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TITLE:

Production of Autologous Platelet-Rich Plasma for Boosting In Vitro Human Fibroblast Expansion

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KEYWORDS:

platelet-rich plasma, PRP, fibroblasts, cell proliferation, cell migration, wound healing, cell culture, cell therapy

SUMMARY:

This protocol presents a device that produces PRP to boost the in vitro expansion of cells in a 100% autologous fibroblast culture system.

ABSTRACT:

There is currently great clinical interest in the use of autologous fibroblasts for skin repair. In most cases, culture of skin cells in vitro is required. However, cell culture using xenogenic or allogenic culture media has some disadvantages (i.e., risk of infectious agent transmission or slow cell expansion). Here, an autologous culture system is developed for the expansion of human skin fibroblast cells in vitro using a patient's own platelet-rich plasma (PRP). Human dermal fibroblasts are isolated from the patient while undergoing abdominoplasty. Cultures are followed for up to 7 days using a medium supplemented with either fetal bovine serum (FBS) or PRP. Blood cell content in PRP preparations, proliferation, and fibroblast differentiation are assessed. This protocol describes the method for obtaining a standardized, non-activated preparation of PRP using a dedicated medical device. The preparation requires only a medical device (CuteCell-PRP) and centrifuge. This device is suitable under sufficient medical practice conditions and is a one-step, apyrogenic, and sterile closed system that requires a single, soft spin centrifugation of 1,500 x *g* for 5 min. After centrifugation, the blood components are separated, and the platelet-rich plasma is easily collected. This device allows a quick, consistent, and standardized preparation of PRP that can be used as a cell culture supplement for in vitro expansion of human cells. The PRP obtained here contains a 1.5-fold platelet concentration compared to whole blood together, with a preferential removal of red and white blood cells. It is shown that PRP presents a boosting effect

in cell proliferation compared to FBS (7.7x) and that fibroblasts are activated upon PRP treatment.

INTRODUCTION:

Regenerative medicine aims to heal or replace tissues and organs damaged by age, disease, or trauma as well as correct congenital defects. In autologous therapy, cells or tissue are withdrawn from a patient, expanded or modified, then reintroduced to the donor. This form of therapeutics has broad potential in the field of dermatology[1]. In autologous fibroblast therapy, a patient's fibroblasts are cultured and reinjected to treat wrinkles, rhytids, or acne scars. As fibroblasts are the main functional cells in the dermis, injection of autologous fibroblasts may be more beneficial than other therapies in facial rejuvenation[2].

In the skin, fibroblasts are responsible for the synthesis and secretion of extracellular proteins (i.e., collagen, elastin, hyaluronic acid, and glycosaminoglycans). They also release growth factors that regulate cell function, migration, and cell-matrix/cell-cell interactions in normal skin homeostasis and wound healing[3]. Dermal fibroblasts have already been introduced as a potential clinical cell therapy for skin wound healing[4], tissue regeneration[5], or dermal fillers in esthetic and plastic surgery procedures[6]. Some studies even suggest that, in the context of regenerative medicine, fibroblasts may be a more practical and effective cell therapy than mesenchymal stem cells[7].

To obtain a sufficient number of fibroblasts for clinical applications, cell expansion is usually mandatory. Ex vivo/in vitro cell culture requires basal medium supplemented with growth factors, proteins, and enzymes to support cell adhesion and proliferation. Fetal bovine serum (FBS) is a common supplement for cell culture media, because fetal blood 1) is rich in growth factors compared to adult blood and 2) presents a low antibody content[8]. As cell therapy progresses, there are concerns about the safety of classical cell culture conditions in which FBS is added to the culture medium. Furthermore, there is now a tendency to replace FBS with alternatives[9]. Several FBS substitutes have shown promising results[10].

The platelet-rich plasma (PRP) alternative has been selected here, and we have developed a medical device to produce a standardized preparation of PRP, named CuteCell-PRP. The intended use of this device is the preparation of autologous PRP to be used as a culture media supplement for in vitro expansion of autologous cells under GMP conditions.

PRP is defined as a concentrated platelet suspension in plasma. Because there are numerous preparation protocols, which differ in 1) the amount of blood needed, 2) types of devices used, and 3) centrifugation protocol, the resulting platelet concentrations vary from slightly above to more than 10x the blood baseline value. In addition, PRP preparations contain variable levels of red and white blood cell contamination. The terminology "PRP" is thus used to describe products that vary greatly in their biological composition and potential therapeutic effects.

In most studies, FBS substitution is achieved using different concentrations of PRP that is activated (by thrombin or calcium). This artificial activation provokes an immediate and

important release of platelet growth factors from 15 min up to 24 h[11]. Therefore, it is believed that platelet activation is undesirable for applications in cell cultures, in which the slow release of growth factors from gradual platelet degranulation is required.

