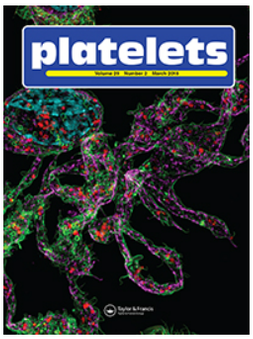

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ORIGINAL ARTICLE

The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte- and platelet-rich fibrin (L-PRF) clot and membrane

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Abstract

L-PRF (leukocyte- and platelet-rich fibrin) is one of the four families of platelet concentrates for surgical use and is widely used in oral and maxillofacial regenerative therapies. The first objective of this article was to evaluate the mechanical vibrations appearing during centrifugation in four models of commercially available table-top centrifuges used to produce L-PRF and the impact of the centrifuge characteristics on the cell and fibrin architecture of a L-PRF clot and membrane. The second objective of this article was to evaluate how changing some parameters of the L-PRF protocol may influence its biological signature, independently from the characteristics of the centrifuge.

In the first part, four different commercially available centrifuges were used to produce L-PRF, following the original L-PRF production method (glass-coated plastic tubes, 400 g force, 12 minutes). The tested systems were the original L-PRF centrifuge (Intra-Spin, Intra-Lock, the only CE and FDA cleared system for the preparation of L-PRF) and three other laboratory centrifuges (not CE/FDA cleared for L-PRF): A-PRF 12 (Advanced PRF, Process), LW-UPD8 (LW Scientific) and Salvin 1310 (Salvin Dental). Each centrifuge was opened for inspection, two accelerometers were installed (one radial, one vertical), and data were collected with a spectrum analyzer in two configurations (full-load or half load). All clots and membranes were collected into a sterile surgical box (Xpression kit, Intra-Lock). The exact macroscopic (weights, sizes) and microscopic (photonic and scanning electron microscopy SEM) characteristics of the L-PRF produced with these four different machines were evaluated.

In the second part, venous blood was taken in two groups, respectively, Intra-Spin 9 ml glass-coated plastic tubes (Intra-Lock) and A-PRF 10 ml glass tubes (Process). Tubes were immediately centrifuged at 2700 rpm (around 400 g) during 12 minutes to produce L-PRF or at 1500 rpm during 14 minutes to produce A-PRF. All centrifugations were done using the original L-PRF centrifuge (Intra-Spin), as recommended by the two manufacturers. Half of the membranes were placed individually in culture media and transferred in a new tube at seven experimental times (up to 7 days). The releases of transforming growth factor β-1 (TGFβ-1), platelet derived growth factor AB (PDGF-AB), vascular endothelial growth factor (VEGF) and bone morphogenetic protein 2 (BMP-2) were quantified using

Keywords

Blood platelets, growth factors, leukocytes, platelet-rich plasma, regenerative medicine, wound healing,

History

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David M. Dohan Ehrenfest and Nelson R. Pinto contributed equally to this work.

All authors participated to the technical design and organization of the study, the treatment of data and to the elaboration of the manuscript. DDE, NP, AP, PJ, BSK, MDC and MQ were in charge of the collection of the materials and raw data.

This study was previously published in 2014 in three articles in the POSEIDO journal [39–41], an international open-access peer-review scientific journal that is not currently indexed. These data were regrouped in this article and submitted for republication in the indexed peer-review database to ensure better visibility of this important work.

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ELISA kits at these seven experimental times. The remaining membranes were used to evaluate the initial quantity of growth factors of the L-PRF and A-PRF membranes, through forcible extraction.
Very significant differences in the level of vibrations at each rotational speed were observed between the four tested centrifuges. The original L-PRF centrifuge (Intra-Spin) was by far the most stable machine in all configurations and always remained under the threshold of resonance, unlike the three other tested machines. At the classical speed of production of L-PRF, the level of undesirable vibrations on the original centrifuge was between 4.5 and 6 times lower than with other centrifuges. Intra-Spin showed the lowest temperature of the tubes. A-PRF and Salvin were both associated with a significant increase in temperature in the tube. Intra-Spin produced the heaviest clot and quantity of exudate among the four techniques. A-PRF and LW produced much lighter, shorter and narrower clots and membranes than the two other centrifuges. Light microscopy analysis showed relatively similar features for all L-PRF types (concentration of cell bodies in the first half). However, SEM illustrated considerable differences between samples. The original Intra-Spin L-PRF showed a strongly polymerized thick fibrin matrix and all cells appeared alive with a normal shape, including the textured surface aspect of activated lymphocytes. The A-PRF, Salvin and LW PRF-like membranes presented a lightly polymerized slim fibrin gel and most of the visible cell bodies appeared destroyed (squashed or shrunk).

In the second part of this study, the slow release of the three tested growth factors from original L-PRF membranes was significantly stronger (more than twice stronger, $p<0.001$) at all experimental times than the release from A-PRF membranes. No trace of BMP2 could be detected in the A-PRF. A slow release of BMP2 was detected during at least 7 days in the original L-PRF. Moreover, the original L-PRF clots and membranes (produced with 9 mL blood) were always significantly larger than the A-PRF (produced with 10 mL blood). The A-PRF membranes dissolved in vitro after less than 3 days, while the L-PRF membrane remained in good shape during at least 7 days.
Each centrifuge has its clear own profile of vibrations depending on the rotational speed, and the centrifuge characteristics are directly impacting the architecture and cell content of a L-PRF clot. This result may reveal a considerable flaw in all the PRP/PRF literature, as this parameter was never considered. The original L-PRF clot (Intra-Spin) presented very specific characteristics, which appeared distorted when using centrifuges with a higher vibration level. A-PRF, LW and Salvin centrifuges produced PRF-like materials with a damaged and almost destroyed cell population through the standard protocol, and it is therefore impossible to classify these products in the L-PRF family.

Moreover, when using the same centrifuge, the original L-PRF protocol allowed producing larger clots/membranes and a more intense release of growth factors (biological signature at least twice stronger) than the modified A-PRF protocol. Both protocols are therefore significantly different, and the clinical and experimental results from the original L-PRF shall not be extrapolated to the A-PRF. Finally, the comparison between the total released amounts and the initial content of the membrane (after forcible extraction) highlighted that the leukocytes living in the fibrin matrix are involved in the production of significant amounts of growth factors. The centrifuge characteristics and centrifugation protocols impact significantly and dramatically the cells, growth factors and fibrin architecture of L-PRF.

1. Introduction

Leukocyte- and platelet-rich fibrin (L-PRF) is one of the four main families of platelet concentrates for surgical use [1–3]. L-PRF is frequently used in oral and maxillofacial surgery as a surgical adjuvant to improve healing and promote tissue regeneration [4–13]. The L-PRF technology is very simple and inexpensive (particularly in comparison to the many kinds of platelet-rich plasma PRP available on the market), and the method was developed as an open-access system [14]: Blood sample is taken in 9 ml tubes without anticoagulant and immediately centrifuged at 2700 rpm during 12 minutes. At the end of the process, a large L-PRF clot can be collected in the middle of each tube. This clot can be used directly to fill a cavity [15, 16] or mixed with a bone material [4], or compressed into a membrane [10] or a fibrin cylinder [17] using the adequate surgical box designed to prepare it without damage (marketed with CE/FDA clearance as Xpression kit, Intra-Lock, Boca-Raton, FL, USA) [18].

The L-PRF clot or membrane contains most of the platelets and half of the leukocytes present in the initial blood harvest [19]. Platelets are mostly activated and serve as a cement to reinforce the strongly polymerized fibrin matrix [19]. Leukocytes (a majority of lymphocytes) are trapped within this fibrin network, but are still alive and ready to move in culture [20]. The platelet growth factors are trapped within the fibrin network [21]. With this architecture, L-PRF is the source of a strong and slow release of growth factors during more than 7 days in vitro [22, 23], through the release of the platelet growth factors trapped within the fibrin gel or through the production of new molecules by the leukocytes of the clot [21]. This intensity and pattern of release were compared with other forms of platelet concentrates (particularly with some platelet-rich plasma gel) [22], and it was claimed that this growth factors slow release profile can be considered as a biological signature of each platelet concentrate gel as a regenerative healing biomaterial [22, 23].

In vitro, the L-PRF membranes have strong effects on the stimulation of the proliferation of most cell types (fibroblasts, keratinocytes, pre-adipocytes, osteoblasts, bone mesenchymal stem cells) [20, 24] and on the differentiation of the bone cells [20]. This result was explained by the growth factors and cell content of the L-PRF [20]. Finally, through its specific natural architecture combining a wide cell population (mostly leukocytes), large quantities of mediators (particularly platelet growth factors) into a strong natural fibrin matrix, L-PRF was considered as a tissue and was often described as an optimized natural blood clot [19]. This specific architecture in itself may explain most of the positive characteristics of this material [25–27].

