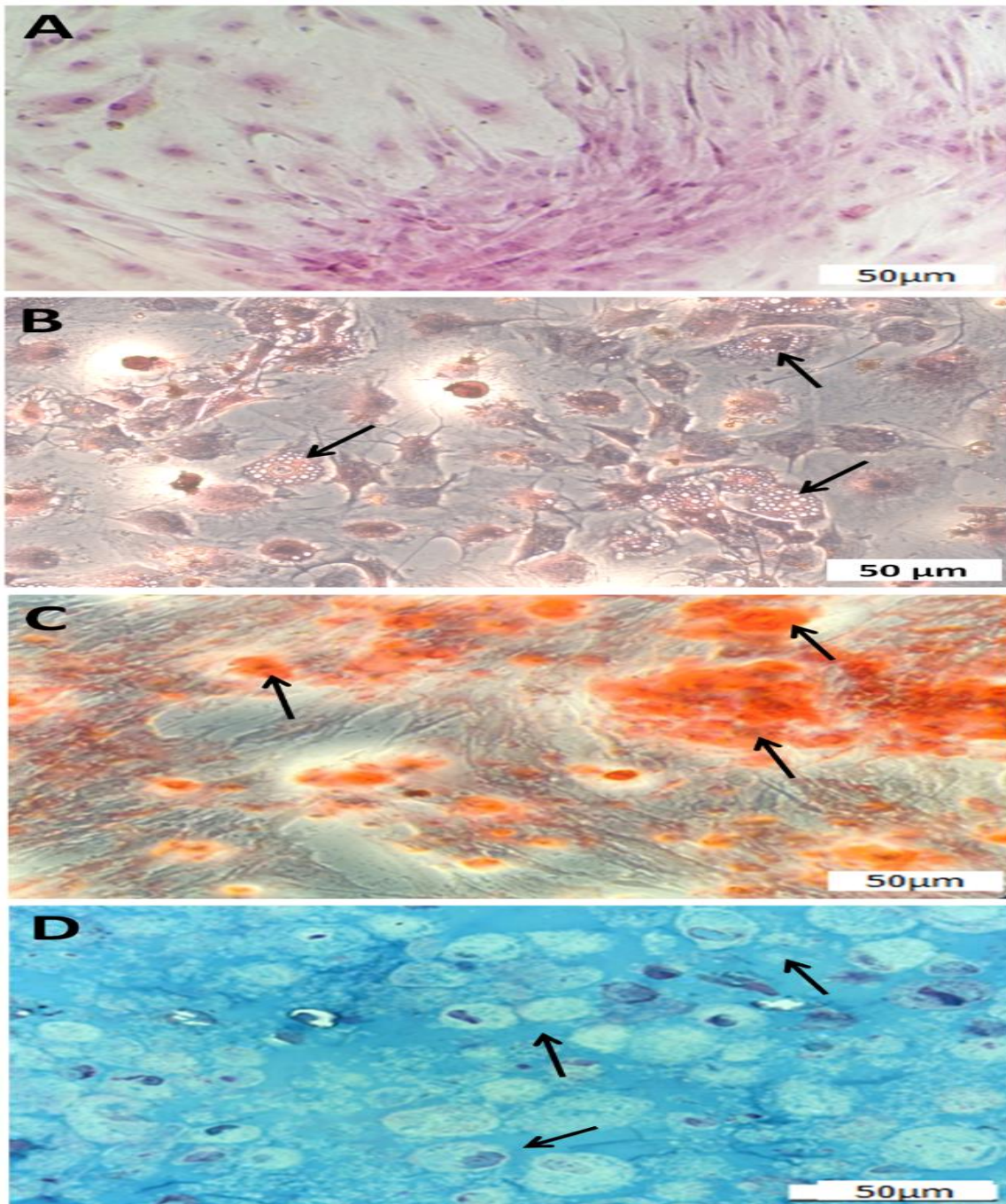
Figure 2. Characterization of ASCs by tri-lineage differentiation before scaffold seeding. (A) negative control stained with H/E; (B) adipogenic differentiation with cells showing red small lipid droplets confirmed by Oil Red O® and H/E staining. (C) osteogenic differentiation confirmed by Alizarin Red S® staining, with cells showing red calcium depots. (D) chondrogenic differentiation with Alcian Blue® staining, showing blue collagen matrix