PRP therapy involves the preparation of autologous platelets in concentrated plasma[12]. The optimal platelet concentration is unclear, and a broad range of commercial devices are available to prepare PRP[13]. This lack of standardization results from inconsistency among studies and has led to a black box regarding the dosage and timing of injection. This protocol describes a procedure to obtain autologous PRP using this dedicated PRP device to expand skin fibroblasts in a 100% autologous ex vivo culture model.

PROTOCOL:

The study protocol complied with the Declaration of Helsinki, and all patients provided written informed consent before participating in the study. Skin samples are obtained from healthy women undergoing abdominoplasty in the Plastic, Reconstructive and Aesthetic Surgery Department at Geneva University Hospitals (Geneva, Switzerland). The procedure conforms to the principles of the Declaration of Helsinki and was approved by the local institutional ethics committee (protocol #3126).

1. Preparation of PRP

NOTE: The CuteCell-PRP tubes (**Table of Materials**) are designed for the rapid preparation of PRP from a small volume of the patient's blood in a closed circuit system.

1.1. Collection of whole blood

NOTE: Harvest autologous blood from a peripheral vein of the arm using a butterfly needle directly connected to the PRP tube, according to the collection protocol of the medical institute. Blood can be directly withdrawn through the venous cannula if the patient is under anesthesia.

1.1.1. Open the tube blister pack. Perform the venous puncture and fill the desired number of PRP tubes with whole blood. The vacuum within the tubes will enable automatic collection of the necessary volume of blood (~10 mL).

1.1.2. Carefully turn the tubes upside down several times to mix the blood with anticoagulant.

1.1.3. Inside a biosafety cabinet (class 2), remove 100 µL of total whole blood using a 1 mL syringe for further blood cell number counts.

1.2. Centrifugation

NOTE: Ensure that the centrifuge is correctly balanced before starting it.

1.2.1. Once the blood is collected in the PRP tubes, if necessary, prepare a counterbalance tube (supplied separately) with water to the same level as the blood in the PRP tube. Place the filled tubes in the centrifuge opposite each other, ensuring that the machine is balanced.

1.2.2. Centrifuge samples at 1,500 x *g* for 5 min.

NOTE: Set the corresponding rpm speed according to the centrifuge manufacturer's instructions. After centrifugation, the blood will become fractionated. The red and white blood cells are trapped under the gel, and platelets settle on the surface of the gel (**Figure 1**).

1.3. Gently invert the PRP tube 20x to resuspend the platelet deposit in the plasma supernatant.

NOTE: Ensure that the platelets are fully detached from the gel. The plasma should change from clear and transparent to turbid. If platelet aggregates are present, they should be collected with the plasma.

1.4. To collect PRP, take the plasma solution from the tube using a syringe transfer device connected to a 10 mL syringe.

NOTE: About 5 mL of PRP will be obtained from each tube. The PRP solution is now ready to mix in the final medium preparation. The solution can be kept at room temperature (RT) under the safety cabinet until the future steps involving hematology analyzer and use.

1.5. Before using the PRP, determine the platelet concentration, mean platelet volume, and number of red and white blood cells using an automated hematology analyzer (**Table of material**). To do so, carefully withdraw 200 µL of the solution and transfer into a 1.5 mL tube.

NOTE: PRP is used as an autologous culture supplement at a concentration between 5–20% v/v. The optimal concentration of PRP needs to be determined for each cell line. To prevent fibrin clot formation, the final culture media should be supplemented with 2 U/mL heparin.

2. Isolation of autologous fibroblasts and culture in FBS- or PRP-supplemented media

2.1. Wash skin samples in phosphate-buffered saline (PBS) by gently shaking in a 50 mL polypropylene tube.

2.2. If the skin sample is relatively large (>10 cm^2), place the sample on the lid of a 150 cm^2 tissue culture dish (epidermal side down). Remove the subcutaneous fat tissue using a pair of forceps and scissors. To prevent the tissue from drying out, rinse the tissue every few minutes in PBS.

NOTE: Use smaller tissue culture dish for smaller samples.

2.3. When fat trimming is complete, cut the tissue into approximately 0.5 cm x 1.5 cm strips using a sterile scalpel.

2.4. Add 5 mL of collagenase-dispase mix (**Table of Materials**; 14 Wünsch units/mL) to a sterile 15 mL tube.

2.5 Transfer the cut tissue to the tube, ensuring that the tissue pieces are submerged in the solution. Cap the tube securely.

2.6. Place the tubes in an incubator at 37 °C with orbital shaking for 150 min. After incubation, place the tube containing the tissue in the biosafety cabinet.

2.7. Place the lid of a sterile 100 mm culture dish upside down in the biosafety cabinet. Transfer the digested tissue solution to the bottom of the 100 mm culture dish, without splashing. If any pieces of tissue remain in the tube, use a sterile 1 mL pipette or sterile forceps to transfer the tissue pieces to the bottom of the 100 mm culture dish.