The original L-PRF was developed as an open-access protocol, but the material and method were tailored with a lot of care in order to reach the best possible clot and result [19]. The protocol was established by using a high-quality table centrifuge, specific glass-coated plastic tubes and a specific protocol (12 minutes, 2700 RPM). The relevant literature on the L-PRF has been published for more than 10 years using this adequate material. The original open-access experimental method and associated devices used in the early phases of the development of this technique have evolved into a regulated medical device system and are nowadays marketed with CE/FDA clearance as the Intra-Spin L-PRF system and kit (Intra-Lock, Boca-Raton, FL, USA). This system is actually the only L-PRF system available on the market with all adequate certifications and using the original protocol and devices.

With the development worldwide of this open-access method, many variations of the original method appeared, using different centrifuges (often less sophisticated and cheaper models) and/or different protocols. The situation is starting to be confusing as all variations of the materials and methods clearly do not offer the same material than the original L-PRF [18, 28–30]. Differences between the original L-PRF and various PRF-like materials are obvious and easily observable (e.g., the size and weight of the clots and membranes), but this simple truth is often not understood because of ignorance and the confusions created by commercial statements and marketing claims [31]. The specific fibrin architecture and cell and growth factors contents of the L-PRF are key characteristics of an original L-PRF clot/membrane as characterized in the literature [19], and any modification of the material and protocol can lead to a different biological signature and clinical result [18].

The exact differences between the various materials and methods to produce L-PRF and the characteristics of the different L-PRF products were not clearly demonstrated and published scientifically up to now. As mechanical instruments, all centrifuges have specific mechanical characteristics that differ significantly among the many possible available models. However, these different characteristics were never evaluated before in the production of PRP/PRF. In the case of small table centrifuges used for L-PRF production, the most relevant parameters to evaluate appeared logically to be the vibrations of the centrifuges during the centrifugation process, the vibration shocks during the acceleration phases and an eventual resonance of the vibrations. All these mechanical characteristics may interfere with the quality and biological signature of the final L-PRF product.

The first objective of this study was to point out the impact of the centrifuge characteristics on the cell, growth factors and fibrin architecture of a L-PRF clot and membrane. For this purpose, the mechanical vibrations (both radial and vertical) appearing during centrifugation were evaluated in four models of commercially available table centrifuges frequently used to produce L-PRF. The exact macroscopic and microscopic (photonic and scanning electron microscopy) characteristics and the cell composition of the L-PRF clots and membranes produced with these four different machines were evaluated, in order to investigate the impact of the vibration parameter on the architecture and cell content of the L-PRF clots.

The second objective of this study was to evaluate how the changes of the protocol alone (for example reduction of the g force) may influence the biological signature of the L-PRF membranes, independently from the characteristics of the centrifuge. To reach this objective, the slow release of some growth factors from an original L-PRF membrane was compared with the slow release from an A-PRF (advanced platelet-rich fibrin) membrane, as both products can be prepared using the same original L-PRF centrifuge.

2. Materials and methods

This study was developed in two parts. The first part evaluated the vibrations of four models of table centrifuges used to produce a form of L-PRF, and the impact of the centrifuges performances on macroscopic characteristics (sizes, weights, etc.), cell content and fibrin architecture of their respective L-PRF clots and membranes.

The second part of this study focused on the growth factors content and slow release (i.e., the biological signature) of two forms of PRF (an original L-PRF and a modified A-PRF protocol) produced with the same devices but different protocols.

2.1. Impact of the centrifuge characteristics on the quality of the L-PRF

2.1.1. Description of the tested centrifuges

In this study, four different centrifuges, found on the market and used to produce L-PRF, were tested (Figure 1). The country of manufacture being used by some companies as a claim for quality, the country of manufacture of each centrifuge and its main components, was checked. The four selected centrifuges were purchased from their manufacturers (or distributors).

The first centrifuge was the original centrifuge used during the early development of the L-PRF open-access method and is nowadays marketed under the name Intra-Spin L-PRF centrifuge (Intra-Lock International, Boca-Raton, FL, USA; Made in Germany). It is actually the only CE marked and FDA cleared system for the preparation of L-PRF clots.

The three other centrifuges are laboratory centrifuges and are not CE/FDA cleared for L-PRF. However, they can be found on the market for this use (marketed for off label usage): centrifuge A-PRF 12 (Advanced PRF, Process for PRF, Nice, France; Country of manufacture not indicated on the label, components inside show “Made in China”), centrifuge LW - UPD8 (LW Scientific, Lawrenceville, GA, USA; Components made in China, assembled in the USA) and centrifuge Salvin 1310 (Salvin Dental Specialties, Charlotte, NC, USA; Made in China).

Figure 1. The four centrifuges used to produce L-PRF clots and tested in this study. From left to right: original L-PRF centrifuge (Intra-Spin, Intra-Lock), A-PRF 12 (Advanced PRF, Process), Salvin 1310 (Salvin Dental) and LW - UPD8 (LW Scientific).

2.1.2. Protocol of analysis of the vibrations

Each centrifuge was loaded with eight blood collection plastic tubes filled with water to the manufacturer's recommended level (approximately 9 ml). The tube weights were measured on a high precision balance device (Sartorius M-Prove high precision balance, Model AY123, Sartorius AG, Goettingen, Germany) to ensure that each tube had a substantially equivalent load of water (full tube weights were measured between 18.41 and 18.43 grams).

Each centrifuge was opened for inspection and the placement of two accelerometers (Wilcoxon: Model 780A-IS, 100 mV/g, Meggitt, Germantown, MD, USA). One accelerometer was used to access radial vibration on the centrifuges when under load and under acceleration. This radial accelerometer was positioned directly on the motor frame of each centrifuge, as close as possible to the bottom of the rotating tube holder. The other accelerometer was used to determine vertical vibration when under load and under acceleration. This vertical accelerometer was positioned on the centrifuge base, as close as possible to the lower edge of the rotating tube holder. The data were collected with a spectrum analyzer-FFT (Fast Fourier Transform) capable and its data processing software (Commtest Model VB7 and software Ascent 2013 Level 2, R3, v13.5.5; Commtest, GE Energy, Christchurch, New Zealand).

Each centrifuge was tested with two configurations: half tube load (three or four tubes depending on capacity) and full tube load (six or eight tubes depending on capacity). For each configuration (half tube load and full tube load), tests were run at the following rotational speeds: 1500, 1800, 2100, 2400, 2700, 3000 and 3300 rpm. Extra rotational speeds were used on some centrifuges. One centrifuge (Salvin 1310) had only one available rotational speed (3400 rpm). For each test, the software documented both radial and vertical vibration. Plotted curves showing vibration (m/s^2) versus frequency (Hz) were obtained from this documentation and recorded.

2.1.3. Preparation of L-PRF

The study was conducted in accordance with the Helsinki Declaration (2000) and approved by the Medical Ethics Committee of the University of the Andes (UANDES). All volunteers provided signed informed consent.

Blood samples were collected at the San Bernardo University of the Andes Health Center from eight healthy volunteers (age range 25–35 years, ASA 1), with no history of recent aspirin intake or any medication neither disease correlated with the coagulation process. For each volunteer, nine tubes of blood were obtained from the antecubital vein. One tube with 2,5 ml of anticoagulant was used for whole blood analysis as a control for normal blood parameters. Eight plastic glass-coated tubes were taken without anticoagulant (with BD Vacutainer Serum 10.0 ml tubes, Becton Dickinson, Franklin Lakes, NJ, USA) for the production of L-PRF clots and membranes.

The blood was collected quickly (22 seconds mean value, less 25 seconds per tube) and immediately (before 1 minute) centrifuged at 400 g during 12 minutes in the four different centrifuges (two tubes were distributed per centrifuge in a randomized way) at room temperature. To standardize exactly the protocol and isolate only the centrifuge vibration parameter, the 400 g centrifugation force used in the original L-PRF method (corresponding to 2700 rpm in the original Intra-Spin centrifuge) was used with all centrifuges, and rpm was adjusted accordingly for each centrifuge, that is, 2400 rpm for the A-PRF machine and 2300 rpm for the LW centrifuge. Salvin centrifuge has only one preset possible speed (3400 rpm), which lead to a centrifugation force higher than 400 g. The temperatures of the surface at the center of the tubes were registered before and after centrifugation with an infrared thermometer (HVACPro, Fluke, Everett, WA, USA).