Immunocytochemistry for fibroblasts stained with anti-collagen type I and vimentin showed positive for all samples analyzed. Even when fibroblasts were seeded into the scaffolds, they showed the same staining standard as when only seeded in culture dishes as seen in Figure 3.
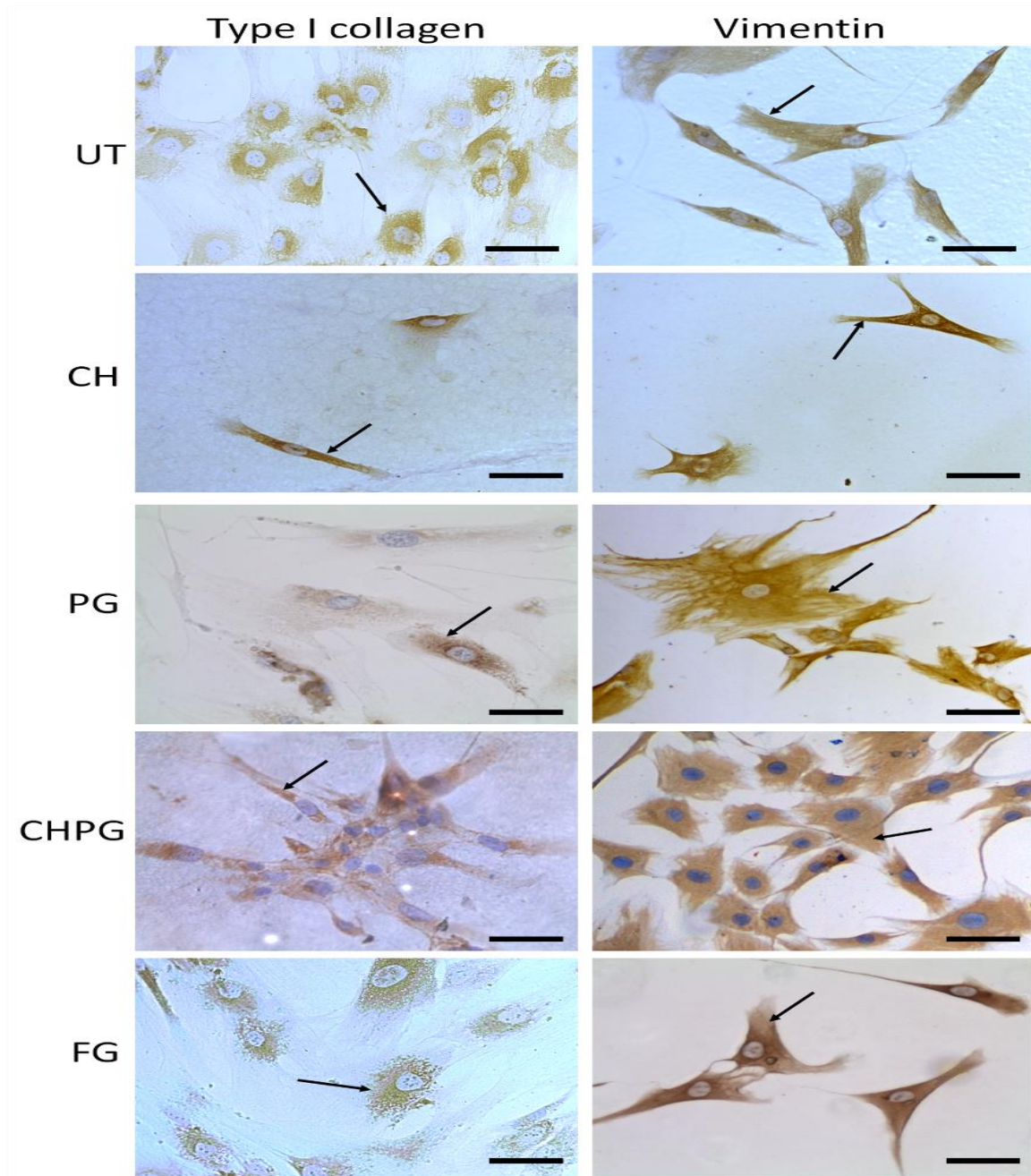
Figure 3. Fibroblasts positive for collagen and vimentin staining before and after scaffold seeding. Immunocytochemistry analysis using anti-collagen and anti-vimentin antibodies in untreated fibroblasts (UT); or seeded into: chitosan (CH); platelet gel (PG); chitosan blended with platelet gel (CHPG); and fibrin glue (FG); demonstrating positive staining (arrows). Scale bar, 10 µm for each panel.