2.8. With the epidermis strip facing up, separate the intact epidermis sheet from the dermis by using two pairs of forceps. Hold the dermis of the tissue strip with a pair of forceps and the edge of the epidermis with another pair of forceps. Peel the dermis and epidermis apart in two pieces, keeping the separated pieces on the same lid. Try to perform this step quickly and repeat the manipulation for each piece of tissue.

2.9. Transfer the dermal pieces to a new 100 mm culture dish containing PBS.

2.10. Using laboratory forceps, place the dermal pieces in a 15 mL polypropylene tube with 3 mL of 0.3% trypsin/PBS. Incubate for 10 min in a 37 °C water bath and invert the tube several times every 2−3 min.

2.11. To stop the enzymatic reaction, add 3−5 mL of ice-cold complete growth medium (Dulbecco's modified Eagle medium [DMEM] or RPMI containing 10% FBS). Vortex the tube vigorously several times. Filter the fibroblast suspension through an 85 µm nylon mesh (placed over the top of a 50 mL tube) to remove dermal debris.

2.12. Centrifuge for 10 min at 150 x *g*, at 4 °C. Aspirate the supernatant and resuspend the pellet in 100−200 µL of complete growth medium.

2.13. Using trypan blue (dilution factor of x2) mixed with the cell suspension, count the number of total and viable cells by filling the chamber of the haemocytometer.

NOTE: Cell viability depends on the conditions used for enzymatic digestion. To increase cell recovery and cell viability, the skin sample may be cut into smaller pieces.

2.14. Plate 3−10 x 10^4 cells in 5 mL of complete FBS growth medium (DMEM, 10% FBS heat-inactivated for 60 min at 56 °C, 1% 1 M HEPES buffer solution, 1% 100x nonessential amino acid mixture, 1% 100x L-glutamine, 1% 100x penicillin/streptomycin, 1% 100x sodium pyruvate) in a

25 cm^2 tissue culture flask. Incubate at 37 °C.

NOTE: Viable fibroblasts will attach to the flask within 24 h and begin to exhibit the spindle-shape in 2−3 days.

2.15. On day 2, aspirate the medium containing nonadherent cells and add fresh medium.

NOTE: As dead cells in the culture medium affect the growth of viable fibroblasts, nonadherent, dead cells must be removed from the culture.

2.16. Change the medium every 3−4 days until the culture reaches 70−80% confluency.

2.17. To harvest fibroblasts, wash with PBS and incubate with trypsin/EDTA solution for 3 min at 37 °C in the incubator.

2.18. To stop the reaction, add 3−5 mL of warm complete growth medium (DMEM or RPMI containing 10% FBS). Take an aliquot of the cell suspension to count the cells with the haemocytometer. Centrifuge the suspension for 5 min at 200 x *g* and remove the medium by aspiration.

2.19. Culture the fibroblast in PRP medium (DMEM, 20% PRP, 1% 1 M HEPES buffer solution, 1% 100x nonessential amino acid mixture, 1% 100x L-glutamine, 1% 100x penicillin/streptomycin, 1% 100x sodium pyruvate, 2 U/mL heparin) in the incubator at 37 °C.

NOTE: The minimum cell density recommended is 4,000 viable cells/cm^2.

2.20. To assess PRP effects on fibroblast proliferation, seed fibroblasts (passage 2) in 24 well plates at a density of 8 x 10^3 cells per well in PRP medium with different PRP concentrations (1%, 5%, 10%, 20%, 30%, 40%, and 50%) and with 2 U/mL heparin or under classical culture medium conditions (10% FBS). After 7 days of culture, add a vital dye (cell proliferation violet) to cells and assess the proliferation by flow cytometry.

2.21. To study cytoskeletal rearrangements, seed cells in 96 well black/clear flat bottom plates at a concentration of 1 x 10^5 cells/mL in complete FBS growth DMEM (see step 2.14) supplemented with 0.5% FBS for 24 h. Treat cells with 10% FBS or different PRP concentrations (1%, 5%, 10%, 20%, 30%, 40%, and 50%) for 7 days.

2.21.1. Fix the fibroblasts with 4% paraformaldehyde for 10 min and permeabilize with 0.1% Triton X-100 for 5 min at RT. Stain the fibroblasts with 50 mL of 5 U/mL phalloidin, wash 2x with PBS, and mark with 50 mL of 1 mg/mL 4',6-diamidino-2-phenylindole (DAPI) for 5 min.

REPRESENTATIVE RESULTS:

This patented technology is a simple, fast, and reproducible medical device used to produce standardized PRP preparations. It is a one-step, fully closed system that allows the preparation

of PRP from venous whole blood after 5 min of centrifugation at 1,500 x *g* (due to the separating gel technology). The PRP obtained after centrifugation is cleared from red and white blood cells, which sit below the gel. After several tube inversions, the platelets that are on top of the gel are resuspended in the plasma, and the PRP is ready to use (**Figure 1**).