A total of 64 L-PRF clots/membranes were obtained: 32 membranes were prepared for scanning electron microscopy (SEM) analysis, and 32 membranes were prepared for light/photonic microscopy.

2.1.4. Macroscopic analysis

After centrifugation, the L-PRF clot was removed from the tube using sterile tweezers and a smooth spatula to gently release the red blood cells clot inside the tube (Figure 2A). The L-PRF fibrin clot obtained was placed on a sterile microscope slide (Figures 2B and 2C) placed in an individual tray for weight and size measurements (Figure 3). The supernatant and red blood cells clot remaining in the tube were also weighted to get the L-PRF fibrin clot/whole blood ratio per tube. Each sterile microscope slide had in every corner a 1 mm rubber stop (Figure 2C) to allow the compression of the clot with another microscope slide using 100 grams constant pressure for two minutes. This standardized method allowed to obtain from each clot 1 mm-thick L-PRF membranes, which were weighted and measured individually (Figure 4).

From each volunteer, two membranes were obtained per each centrifuge, and after macro-analysis (weight, size measurements) were prepared for histologic procedures. One membrane was prepared for SEM evaluation and the second one for light-microscopy analysis. The membranes were kept between the microscope slides during fixation to avoid distortions.

2.1.5. Light microscopy and scanning electron microscopy (SEM) procedures

For light microscopy analysis, the membranes were fixed in 10% neutrally buffered formalin for 24 hours at room temperature for

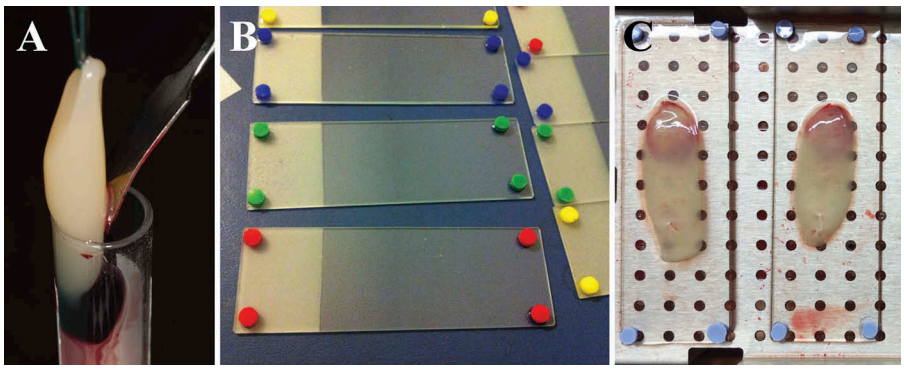

Figure 2. Material for PRF clot handling. L-PRF clots were collected in each tube, and the red blood cell part was gently removed with a smooth instrument and a light lateral pressure **(A)**. Standard glass histological slides were use to support the clots during the macroscopic evaluation **(B)**. Rubber stops were placed on each corner of the slides, in order to perform a standardized compression of all the samples into membranes between two glass slides **(C)**. The same procedure was applied for all clots produced during this study, even if the handling was often more difficult with the A-PRF, LW and Salvin products.

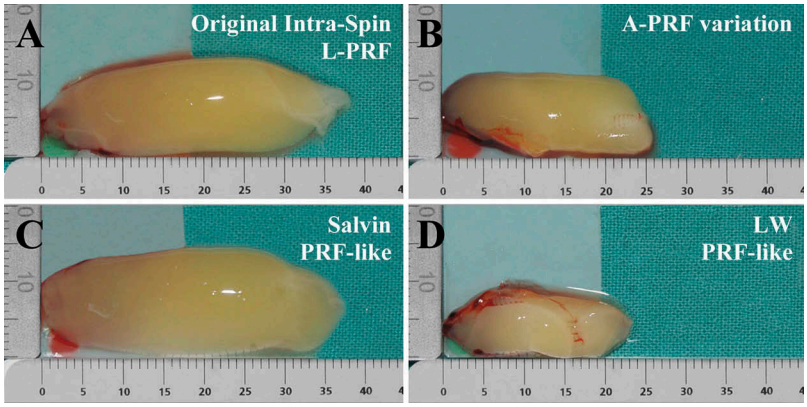

Figure 3. Macroscopic evaluation of the PRF clots produced with the four different centrifuges: original Intra-Spin L-PRF system **(A)**, A-PRF system **(B)**, Salvin centrifuge **(C)** and LW centrifuge **(D)**. Obvious differences can be observed in terms of size and aspect, the original L-PRF **(A)** being always denser and heavier (and in most cases larger) than the others.

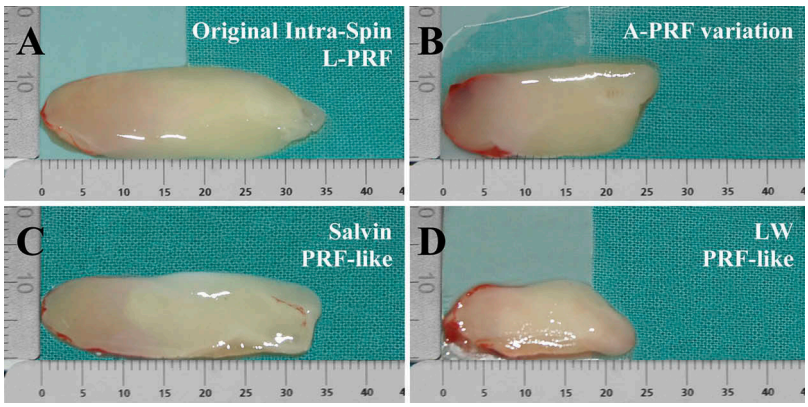

Figure 4. Macroscopic evaluation of the PRF membranes produced with the four different centrifuges: original Intra-Spin L-PRF system **(A)**, A-PRF system **(B)**, Salvin centrifuge **(C)** and LW centrifuge **(D)**. Obvious differences can be observed in terms of size and aspect, the original L-PRF **(A)** being always denser and in most cases larger than the others.

paraffin inclusion. Successive sections of four microns were performed along the center of the long axis of the membranes and were stained with hematoxylin-eosin. Each section was divided in three areas of equal size: Proximal (Head & Face), Center (Body), Distal (Tail). Each area of these sections was observed through light microscopy and analyzed by counting the visible cell bodies (marked in dark purple, mostly leukocytes) in the center of each area observed with a 40X magnification. The total numbers of counted cell bodies were used to correlate their distribution among the three areas of the membrane (head & face, body and tail). Most of the cells were concentrated in the proximal area (head & face).

For the morphologic evaluation of the L-PRF membranes with a scanning electron microscope (SEM), the membranes were fixed in 2.5% glutaraldehyde for 24 hours at 4°C and treated for gradual desiccation. The specimens were sputter coated with 20 nm gold (Edwards S-150, Crawley, UK) and examined in a scanning electron microscope (JEOL JSM-6380LV, JEOL Ltd, Tokyo, Japan). Photographs were taken with 15–20 kV using 2,000–5,000X magnifications. This study was mainly descriptive.

2.2. Comparison of growth factors content and slow release (biological signature) of original L-PRF Versus A-PRF

2.2.1. Preparation of L-PRF and A-PRF

For the production of L-PRF and A-PRF clots and membranes, blood collection was carried out on six volunteer donors, three males and three females, non-smokers, aged between 30 and 40 years old, with no history of recent aspirin intake or any medication neither disease correlated with the coagulation process. For each volunteer, eight tubes of blood were obtained without anticoagulant from the antecubital vein, respectively, 4 Intra-Spin 9 ml glass-coated plastic tubes (Intra-Lock International Inc., Boca-Raton, FL, USA) and 4 A-PRF 10 ml glass tubes (Process, Nice, France).

The blood was collected quickly (17 seconds mean value, less than 20 seconds per tube) and immediately (before 1 minute) centrifuged at room temperature at 2700 rpm (around 400 g) during 12 minutes to produce L-PRF clots or at 1500 rpm during 14 minutes to produce A-PRF clots. All centrifugations were done using the original L-PRF centrifuge (Intra-Spin system, Intra-Lock, Boca Raton, FL, USA), as recommended by the manufacturers of both A-PRF (Process) and Intra-Spin L-PRF (Intra-Lock). The A-PRF was initially developed on the original centrifuge (Intra-Spin), before to become an independent technique with its own centrifuge. The use of the same centrifuge allowed to neutralize the parameter related to the quality of the centrifuge (particularly vibrations).