Cell proliferation

All scaffold types were successfully assembled, but after four days of experiments, platelet gel (PG) and fibrin glue (FG) scaffolds lost stiffness and acquired a gelatinous consistency. Chitosan (CH) and chitosan blended with platelet gel (CHPG) scaffolds remained a membrane state throughout the 7 days as seen in Figure 4.
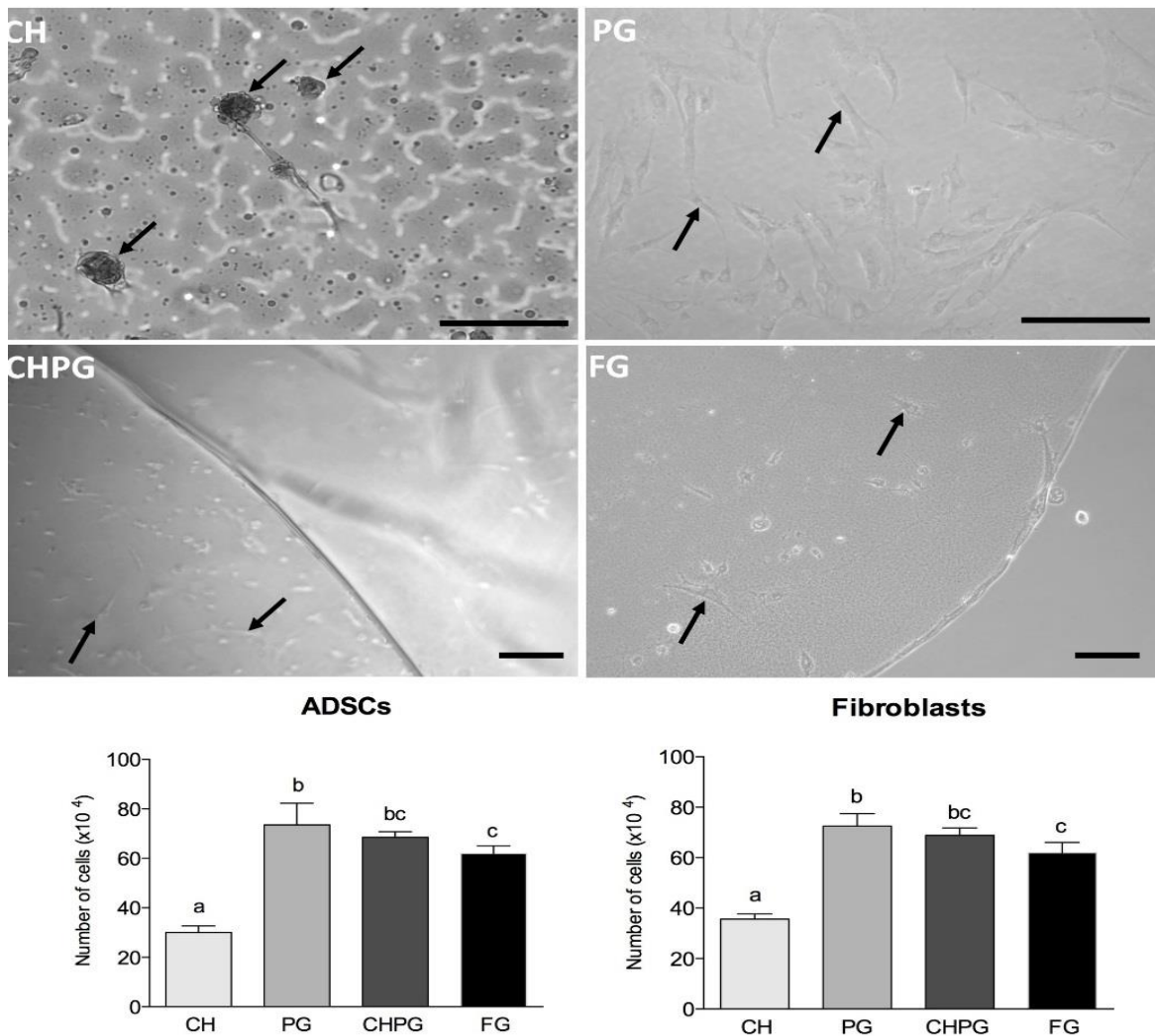
Figure 4. Morphology and cell viability of ASCs and fibroblasts after scaffold seeding. Inverted microscopy of: CH − chitosan control; PG − platelet gel; CHPG - chitosan blended with platelet gel; and FG - fibrin glue. Only the ASCs seeded in CH did not assume fibroblastoid morphology, assembling in a rounded shape. Scale bar, 20 µm for each panel. Arrows indicates cell morphologies. The ASCs and fibroblast viabilities were assessed by Tripan Blue staining. Values are expressed by mean ± SD; means with different letters are significantly different (p<0.05).