To assess the reproducibility of the blood cell content in the preparation, whole blood and PRP samples were analyzed from 10 different patients with a hematology analyzer. After processing of the whole blood with the device, the majority of the red blood cells (RBCs) and white blood cells (WBCs) were removed. The mean platelet concentration showed a 1.5-fold increase when compared to the physiological concentration found in the whole blood (**Figure 2**).

In the autologous culture set-up, a patient's isolated fibroblasts were cultivated in the presence of increasing PRP concentrations (1−50%), compared to the classical culture medium condition (10% FBS). After 7 days of culture without changing the media, the cultures primed with 20% PRP exhibited a higher number of viable fibroblasts (**Figure 3A,B**; 7.7x higher compared to the control condition). Phalloidin staining evidenced that the morphological change observed upon-PRP activation was related to F-actin reorganization, which is a signature of fibroblast activation (**Figure 4**).

FIGURE LEGENDS:

Figure 1: Preparation of autologous PRP to be used as a culture media supplement for in vitro expansion of autologous cells under GMP conditions.

Figure 2: Blood cell and platelet counts. PRP prepared with the commercial device displays highly reproducible blood cell content despite inter-donor variations. PLT: platelets (x 10^5); WBCs: white blood cells (x 10^3); RBCs: red blood cells (x 10^6), n = 10 patients. This figure has been modified from Berndt et al.[14].

Figure 3: Assessment of PRP proliferative effects on autologous fibroblast culture. (A) Representative brightfield microscopic images demonstrating the boosting effects of 20% PRP medium added to fibroblasts isolated from the same patient after 7 days of culture. Scale bar = 100 µm. (**B**) Assessment of PRP proliferative effect by flow cytometry using vital dye. Proliferative effects of increasing PRP concentrations in comparison (1−50%) with 10% FBS (n = 10 patients) on normal human dermal fibroblast (NHDF) for 7 days without medium change in a complete autologous system (cells and PRP from the same patient; *p < 0.05, **p < 0.01, ***p < 0.001). This figure has been modified from Berndt et al.[14].

Figure 4: Cytoskeleton visualization through F-actin staining. After 7 days grown in 20% PRP (right panel), fibroblasts are activated and reorganize their cytoskeleton as compared to control culture conditions with 10% FBS (left panel). Scale bar = 200 µm. This figure has been modified from Berndt et al.[14].

DISCUSSION:

The advantages of using autologous fibroblasts as a natural alternative compared to other filler materials in wound cell therapy include good biocompatibility, minimal side effects, and easiness of harvesting and use. However, before using these therapeutics in a daily clinical setting, proper preclinical studies are necessary to identify the growth features and assess the biological function and safety of isolated fibroblasts both before and after transplantation. Thus, directly after the isolation process, in vitro expansion of the cells must be performed quickly in order to limit the number of cell divisions. Culture conditions must be tightly controlled to ensure the safety and efficacy of the final autologous biological product.

In published preclinical studies, cells at passages 3−4 have typically been chosen to be injected, as they are most suitable in terms of cell quantity and proliferative/secretory activity[15]. Most preclinical research has been performed with culture media in which FBS is the source of nutrients and growth factors[16]. However, this xenogeneic growth supplement presents a possible risk of infection and immunological reactions. Due to the occurrence of bovine spongiform encephalopathy (BSE) in herds worldwide, there is considerable regulatory concern regarding the use of FBS as a cell culture supplement because of the risk of introducing xenogeneic material into cell therapy cultures[10].

Usually, proliferation of fibroblast or mesenchymal stem cells (MSC) are achieved using FBS-supplemented growth media. While FBS has been an adequate nutrient supply for cell culture, it poses additional hindrances besides the risk of zoonose contamination. First, FBS products present huge manufacturing cost compared to human blood-derived products, partly due to reduced proliferation rates and an extended culture expansion period[9]. Second, FBS is a source of xenoproteins that interact with the isolated cells, thereby increasing the risk of immune reaction because of the appearance of antibodies against surface protein complexes on transplanted cells[10].

To address the potential drawbacks of FBS as a nutrient for clinical expansion of cells, human blood-derived products have been introduced as alternatives. Some studies have proposed replacing FBS in culture media with autologous human serum for cell expansion, but since cell proliferation is slow, it still requires a long culture time (3 weeks) and several cell passaging before transplantation[17,18].