Four Intra-Spin L-PRF clots were produced for each donor: Two were used to quantify the release of molecules during the experiment, and two were used for immediate extraction by force and quantification. Four Process A-PRF clots were produced for each donor: Two were used to quantify the release of molecules during the experiment, and two were used for immediate extraction by force and quantification. The clots were finally collected carefully into a sterile adapted surgical box (Xpression kit, Intra-Lock, Boca-Raton, FL, USA) and compressed into membranes for the next step of the study.

2.2.2. Sample preparation

In the release quantification group, each L-PRF or A-PRF clot was gently pressed into a membrane and placed in a 10 mL tube with 4 mL of sterile DMEM (Dulbecco's Modified Eagle's

Medium). Then, at each experimental time, the membrane was transferred in a new tube of 4 mL sterile DMEM, and the previous 4 mL were stored at −80°C before ELISA quantification. The membrane transfer was done at seven experimental times: 20 minutes, 1 hour, 4 hours, 24 hours (day 1), 72 hours (day 3), 120 hours (day 5) and 168 hours (day 7). This procedure was done separately for the four membranes (2 L-PRF, 2 A-PRF) of each donor, and thus, 24 membranes were separately treated, in order to calculate means and standard deviations.

In the group for immediate extraction by force, each L-PRF or A-PRF membrane was cut in small pieces and homogenized in 1 mL sterile DMEM using a Polytron extraction-dispersing machine (Polytron, Kinematica AG, Lucerne, Switzerland). Then, a final centrifugation (15000 rpm during 10 minutes) was performed in order to remove residual particulates. About 1 mL of solution was then collected and stored at −80°C before ELISA quantification. This procedure was done separately for the four membranes (2 L-PRF, 2 A-PRF) of each donor, and thus, 24 membranes were separately treated, in order to calculate means and standard deviations.

2.2.3. *ELISA quantification and data collection*

When all the samples were collected, quantifications of four molecules were performed by using classically available ELISA kits (Quantikine, R&D Systems, Minneapolis, MN, USA): transforming growth factor β-1 (TGFβ-1), platelet derived growth factor AB (PDGF-AB), vascular endothelial growth factor (VEGF) and bone morphogenetic protein 2 (BMP-2). Absorbances were read using a microplate reader ELISA X500, and then, concentrations were calculated. For intra- and inter-study comparisons, all the results were finally referred to a 1 mL volume and then expressed as total weight of molecules (nanograms for TGFβ-1 and PDGF-AB, picograms for VEGF, picograms for BMP2). For each molecule and each experimental period, means and standard deviations were calculated. Differences at each time between L-PRF and A-PRF data were assessed using a paired t-test ($p<0.01$).

Finally, for each tested molecule, the total released amounts were calculated and these results were then compared to the initial amount forcibly extracted from the membrane soon after L-PRF and A-PRF preparation. The ratio between the total released quantity and the initial extracted quantity was calculated.

3. Results

3.1. Vibration shocks of the four models of table centrifuges for L-PRF

All plotted curves for the tested centrifuges demonstrated a high level of acceleration in a very narrow range of frequencies. These were centered on the excitation frequency (rotational speed). Almost no vibration at other frequencies was noted. Since the software has the capability of combining several curves on a single chart, we were able to obtain for each machine and for a given configuration (half–full or full) a set of curves from which we were able to derive an envelope curve showing the level of vibration versus the rotational speed. Therefore, all envelope curves could be combined on a single and final chart: one chart for the half–full configuration and one chart for the full configuration. These two final charts allowed us to compare easily all machines tested.

This experiment highlighted two clear results (Figures 5 and 6). First, all centrifuges experienced an increase in the level of vibrations when the rotational speed was increasing. Second, very significant differences in the level of vibrations at each rotational speed were observed between the four tested machines. Each machine had its clear own profile of vibrations depending on the rotational speed. The test curves of the four machines never crossed. These results were observed for both experimental configurations (half or full tube load).

The original L-PRF machine (Intra-Spin) presented the lower level of vibrations at all speeds in both experimental configurations, and the increase in the vibrations remained very limited when the speed was increasing. This was clearly the most stable machine on this aspect. As this machine served for the development of the L-PRF protocol and significant literature, these values can serve a standard of comparison with other machines.

The LW centrifuge presented a very strong increase in vibrations when the rotational speed was increasing. The vibrations of this centrifuge are 4.5 times higher than the vibrations of the Intra-Spin centrifuge for the production of L-PRF (2700 rpm) in full-load configuration, and the difference was even stronger in half-load configuration (5.2 times higher).

The Salvin centrifuge offered only one speed of centrifugation (3400 rpm), what was therefore the speed used to produce L-PRF with it. The vibrations of this centrifuge were six times higher than the vibrations of the Intra-Spin centrifuge for the production of L-PRF in full-load configuration, and the difference was a bit stronger in half-load configuration (6.3 times higher).

The A-PRF centrifuge presented the strongest increase in vibrations when the rotational speed was increasing. The vibrations of this centrifuge were six times higher than the vibrations of the Intra-Spin centrifuge for the production of L-PRF (2700 rpm) in full-load configuration, and the difference was even stronger in half-load configuration (6.8 times higher).

The results of this study were very clear and highlighted that each centrifuge had its own vibration profile and that devices can have considerable differences in terms of intensity of the vibrations.

3.2. Macroscopic analysis of the clots and membranes from four different table centrifuges for L-PRF

All the macroscopic results are presented in the Table I. The numeric values are clearly illustrated by the observation of the clots and membranes in the Figures 3 and 4.

For the temperature of the tubes, Intra-Spin allowed to keep the lowest temperature among the four tested machines. A-PRF and Salvin were both associated with a significant increase in temperature in the tube.

For the clot and exudate weights, Intra-Spin produced by far the heaviest clot and quantity of exudate among the four techniques. Salvin remains high but far behinds. Finally, A-PRF and LW produced very light and small clots. For the membranes weights, Intra-Spin and Salvin presented similar weight. The A-PRF and LW membranes were significantly lighter.

In terms of clot and membrane length and width, the clots and membranes from Intra-Spin and Salvin presented similar sizes. The A-PRF and LW clots and membranes were significantly shorter and narrower.

Finally, the Intra-Spin L-PRF clot was the heaviest clot to be produced with an initial blood harvest of 9 ml.

3.3. Light microscopy analysis of the membranes from four different table centrifuges for L-PRF

In light microscopy (Figure 7), most cell bodies (stained in dark purple for the nuclei) were concentrated in the proximal (head-face) area of each membrane: With Intra-Spin, A-PRF and Salvin, the 3/4 of the cell bodies were observed in the proximal area, and the last 1/4 was observed in the center; the distal part had only residual traces of cell bodies. With LW, the cell bodies appeared more spread all over the membrane (40% proximal, 48% center

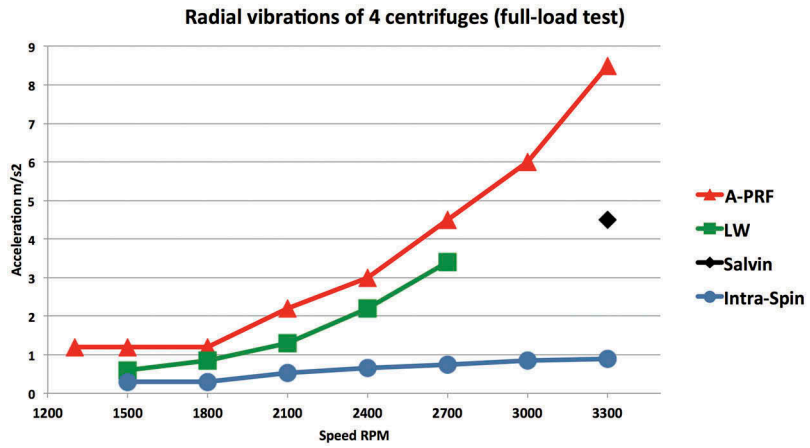

Figure 5. Radial vibrations of the four centrifuges during the full-load test. The curves showed the intensity of vibrations at each rotational speed (in RPM, Revolutions Per Minute). The lowest intensity of vibrations was observed always with the original L-PRF Intra-Spin centrifuge. At the classical speed of production of L-PRF, the level of undesirable vibration on the Intra-Spin centrifuge was between 4.5 and 6 times lower than with other centrifuges. Moreover, Intra-Spin always remained under the threshold of resonance, unlike the three other tested machines.