ASCs and fibroblasts have similar proliferative cell behavior, but they were scaffold-dependent. After one week, ADSCs and fibroblasts presented significantly more cells in the PG scaffold (7.3×10^4 cells/ml and 7.2×10^4 cells/ml, respectively), followed by the CHPG scaffold (6.85×10^4 cells/ml for ADSCs and 6.88×10^4 cells/ml for fibroblasts) and FG scaffold (6.19×10^4 cells/ml for ASCs and fibroblasts). The CH scaffold showed the lowest number of cells compared to the others, with 3×10^4 cells/ml for ASCs and 3.56×10^4 cells/ml for fibroblasts as seen in Figure 4 graphs.

Cytotoxicity analysis

As can be seen in Table 1, apoptosis assays revealed that CH scaffold had the highest rates of early apoptosis (4.7%) and late apoptosis (13.9%). The PG scaffold presented a 0.87% rate of early apoptosis and a significantly lower rate of late apoptosis (5.91%) when compared to the CH scaffold. The CHPG scaffold showed similar rates of early (4.73%) and late (8.75%) apoptosis compared to the CH scaffold. The FG scaffold revealed the lowest rates, with 0.40% early apoptosis and 0.04% late apoptosis.

Table 1. The apoptosis percentages measured by Anexin V/Propidium iodide by flow cytometry analysis.

Variables	CH	PG	CHPG	FG
Early apoptosis rate (%)	4.75 ± 0.04	0.87 ± 1.00	4.73 ± 1.62	0.40 ± 0.25
Late apoptosis rate (%)	13.99 ± 7.39[b]	5.91 ± 4.42[a]	5.91 ± 4.42[a]	0.04 ± 0.05[a]

Note: Values are expressed by mean ± SD, means with different letters are significantly different (p<0.05).

DISCUSSION

In this study, we compared the performance of three blood-derived scaffolds with a CH control scaffold in maintaining the cell culture of ASCs and human dermal fibroblasts. The benefit of using scaffolds derived from blood components is that they can mimic the physiological microenvironment of cells, since they contain growth factors, cytokines, and a number of physiologically relevant and bioactive proteins, such as fibronectin (Makogonenko et al. 2002; Puente and Ludeña 2013; Erich, Helmholtz, and Jenne 2016). The need for 3D scaffolds in tissue engineering can be traced back to their ability to maintain the cell phenotype of differentiated cells, while also being able to maintain the undifferentiated status of stem cells, which are paramount for the quality of the assembled tissue, their interaction with the host organism, and their stability.