Other groups have used platelet lysate (PL), a cell-free supernatant rich in growth factors that are released from platelets after freeze-thawing disruption of the platelet concentrates. The main advantage of PL compared to PRP is its frozen storage potential. Therefore, the same lot can be used for consecutive cell culture applications. Furthermore, as an off-the-shelf product, it can be standardized by analyzing the growth factor content and other biological features before use. Some disadvantages are that it is allogenic, and batches are pooled from several donors. Moreover, since PL does not contain any living platelets, which can produce cytokines and growth factors for up to 10 days, culture media containing PL has to be changed every 3 days (instead of 7−10 days when culture is supplemented with PRP)[19]. Expanding the cells in vitro in their own source of growth factors and nutrients provided by this PRP preparation is certainly

advantageous. The preparation is free of any xenogeneic supplements in the cell culture that could cause rejection, allergy, or transmission of infectious disease.

In previous studies, it has been demonstrated that autologous PRP can be used as a safe, efficient, and cost-effective culture medium for adipose-derived mesenchymal stem cell (ADSC) proliferation[20]. Media supplied with 20% autologous PRP increased cell proliferation up to 13-fold without changing the cell phenotype. In clinics, PRP is considered to have powerful rejuvenation and regeneration capacities.

Currently, PRP obtained from a patient's blood is already used efficiently in the clinical setting for wound healing alone[21] or in combination with hyaluronic acid[22,23] or bone regeneration[24,25]; or skin rejuvenation alone[26] or in combination with adipose-derived stromal vascular fraction cells[27-29]. This prompted the investigation of using a patient's own PRP to promote expansion of autologous fibroblasts.

For this purpose, and to ensure reproducibility of the results obtained with the PRP preparation, we developed a special PRP device. This closed system allows the standardization of the PRP preparations, which is a prerequisite to minimize fluctuations from a patient's donor (regarding platelet concentration and operator-dependent variations). The blood cell counts obtained from 10 patients showed that the protocol is highly reproducible (**Figure 2**). Moreover, it was shown that PRP had potent effects as a proliferation booster, up to 7.7-fold compared to FBS (**Figure 3B**). This is important in order to reduce the costs and time required for the expansion process. In a recent study, we further analyzed how PRP affects fibroblast proliferation and differentiation. The 20% PRP displayed the best efficacy without affecting the cell phenotype or genotype[14].

A limitation of the method is that the medium containing PRP must be prepared with heparin to prevent clot formation. Even though standard plasma anticoagulant (a sodium citrate anticoagulant and calcium chelator) is used in this device, fibrin clots formation can occur when plasma is added to culture media that contains calcium[30]. Heparin addition to the PRP preparation prevents this fibrin clot formation. However, heparins can bind growth factors and may interfere with cell growth[30]. For example, heparin was shown to negatively affect dermal fibroblast proliferation[31]. However, the concentrations used were 4x more concentrated compared to the concentration used in this study (2 U/mL).

Moreover, commercial heparin is mainly purified from porcine sources; therefore, because of its animal origin, it represents a limitation in the development of completely xeno-free medium. Although porcine heparin has been approved for human therapy, hypersensitivity to the molecule has been reported[32]. Another limitation is that PRP must be used as a fresh biological supplement and cannot be frozen. Concerning the half-life of the platelets, it has been previously shown that after 10 days, half of the platelets present in the PRP are still viable[33]. For this reason, the PRP is kept at room temperature and used for 10 days. Moreover, for future cell therapies that require the production of massive numbers of cells, the main limitation is the total amount of blood that can be withdrawn from the patient.

In summary, this protocol demonstrates that replacing FBS with PRP results in a rapid expansion of fibroblasts. The platelets present in the PRP slowly release an orchestrated mix of growth factors during the culture phase of expansion. The presented device is used during a standardized procedure that reduces the complexity and hands-on time during the preparation of PRP. This system is thus deemed an appropriate method when a quick and safe ex vivo expansion is needed for autologous cell therapy applications in GMP conditions.

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DISCLOSURES:

This project has been funded by Regen Lab SA. Sarah Berndt is the cell therapy head project manager for Regen Lab and is employed by Regen Lab SA. Antoine Turzi is the CEO of RegenLab.