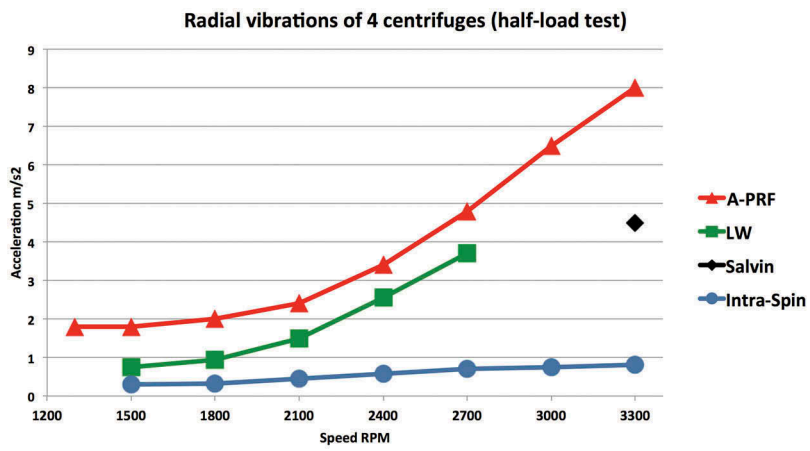

Figure 6. Radial vibrations of the four centrifuges during the half-load test. Half-load test was closer from the clinical reality, as clinicians often only use 3–4 tubes of L-PRF for a daily life small surgery. The curves showed the intensity of vibrations at each rotational speed (in RPM, Revolutions Per Minute). The lowest intensity of vibrations was observed always with the original L-PRF Intra-Spin centrifuge. At the classical speed of production of L-PRF, the level of undesirable vibration on the Intra-Spin centrifuge was between 5.2 and 6.8 times lower than with other centrifuges. Moreover, Intra-Spin always remained under the threshold of resonance, unlike the three other tested machines. The results were very similar to the full-load test, but the differences between machines were even more marked in this half-load configuration.

Table I. Results of the macroscopic analysis of the clots and membranes produced with the four tested centrifuges. Values expressed in Mean and Standard Deviation (SD).

Variable	IntraSpin Mean (SD)	A-PRF Mean (SD)	Salvin Mean (SD)	LW Mean (SD)
Final T° of tube (°C)	27.5 (0.66)	28.83 (0.67)	28.8 (0.66)	27.88 (0.57)
Clot weight (g)	2.09 (0.19)	1.38 (0.24)	1.73 (0.27)	0.74 (0.15)
Membrane weight (g)	0.62 (0.15)	0.48 (0.17)	0.6 (0.19)	0.3 (0.25)
Exudate weight (g)	1.47 (0.13)	0.9 (0.21)	1.12 (0.27)	0.44 (0.26)
Clot length (mm)	35.69 (3.43)	26.56 (4.25)	35.25 (4.1)	20.12 (4.29)
Clot width (mm)	12.81 (0.75)	10.93 (1.08)	13.06 (0.94)	9.12 (1.13)
Membrane length (mm)	34.81 (2.95)	26.81 (3.38)	34.43 (2.87)	21.5 (2.39)
Membrane width (mm)	12.25 (0.71)	10.37 (0.92)	11.93 (0.78)	9.12 (0.64)
Weight ratio(%) clot/blood sample 10 ml	20.94 (2.4)	13.98 (2.6)	17.42 (2.63)	7.41 (1.45)

and 12% distal), as the clot and membranes were particularly small and shrunk. Light microscopy did not allow observing in more details the exact state of these cell bodies.

3.4. SEM analysis of the membranes from four different table centrifuges for L-PRF

The SEM analysis allowed to evaluate in details the aspect of the fibrin network and of the cell content of each membrane (Figures 8 and 9).

The original L-PRF produced through Intra-Spin presented a strongly polymerized fibrin matrix with thick fibrin fibers. Moreover, all observed cells appeared alive with a normal shape. Lymphocytes presented typical textured surface aspect observed in activated lymphocytes. This observation corresponds to the exact characterization of an original L-PRF clot done in previous works and can serve as a standard to evaluate the three other types of L-PRF produced in this study.

The A-PRF, Salvin and LW PRF-like membranes presented a lightly polymerized fibrin gel with slim fibrin fibers, clearly very different from the original L-PRF. Moreover, all the visible cell

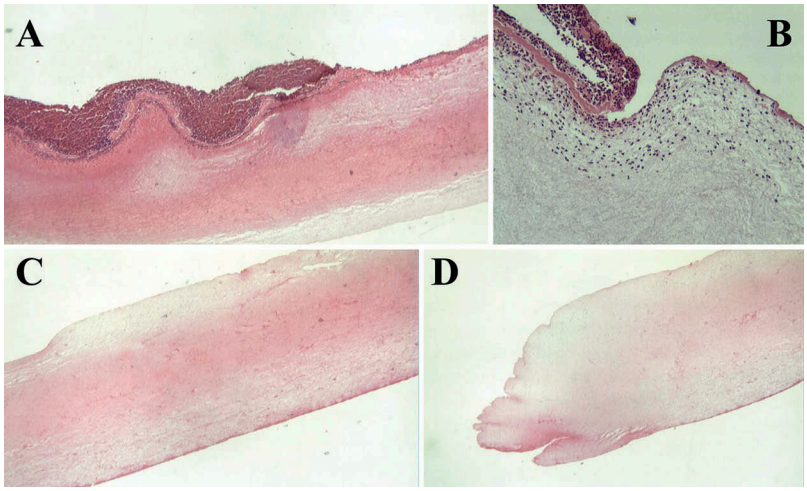

Figure 7. Microscopic evaluation of the PRF membranes produced with the four different centrifuges in light microscopy (hematoxylin eosin). The different membranes showed similar organization in light microscopy, with a concentration of most visible cell bodies (75%) in the first 1/3 proximal part of the membrane (A, x2; B, x80), the remaining in the central 1/3 part (C, x2) and only residual bodies in the last 1/3 distal part (D, x2). Illustration obtained here from an original L-PRF membrane (Intra-Spin). The LW PRF-like membrane was the only one with a different distribution, mostly due to the strong shrinking of the membrane.

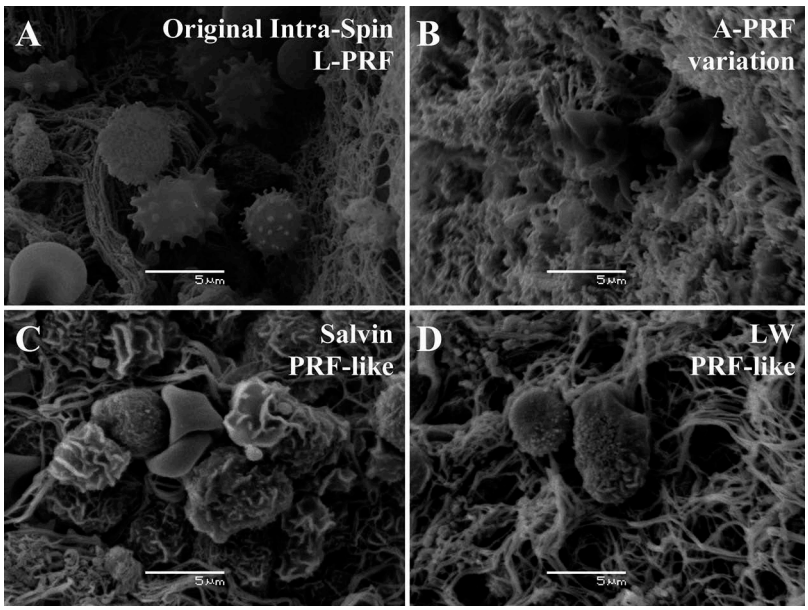

Figure 8. SEM Microscopic evaluation of the PRF membranes produced with the four different centrifuges. The different membranes showed very different aspects during SEM analysis. The original L-PRF membrane (Intra-Spin, **A**) presented a strongly polymerized fibrin network and the presence of a large living cell population appearing in good shape. The PRF-like membranes produced with the A-PRF **(B)**, Salvin **(C)** and LW **(D)**, all presented a slimmer and more disorganized fibrin network, and all cells appearing severely damaged, shrunk or squashed.