In this sense, a scaffold with fibrin is a great alternative. This protein plays important roles in numerous biological processes and seeded cells can adhere to it either directly via integrin receptors (mainly integrin αvβ3), or indirectly based on the capacity of the fibrin to bind to extracellular matrix proteins, such as fibronectin (Makogonenko et al. 2002; Puente and Ludeña 2013) and vitronectin (Erich; Helmholtz; Jenne, 2016), which also interacts with cells. It is also important to note that when fibronectin and vitronectin binds to fibrin, the whole scaffold compound becomes more bioactive, given that these proteins can further stimulate gene and protein expression, as previously described by our group (Moroz, et al. 2013). Fibrin also provides a three dimensional structure which is similar to the native extracellular matrix architecture (Burnouf et al., 2009) and, in this sense, it can positively maintain cell differentiation and phenotype. Platelet gel is also a promising alternative for producing a bioactive scaffold, because of the growth factor content released during its production, such as PDGF and VEGF, which contribute to angiogenesis, and TGF-β, which contributes to cell differentiation of stem cells (O´Brien 2011; Moroz, et al. 2013). Furthermore, one must bear in mind that the growth factors are not the only bioactive compounds; platelet gel is also known to contain a large quantity of other proteins, such as fibronectin, which may also modulate cell behavior, as mentioned (Borzini; Mazzucco, 2007).

However, for a scaffold to be considered efficient, it has to be feasible to cells, allowing them to adhere, scatter, communicate, proliferate, and differentiate. The scaffold itself must be non-toxic, biodegradable, and must allow neovascularization (Swinehart; Badylak, 2016). To ensure that our scaffold had all these characteristics, we chose to compare them with a CH scaffold, which alongside sodium alginate is one of the mostly widely used (Rodriguez-Vázquez et al., 2015). It must be highlighted that comparing them to regular cell cultures in treated plastic such as 2D mono layers would be a mistake, as this culture condition does not provide the 3D architecture present in our scaffolds. CH was chosen as the control scaffold based on having previously found to be biodegradable, biocompatible, and an excellent hemostatic and analgesic agent, presenting antioxidant properties (Muzzarelli; Tanfani; Scarpini, 1980). Additionally, CH enhances the functions of inflammatory cells and the deposition of growth factors, thereby promoting granulation and remodeling of damaged tissues (ARANAZ et al., 2009). The three blood derived scaffolds (PG, FG and CHPG), were therefore compared to the CH scaffold to analyze their interaction with ASCs and human dermal fibroblasts in terms of cell adherence, maintenance of morphology, proliferation behavior and apoptosis rates, with a future focus on tissue engineering for wound healing.

Firstly, we demonstrated that ASC and fibroblast cultures seeded in CHPG, PG and FG scaffolds presented a classic fibroblastic/spindle like morphology, the first parameter for cell phenotype, while cells seeded in control CH scaffolds did not assume this morphology, assembling in a rounded shape, which indicates a lower adhesion to the substrate (Wankhade et al., 2016) and loss of their phenotypes.

Besides this, our results also showed that both cells had acceptable viability rates among the studied scaffolds. ASCs and fibroblasts were significantly more abundant in the PG scaffold, followed by the CHPG and FG scaffold. The control CH scaffolds had the lowest number of cells, which indicates that our scaffolds are more

physiologically stable than the control scaffold. In the apoptosis assay, our proposed scaffolds also exhibited good results, displaying low levels of early and late apoptosis, while the control CH scaffold presented higher apoptosis levels. Furthermore, the cells were more evenly distributed in the proposed scaffolds (homogeneously concentrated) when compared to the control CH scaffolds, which is indicative of good cell mobility and migration. We may therefore conclude that our proposed scaffolds presented the initial qualities required for the maintenance of cells, which include allowing cells to proliferate and migrate while maintaining cell apoptosis at controlled rates.