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Figure 1

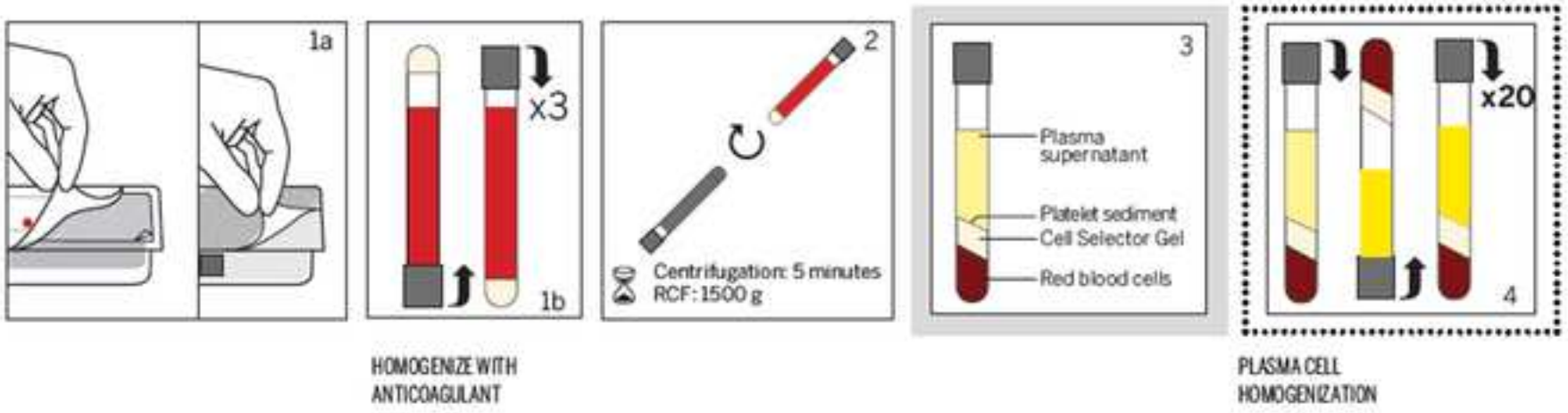

Figure 2

Click here to access/download;Figure;Figure 2 JoVE.psd

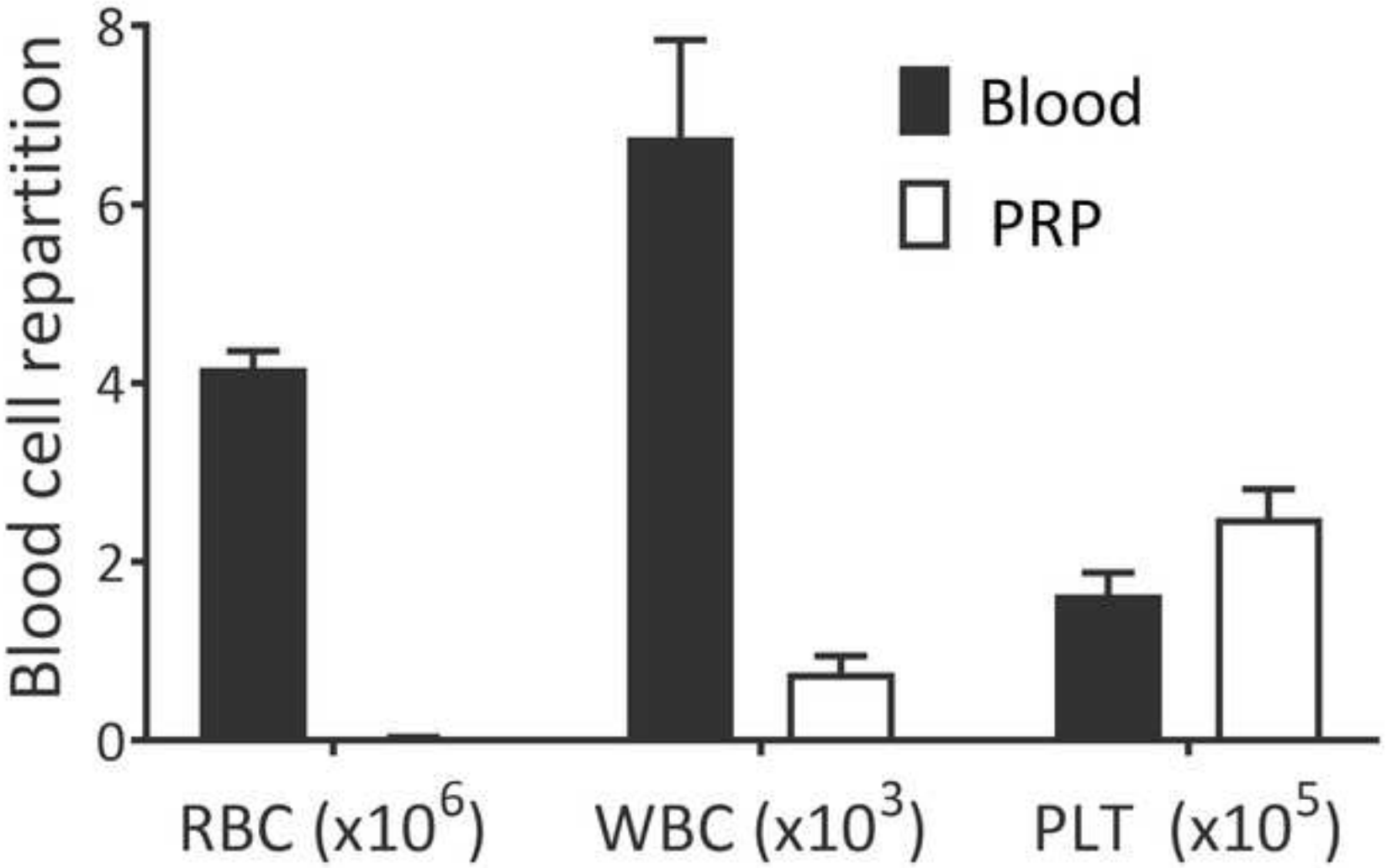

Figure 3

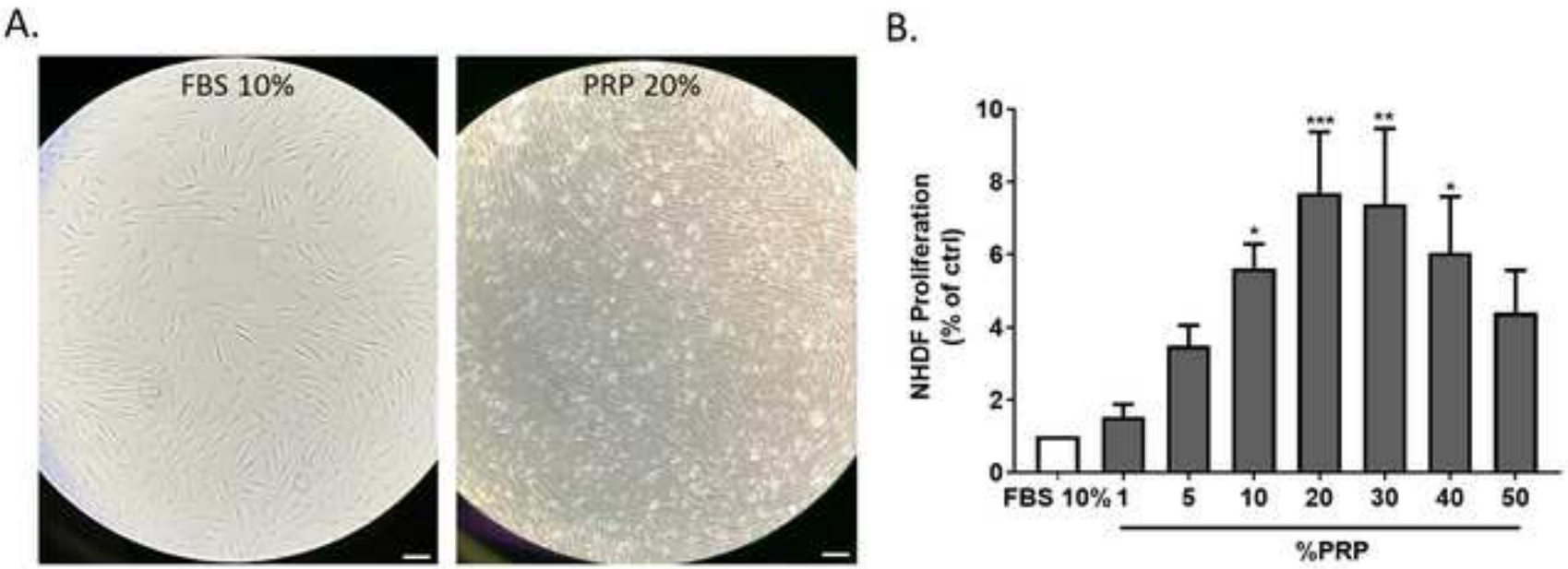

Figure 4

Click here to access/download;Figure;Berndt et al_Figure 4.psd

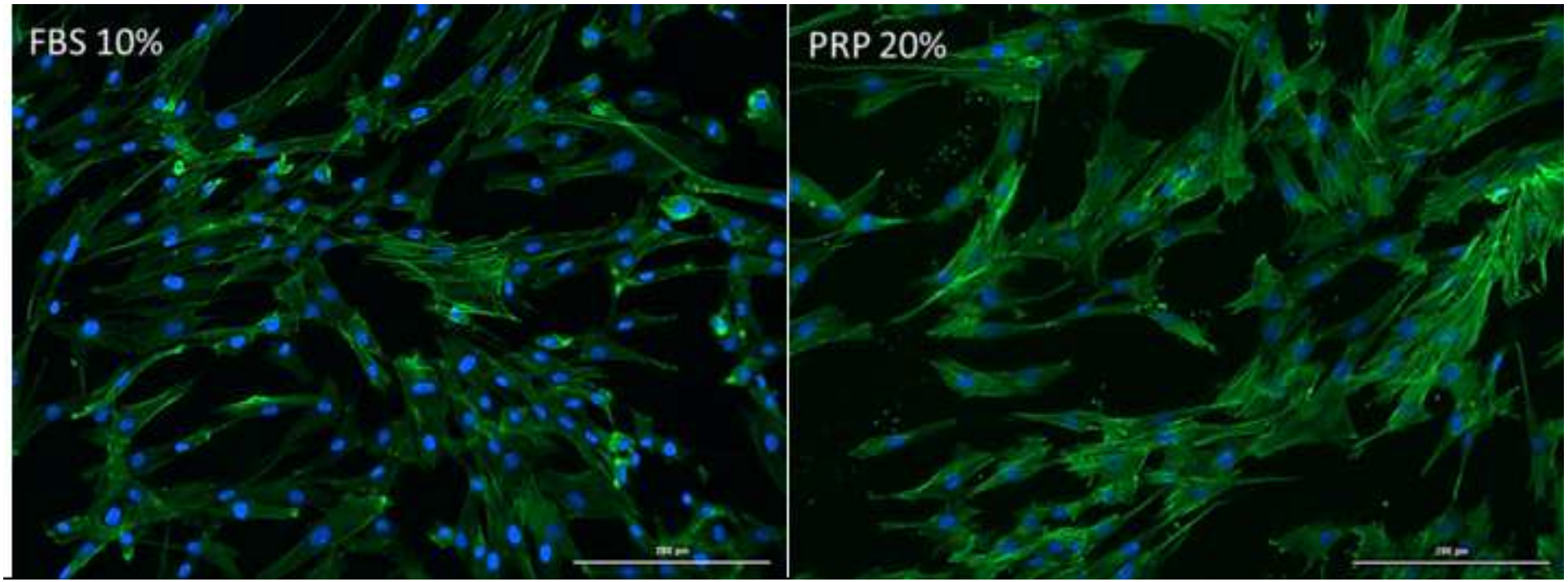

Table of Materials

Click here to access/download;Table of Materials;60816_Berndt etal_Jove_Table_of_Materials_R2.xlsx

Name of Material/Equipment	Company	Catalog Number	Comments/Description
96 well black clear flat bottom	BD Falcon	353219	32/case
Cell trace Violet Dye	Thermo Fischer Scientific	C34557	180 assays
CuteCell PRP	Regen Lab SA	CC-PRP-3T	3 tubes per package
DAPI	Sigma	D9542	1 mg
DMEM	Gibco	52400-025	500 mL
FBS	Gibco	10270106	500 mL
Glutamine 200 mM	Gibco	25030024	100 mL
Hematology Counter	Sysmex	KK-21N	
Heparin 5000E Liquemine	Drossapharm AG		0.5 mL
HEPES Buffer Solution 1M	Gibco	15630-056	100 mL
Liberase DH	Roche	5401054001	2x 5 mg per package
MEM NEAA 100x	Gibco	11140-035	100 mL
Na Pyruvate 1mg/mL	Gibco	11360-039	100 mL
Penicillin streptomycin	Gibco	15140122	100 mL
Phalloidin alexa Fluor 488	Molecular Probes	A12379	300 units