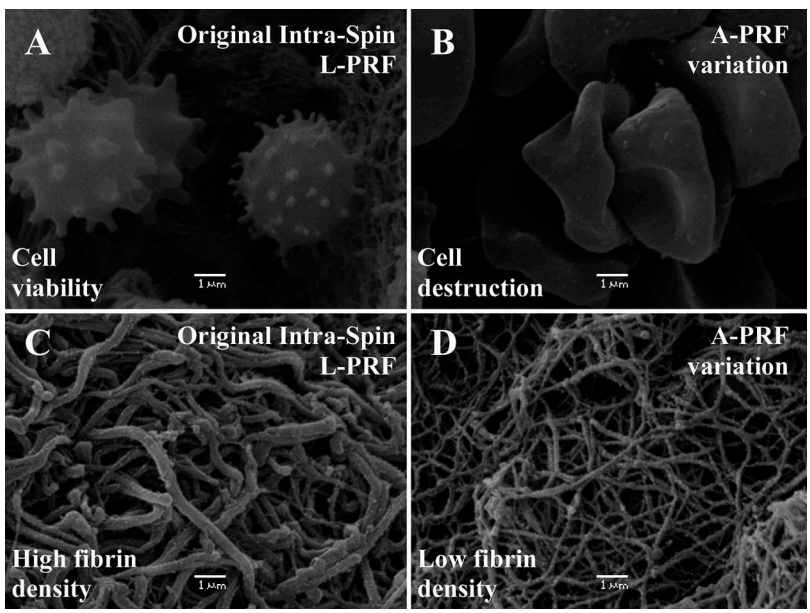

Figure 9. SEM Microscopic evaluation and comparison of the PRF membranes produced with two different centrifuges. The original Intra-Spin L-PRF membranes **(A, C)** presented a large cell population **(A)**, and all observed cells appeared alive with a normal shape. Lymphocytes presented typical textured surface aspect observed in activated lymphocytes. Moreover, the fibrin matrix appeared strongly polymerized with thick fibrin fibers **(C)**. On the contrary, in the A-PRF membranes **(B, D)**, all the visible cell bodies appeared squashed or shrunk **(B)**, and the fibrin gel presented a lightly polymerized fibrin matrix with slim fibrin fibers **(D)**.

bodies appeared squashed or shrunk. No cell body with a normal cell shape or even an activated cell shape could be detected. It was considered that the whole cell population was completely damaged and almost destroyed.

3.5. Original L-PRF Versus A-PRF: comparison of growth factors content and slow release (biological signature)

As a first macroscopic observation, the original L-PRF clots and membranes produced with 9 ml blood were always much larger than the A-PRF clots and membranes produced with 10 ml blood (Figure 10). It appeared systematically that the L-PRF was at least 30% bigger than the A-PRF clots and membranes.

During the test, the original L-PRF membrane remained in good condition up to the last experimental time (7 days), while the A-PRF membrane completely dissolved in the medium between the first and the third day. For this reason, the last A-PRF value was measured at the day three experimental time. Significant amounts of TGFβ-1, PDGF-AB and VEGF were found at each experimental times, even 7 days after production with the original L-PRF membrane and up to 3 days with the A-PRF membrane (Figure 11). These amounts of molecules presented a specific slow release kinetic. TGFβ-1, PDGF-AB and VEGF releases showed similar general profiles, characterized by a quick increase in the release during the first 24 hours. For original L-PRF, a significant but slower release until day 5 (120 h) was observed; during the last 2 days of the experiment, membranes continued to release significant amounts of these molecules, but very slowly. For the A-PRF, the release also slowed down after the first day, but the release stopped quickly thereafter with the complete dissolution of the A-PRF membrane.

The slow release of TGFβ-1, PDGF-AB and VEGF from an original L-PRF membrane (Intra-Spin) was always significantly much stronger ($p < 0.001$) at all experimental times than the release from an A-PRF membrane. All results were presented as graphs (Figure 11) to follow the cumulative released mean amounts of each molecule during the first 168 hours after L-PRF and A-PRF membranes preparation, respectively. The gradient of the curves revealed the force of the slow release during the experimental periods. These curves were defining the biological signatures of the original L-PRF membrane and of the A-PRF membrane. The original L-PRF signature was always more than twice stronger than the A-PRF signature.

For the evaluation of BMP2, no traces of BMP2 could be detected in the A-PRF membrane, and the values represented in the Figure 11 are considered as the unavoidable experimental background noise. On the contrary, a slow release of BMP2 was clearly detected during at least 7 days in the original L-PRF, even if the quantities remained quite small.

For the original L-PRF, the total quantity of released factors was in all cases significantly higher than the total amounts extracted just after membrane preparation (Table II). However, the ratios between these values (total slow release/initial quantity) were very different according to the molecule: TGFβ-1, VEGF and BMP2 following the same high ratio (around 7), while PDGF-AB ratio was much closer to 1.

For the A-PRF, the total quantity of released factors and the total amounts extracted just after membrane preparation were significantly smaller than for the L-PRF membrane (Table II). In A-PRF, the ratios between these values (total slow release/initial quantity) were also very different according to the molecule: TGFβ-1 and VEGF following the same high ratio (around 4.5 or 5, lower than for L-PRF), while PDGF-AB ratio was much closer to 1 (similar to L-PRF).

4. Discussion

4.1. Impact of the centrifuge characteristics

Since the early phases of development of the L-PRF technology, scientists observed easily that the choice of the centrifuge and the protocol of centrifugation was affecting the final aspect (weight and size) of the L-PRF clot [19, 22]. Good sense observations could reveal that the machines have different levels of vibrations, as it can be perceived easily by simply hearing them and placing a hand on the centrifuge during the centrifugation process. Despite this observation, no one investigated or even considered the quality of the centrifuge as an important parameter in the production of PRP and PRF. PRP are often produced with larger and heavier centrifuges than L-PRF [22], and this may explain why this parameter was not investigated before. However, in the case of L-PRF, this parameter is very perceptible as the L-PRF technique was designed to be used easily in daily clinical practice and

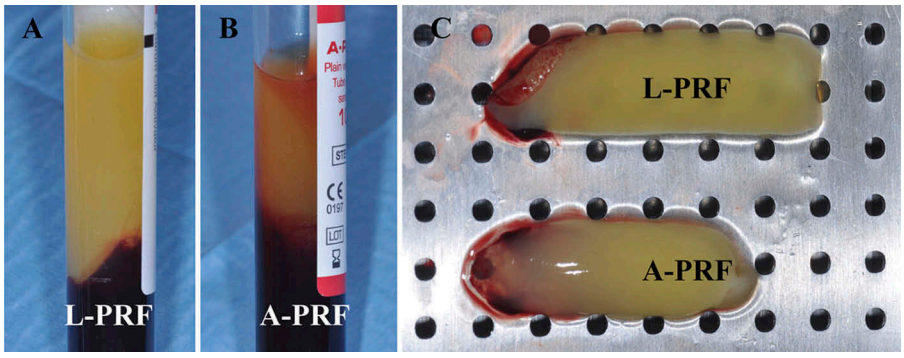

Figure 10. Original Intra-Spin L-PRF **(A)** and A-PRF **(B)** clots in their respective official tubes just after centrifugation. The aspect of the two kinds of clots in the tube was since the beginning very different. Clots were then collected and placed in the PRF surgical box (Xpression, Intra-Lock) for compression into membranes **(C)** to be used for the slow release test. The original Intra-Spin L-PRF membrane was produced with the original L-PRF protocol (2700 rpm, 12 minutes) and 9 mL blood. The A-PRF membrane was produced with the specific A-PRF protocol (1500 rpm, 14 minutes) and 10 mL blood. However, in comparison to the original L-PRF membrane, the A-PRF clots and membranes appeared obviously much smaller, more fragile and not so clearly separated from the red blood cell part.