However, we conducted these experiments because we aim to use these scaffolds in the future to enhance the healing processes in damaged tissue, and so the proposed scaffolds should also allow stem cells(ASCs) to preserve their full differentiation/plasticity potential, and to allow fibroblasts to continue to produce high amounts of type-I collagen, while also expressing vimentin. To verify whether the scaffolds will be able to fulfill the above mentioned requirements, future researchers should check whether ASCs will continue to exhibit a high expression of positive cell surface markers (CD90, CD44, CD105, Stro-1 and CD73) after cell culture, and whether fibroblasts continue to express high rates of CD90 and CD44. And about the medium expression rate of CD45 found, this could be explained by the fact that when working with primary cell culture, it´s mandatory that not always is possible to get a specific cell high purity.

Regarding cell plasticity, it is interesting to note that both ASCs and fibroblasts have been previously described as able to differentiate into adipogenic, osteogenic and chondrogenic lineages, which may seem strange at first, given that fibroblasts are themselves considered differentiated cells, as described by Lorenz and colleagues (2008) (Lorenz et al., 2008). These authors published a study demonstrating such evidence by proving that human dermal fibroblasts not only express the same cell surface markers as ASCs, but that they were also able to differentiate into adipogenic and osteogenic lineages. They also showed that these cell lines are similar regarding cytoskeleton and matrix composition (LORENZ Et al., 2008). Other evidence that fibroblasts and ASCs may have the same phenotype has been provided by Hematti (Hematti, 2012), who published a review summarizing all the reports described so far, showing indistinguishable differences in culture characteristics, morphology, cell surface marker expression pattern, differentiation potential, and gene expression signature. The author also concluded that *ex vivo* cultures of expanded mesenchymal stem cells are in fact very similar to fibroblast cultures. These facts reinforce our findings, as both cell types we tested performed well in all the proposed scaffolds compared to the control scaffold.

CONCLUSION

In conclusion, our findings suggest that the proposed blood-derived scaffolds provide an efficient environment for ASCs and fibroblasts, but PG and FG scaffolds seems to be the best suited to cultivation of these cells in terms of cell proliferation, apoptosis levels and maintenance of phenotype.

ACKNOWLEDGEMENTS

REFERENCES

Aranaz I. et al. (2009). Functional characterization of chitin and chitosan. Current Chemical Biology. 3: 203-230. https://doi.org/10.2174/2212796810903020203

Birchall, M and Macchiarini, P (2008). Airway transplantation: A Debate Worth Having? Transplantation. 85: 1075-1080. https://doi.org/10.1097/tp.0b013e31816a10e4

Borzini P and Mazzucco I (2007). Platelet-rich plasma (PRP) and platelet derivatives for topical therapy. What is true from the biologic view point? ISBT Science Series. 2: 272-281. https://doi.org/10.1111/j.1751-2824.2007.00085.x

Burnouf T. et al. (2009). Blood-derived biomaterials: fibrin sealant, platelet gel and platelet fibrin glue. ISBT Science Series, 4: 136-142. https://doi.org/10.1111/j.1751-2824.2009.01222.x

Busser H, et al. (2015). Isolation and characterization of human mesenchymal stromal cell subpopulations: comparison of bone marrow and adipose tissue. Stem Cells and Development. 24:2142-2157. https://doi.org/10.1089/scd.2015.0172

Calloni R (2013). Reviewing and updating the major molecular markers for stem cells. Stem Cells and Development. 22: 9. https://doi.org/10.1089/scd.2012.0637

Erich D, Helmholtz J and Jenne DE (2016). Vitronectin: A new molecular connection in haemostasis. Thrombosis and Haemostasis. 66: 2. https://doi.org/10.1111/j.1365-2958.2010.07373.x

Green, David M and Klink B (1998) Platelet gel as an intraoperatively procured platelet-based alternative to fibrin glue. Plastic and Reconstructive Surgery. 4: 101. https://doi.org/10.1097/00006534-199804040-00067