RPMI	Gibco	31966-021	500 mL
Trypsin 1x 0.25%	Gibco	25050-014	100 mL
Trypsin EDTA 0.25%	Gibco	25200056	100 mL

Answers to Editorial Decision

Revisions required for your JoVE submission JoVE60816R2

Title: **A Standardized Method to Produce Autologous Platelet-Rich Plasma for Boosting In vitro Human Fibroblasts Expansion**

Comments to the editor Xiaoyan Cao:

Editorial comments:

Changes to be made by the author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

The manuscript was carefully revised and proofread.

2. Please revise lines 67-69, 186-188, 196-198, 279-280, 289-290, 293-295, and 347-349 to avoid textual overlap with previously published work.

These lines were revised and rephrased when necessary.

3. JoVE policy states that the video narrative is objective and not biased towards a particular product featured in the video. The goal of this policy is to focus on the science rather than to present a technique as an advertisement for a specific item. To this end, we ask that you please reduce the number of instances of " CuteCel" to 2-3 within your text. The term may be introduced but please use it infrequently and when directly relevant. Otherwise, please refer to the term using generic language.

We checked in the final version of the manuscript and only 3 CuteCell instances are occurring.

4. Please address specific comments marked in the attached manuscript.

All the comments were resolved or annotated. The new submitted version comprises the track changes of all the modifications made.

5. Figure 2: Please remove CutecellTM from the figure. Please change "X" to lowercase "x".

The new submitted Figure 2 was modified as asked.

6. Figure 3: Please submit multipanel figures (A, B) as a single image file that contains the entire figure. Please define error bars in the figure legend.

The new submitted Figure 3 was modified as asked. Definition of error bars appears in the Figure Legend.

Mary Ann Liebert, Inc. publishers

Sarah Berndt
Regen Lab SA
En Budron B2 CH-1052
Switzerland

Dear Sarah:

RE: "Autologous Platelet-Rich Plasma (CuteCell PRP) Safely Boosts In Vitro Human Fibroblast Expansion"
Sarah Berndt, Antoine Turzi, Brigitte Pittet-Cuénod, and Ali Modarressi
Tissue Engineering Part A, Volume: 25 Issue 21-22
DOI: 10.1089/ten.tea.2018.0335

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Warm regards,

Karen Ballen
Manager, Copyright Permissions
Mary Ann Liebert, Inc., publishers

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