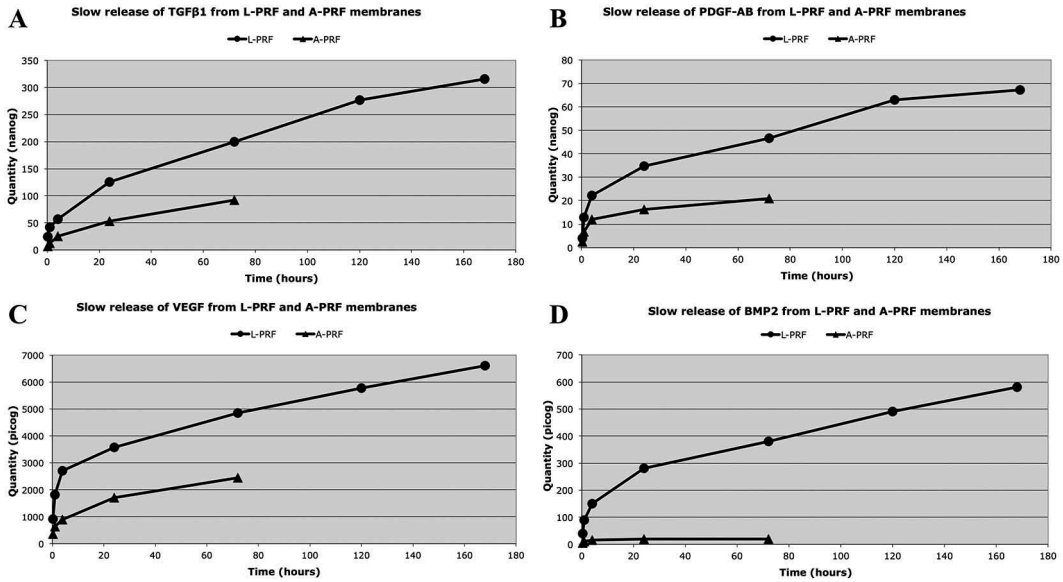

Figure 11. Slow release of TGFβ1 **(A)**, PDGF-AB **(B)**, VEGF **(C)** and BMP2 **(D)** from an original L-PRF membrane and from an A-PRF membrane during 7 days in vitro. Values are expressed as the cumulative mean quantity of molecules at 20 minutes, 1 hours, 4 hours, 24 hours, 72 hours (3 days), 120 hours (5 days) and 168 hours (7 days).

Table II. Comparison between the total released quantity at the end of the experimental time (after 168 hours) and the initial extracted quantity of each tested molecule in an original L-PRF membrane and in an A-PRF membrane. Results are expressed as means and standard deviations.

Tested molecule		TGFβ-1 (nanog)	VEGF (picog)	PDGF-AB (nanog)	BMP-2 (picog)
Total released after 168h	L-PRF	315.5 (±21.1)	6602 (±704)	67.1 (±9.8)	580 (±73)
(sum of the amounts measured at each experimental time)	A-PRF	92.1 (±25.4)	2445 (±782)	20.9 (±8.1)	NA
Total extracted at t0 from the membrane	L-PRF	44.4 (±3.7)	994 (±159)	44.4 (±4.2)	79 (±10)
	A-PRF	18.6 (±4.8)	514 (±188)	15 (±5.8)	NA
Ratio between	L-PRF	7.1	6.64	1.51	7.34
slow released and extracted molecules	A-PRF	4.95	4.76	1.39	NA

therefore with a small and light table centrifuge—therefore with a highest risk of vibrations and resonance during the centrifugation.

This study is the first research evaluating scientifically the intrinsic characteristics of the table centrifuges used to produce platelet concentrates for surgical use. It proves that the devices found on the market have very significant difference in terms of vibrations and that all tested devices have much higher intensity of vibrations than the original L-PRF centrifuge (Intra-Spin). Moreover, when radial vibrations rise above 1, there is a serious risk that resonance occurs in the centrifuged tubes, what can provoke significant damage to the blood cell content of the tubes. At the speed commonly used to produce L-PRF (2700 rpm, or 3400 rpm in the case of Salvin), all tested centrifuges (except Intra-Spin) are largely above this threshold of one for resonance, and it was necessary to evaluate in details the effects of these vibrations on the cell content and fibrin architecture of each L-PRF clot produced with these machines [19].

The A-PRF centrifuge (advanced platelet-rich fibrin) is an interesting case, as it was suggested to be used with a very low speed (1300 rpm) in order to produce a PRF-like clot called A-PRF. This is actually a quite rare approach, as a too low centrifugation force does not allow a good separation of the blood components and the activation of leukocytes. Moreover, the vibrations of this machine at this low speed are already above the threshold of one marking the theoretical limit of resonance. In theory, the best configuration for L-PRF would be to have a reasonable speed (around 2700 rpm, i.e., 400 g) for adequate blood separation and no vibration or resonance to protect the cell content [19], as the adequate collection of the leukocyte appeared as an importance parameter for the clinical effect of these technologies [25, 27]. Therefore, A-PRF could serve as an interesting example to compare with the original L-PRF and to illustrate the impact of speed/g force and vibrations on the final aspect and content of a PRF clot.

Finally, this study raises very serious concerns about the whole PRP literature. PRP centrifuges are in general larger and heavier than PRF centrifuges, using sturdy motors and well-balanced rotors and should therefore in theory present a lower risk of vibrations. However, the centrifuges tested in this study are also used to produce PRP through another protocol, and the inadequate conception of larger centrifuges can lead to the same risk of vibrations integrated to the level of resonance, whatever their size and weight. Moreover, many PRP methods are also using much higher centrifugation speeds and sometimes g forces [4, 5] than the L-PRF method (often considered to be a very soft method), as they are supposed to make a very sharp separation of the blood components. For all these reasons, it would be interesting to evaluate more seriously the vibrations of all centrifuges available for PRP on the market, in order to evaluate if this has an impact on the final cell content of the PRP and its biological effects.

4.2. Impact of the vibrations on the fibrin polymerization and cell content

The original L-PRF materials and protocols were carefully selected in order to reach the best possible result. The

development was not empirical, but based on a significant feedback of observation and experience. In the literature, much confusion started to appear [18, 28–30], as many studies did not use the same hardware (centrifuge and tubes) and did not get the same product, even if the protocols appeared identical (same g force and centrifugation time).

In this study, we tried to highlight how the centrifuge characteristics may impact the L-PRF architecture and composition. Blood collection materials, tubes and protocol were strictly identical. The centrifugation parameters were also standardized (same g force than the original L-PRF protocol, calculated to fit each machine, and same centrifugation time). Therefore, the only difference between the four products was the hardware (the centrifuge). After blood centrifugation in the four different centrifuges was completed, L-PRF clots were observed to be not identical in terms of weight, volume, fibrin architecture and cell content.

Having verified that the g forces were almost identical in the four centrifuges, the hypothesis was that mechanical vibrations might be responsible of differences between the final products. This vibration variable appeared as the main (and most logical) parameter to evaluate. It was proven in the first part of this study that the vibration levels (both radial and vertical) were very different between the commercially available centrifuges used for L-PRF. As vibrations level was the only variable between the four products, it is therefore possible to associate the differences of L-PRF weight, volume, fibrin architecture and cell content between the four systems to this level of vibrations, even if other parameters may be considered in the future.

In this study, all membranes were produced using a 400 g centrifugation force. This corresponds to a 2700 rpm with the original L-PRF centrifuge (Intra-Spin), resulting in a parasite acceleration (vibration) level of 0.75 $m.s^{-2}$, so far under the threshold of 1. For the three other centrifuges, the rpm speed used to stay in the 400 g centrifugation forces was all associated with a vibration level much higher than 1: 2.2 $m.s^{-2}$ (LW), 3 $m.s^{-2}$ (A-PRF) and 4.5 $m.s^{-2}$ (Salvin). It is interesting to point out that the PRF-like products created with these three machines had all in common the damage or destruction of the cell content. This observation reinforces logically the theory that there is an integrated mathematical threshold for resonance located around 1 $m.s^{-2}$ in parasite acceleration and that this limit should be avoided as much as possible to avoid the destruction of the cell content within the tube. The triggering moment for a resonance phenomenon within the tubes that could damage the cell content and damage the fibrin organization is anyway clearly located in this range of vibrations.

4.3. Without cells, A-PRF, Salvin and LW are in fact not L-PRF

The wide and diverse cell content living within the strong fibrin matrix is one of the most important characteristics of a L-PRF clot [25, 27]. It was clearly pointed out in vitro through various cell studies where the significant tissue engineering results obtained with L-PRF were clearly connected to the slow release of growth factors [21], direct contact induction of the fibrin and the interactions of cells in coculture with leukocytes [20, 24]. The presence of activated cells is also what make the L-PRF to be considered as a real tissue that can be used in tissue engineering approach (what was termed leukocyte-driven tissue engineering) [19]. Moreover, the biological signature of the L-PRF presented a strong slow release of growth factors [22], and it was shown that this release was probably even increased by a mediator production from leukocytes [21]. Therefore, the damage or destruction of all cells within a L-PRF clot raises very significant concerns about its biological and clinical potential [25].

Finally, in case, all cells are not destroyed but only damaged, it raises even deeper concerns as damaged cells are releasing per definition many pro-inflammatory mediators. While L-PRF activated and preserved cell content was considered clinically to regulate the inflammatory process, it is impossible to know the effects of a damaged cell population, and it is anyway difficult to claim a necrotic cell population as a positive characteristic.