Hematti P (2012). Mesenchymal stromal cells and fibroblasts: A case of mistaken identity? Cytotherapy. 14: 516-521. https://doi.org/10.3109/14653249.2012.677822

Janmaat CJ (2015). Human dermal fibroblasts demonstrate positive immunostaining for neuron- and glia- specific proteins. PLoS ONE. 10: 1-14. https://doi.org/10.1371/journal.pone.0145235

Lorenz K (2008). Multilineage differentiation potential of human dermal skin-derived fibroblasts. Experimental Dermatology, 17: 925-932. https://doi.org/10.1111/j.1600-0625.2008.00724.x

Makogonenko E (2002) Interaction of fibrin (ogen) with fibronectin: Further characterization and localization of the fibronectin-binding site. Biochemistry, 41: 7907-7913. https://doi.org/10.1021/bi025770x

Andrei M and Bittencourt RA (2009) Platelet gel: 3D Scaffold for cell culture. Acta Ortopedica. 17: 43-45.

Moroz A (2013). Platelet lysate 3D scaffold supports mesenchymal stem cell chondrogenesis: An improved approach in cartilage tissue engineering. Platelets. 24: 219-225. https://doi.org/10.3109/09537104.2012.686255

Moroz A, et al. (2014). Platelet and plasma bioactive scaffolds for stem cell differentiation: What are we missing? Platelet and plasma bioactive scaffolds for stem cell differentiation: What are we missing? Platelets. 7104: 7. https://doi.org/10.3109/09537104.2013.836748

Moroz A and Deffune E (2013). Platelet-rich plasma and chronic wounds: remaining fibronectin may influence matrix remodelling and regeneration success. Cytotherapy. 1: 1-4. https://doi.org/10.1016/j.jcyt.2013.05.019

Muzzarelli R, Tanfani AA and Scarpini F (1980) The degree of acetylation of chitins by gas chromatography and infrared spectrometry. Journal of Biochemical and Biophysical Methods, 2: 299-306. https://doi.org/10.1016/0165-022x(80)90054-8

O´Brien FJ (2011). Biomaterials and scaffolds for tissue engineering. Materials Today, 14: 88-95.

Obara K, et al. (2005). Acceleration of wound healing in healing-impaired db/db mice with a photo cross linkable chitosan hydrogel containing fibroblast growth factor-2. Wound repair and regeneration, 13: 390-397. https://doi.org/10.1111/j.1067-1927.2005.130406.x

Puente, Pilar DL and Ludeña D. (2013) Cell culture in autologous fibrin scaffolds for applications in tissue engineering. Experimental Cell Research. 322: 1-11. https://doi.org/10.1016/j.yexcr.2013.12.017

Rinaudo M (2006) Chitin and chitosan: Properties and applications. Progress in Polymer Science, 31: 603-632. https://doi.org/10.1002/chin.200727270

Rodbell M (1964). Localization of lipoprotein of rat adipose lipase in fat cells. Journal of Biological Chemistry. 3: 239

Rodriguez-Vázquez M (2015). Chitosan and its potential use as a scaffold for tissue engineering in regenerative medicine. BioMed research international, 1: 1-15.

Sierra DH (1993). Fibrin sealant adhesive systems: A review of their chemistry. Material Properties and Clinical Applications. Journal of Biomaterials Applications, 309: 7. https://doi.org/10.1177/088532829300700402

Swinehart IT and Badylak SF (2016). HHS Public Access. 245: 351-360.

Ueno H, Mori T and Fujinaga T (2001). Topical formulations and wound healing applications of chitosan. Advanced Drug Delivery Reviews. 52: 105-115. https://doi.org/10.1016/s0169-409x(01)00189-2

Wankhade UD, et al. (2016). Advances in adipose-derived stem cells isolation, characterization, and application in regenerative tissue engineering. Stem Cells International. 2016. https://doi.org/10.1155/2016/3206807

Whitman H, Green DM and Berry L (1997) Platelet gel: An autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. Journal of Oral and Maxillofacial Surgery. 55: 1294-1299. https://doi.org/10.1016/s0278-2391(97)90187-7