These observations of the cell content in fact allow to claim that the PRF-like products obtained with the A-PRF, LW and Salvin machines cannot be classified in the L-PRF family [1]. Without preserved cell content, they are more likely to be classified as a kind of pure platelet-rich fibrin (P-PRF), therefore from the same family than the Fibrinet PRF matrix (Cascade Medical, Wayne, NJ, USA) for example [2]. In all cases, the literature about L-PRF cannot be applied to the products created with the A-PRF, LW and Salvin devices, and this should be clear for all readers to avoid more confusion in the scientific literature. A-PRF, LW and Salvin centrifuges are not suitable for the production of original L-PRF clots and membranes.

Finally, this result opens a considerable debate about the way PRPs and PRFs have been produced and tested since years, as it is the first time that it is proven that the quality of the hardware is directly impacting the architecture and composition of the platelet concentrates, and therefore also their own definition, type, biological and clinical characteristics. This observation may point out a major flaw in a large quantity of the publications in this field [32, 33].

4.4. Different protocol, different PRF, different growth factors content and release

This study compared accurately the biological signatures of two kinds of L-PRF materials, the original L-PRF (Intra-Spin) and the modified protocol A-PRF. A-PRF is in fact a variation of the original L-PRF using a much lower centrifugation speed, a slightly longer centrifugation time, and glass tubes [34]. This technique was initially proposed on the original L-PRF centrifuge (using 1500 rpm) before it was definitively associated with the specific A-PRF centrifuge (using 1300 rpm) tested in the first part of this study. It was therefore a perfect model to compare the impact of the change of protocol alone on the biological signature of a PRF membrane, as both original L-PRF and A-PRF can be produced using the same centrifuge.

The main observation of this experiment was that A-PRF clots showed a much lower release of growth factors and a weaker biological signature than the original L-PRF. Moreover, the A-PRF clots dissolved quickly in the tubes, while the original L-PRF remained in good condition even after 7 days in vitro. The second observation was that all A-PRF clots and membranes (produced with 10 ml blood) were at least 30% smaller than the original L-PRF clots and membranes (produced with 9 ml blood). It was previously proven in this article that the vibrations of the A-PRF centrifuge were leading to the formation of a much smaller clot and membrane. In this study, the same stable original centrifuge (Intra-Spin) was used to produce both L-PRF and A-PRF clots to neutralize the centrifuge vibrations variable; the main difference that could explain these differences of size of the clots and of biological signature of membranes was the change of the protocol, mostly the forces of centrifugation, but also the proprietary type of tubes and the time of centrifugation.

In a previous work, it was shown that the production of L-PRF clots did not seem to be affected by the use of glass tubes or glass coated plastic tubes [19]; therefore, the differences of tubes between A-PRF and L-PRF may not explain the observed differences. However, this shall be confirmed in future research, as there are many kinds of glass tubes and glass coated plastic tubes.

The Intra-Spin tubes were selected very specifically following the long L-PRF experience to fulfill CE and FDA clearance, while nothing is known about the source of the A-PRF tubes (except that they are for "in vitro diagnostic only" and made in China). This difference may have an impact in the results and should be investigated. Moreover, it was shown that there is very little impact in using longer centrifugation time with an original L-PRF, as it is common to centrifuge during 18 minutes when patients are under anticoagulant treatment [17]. The increase in centrifugation time mostly gives a bit more time for a fibrin clot to polymerize. It was not needed to last longer than 12 minutes for the original L-PRF in most cases, but A-PRF seems to need this supplementary time to finish its gel polymerization (14 minutes in total).

As a conclusion, these differences of size, aspect and biological signature of the clots and membranes between the original L-PRF and A-PRF can be probably associated with the change in the centrifugation forces. It confirms the need for using forces around 400 g (2700 rpm in the original centrifuge), in order to do a proper separation of the blood constituents with an adequate gradient of centrifugation and collect a large and proper L-PRF clot. The use of a lower g force and speed (1500 rpm for A-PRF) did not seem enough for a proper separation of the blood constituents, and it led to the preparation of a clot (A-PRF) of much smaller size, weaker biological signature and lower fibrin polymerization, even when the tubes were larger (10 ml) and if an adequate stable centrifuge was used.

In previous publications, it was advocated that the cell population of a L-PRF membrane was responsible of the production of new growth factors [21]. Indeed, the total released quantities after 7 days of many growth factors were always much higher than the total quantities detected after forcible extraction from the whole membrane just after preparation [21]. In this new study, the same observation can be done for L-PRF, and the result obtained with A-PRF somehow confirmed it. The comparison of the results between L-PRF and A-PRF also highlighted that cells must be placed in a specific environment to massively produce more molecules. TGFβ-1, VEGF and BMP2 presented the same high ratio (around 7 for L-PRF) between total slow release/initial quantity, revealing somehow the activity of production of these molecules by the cells within the clots. The lower ratios of A-PRF revealed also a lower production activity. On the contrary, PDGF-AB ratio was quite stable around one for both products, as this molecule is mostly contained and released by platelets initially collected in the sample. Therefore, this study also confirmed the need to protect the viability of the cells and even pointed out the need for an activation of the cells by the centrifugation process. This notion of activation is the most logical explanation for the very strong differences of biological signatures between original L-PRF and A-PRF, particularly the interesting result with BMP2.

BMP-2 is an important osteoinductive molecule belonging to the TGF-β superfamily of proteins and playing particularly an important function in bone development. For this reason, recombinant forms of this molecule were marketed in a few countries for the treatment of bony defects in orthopedic and maxillofacial surgery, with mixed or controversial results [35] related to the difficult control of the effects of this molecule in a direct therapeutic approach. The release of small quantities of BMP2 from the original L-PRF probably contributed in some way to the stimulation of bone cell proliferation and differentiation observed in vitro by the L-PRF [20, 24] and to the positive clinical effects of L-PRF during bone regeneration [15]. However, its importance in the global equation of the L-PRF (combining many cells and growth factors into a specific fibrin matrix) is impossible to point out at this time [20, 22, 25].

It is interesting to notice that BMP2 was not detected with A-PRF (detected as a noise, probably under the detection threshold of 29 pg/mL), while the company marketing it used the release of BMP2 as a commercial argument for the A-PRF protocol. The exact origin of the BMP2 detected in the L-PRF is difficult to point out, as BMP2 is a molecule specific to bone cells; the ELISA kit itself was designed mostly for bone tissue extracts and bone cell culture supernatants. Small quantities of BMP2 can be detected in the blood in some conditions [36], but its overexpression in blood is often associated with various pathologies [37, 38]. As BMP2 is not supposed to be released by platelets, consequently the different cell populations (mostly leukocytes) living in the L-PRF clot released BMP2. As BMP2 levels were quite low after forcible extraction from the initial L-PRF clot, consequently the L-PRF cells released and produced this molecule step by step during the experiment. The combination of these observations in both L-PRF and A-PRF supported the conclusion that the quantity and state of the cell population within the L-PRF clot defines a large part of its biological signature.

Finally, the evaluation of the slow release of key growth factors from a PRP gel or a L-PRF membrane appeared again as a quite simple method of characterization of the biological signature of an activated platelet concentrate gel. It was already used in several publications [22, 23], and it illustrated quite well the differences of growth factor content, cell content and fibrin architecture of different products. The slow release pattern is so characteristic from a product that it should be evaluated systematically in all kinds of products to define their exact biological signature prior to compare them or analyze their clinical effects.

5. Conclusion

This article is the first study analyzing the intrinsic differences between four L-PRF centrifuges available on the market and its consequences on the quality of the platelet concentrates. At the classical speed of production of L-PRF, the level of undesirable vibration on the original L-PRF centrifuge (Intra-Spin) is between 4.5 and 6 times lower than with other centrifuges. Moreover, Intra-Spin always remains under the threshold of resonance, unlike the three other tested machines. The original L-PRF clot (Intra-Spin) used and validated since years presented very specific characteristics, which appeared completely distorted when using centrifuges with a higher vibration level. A-PRF, LW and Salvin centrifuges produced PRF-like materials with a damaged and almost destroyed cell population through the standard protocol developed initially for the L-PRF, and it is therefore impossible to classify these products in the L-PRF family. A-PRF, LW and Salvin centrifuges are not suitable for the production of original L-PRF clots and membranes.

In the second part of this work, the slow release of the four tested growth factors from original L-PRF membranes was much more intense (a much stronger biological signature) than the release from A-PRF membranes. Moreover, the original L-PRF clots and membranes (produced with 9 mL blood) were always significantly larger than the A-PRF clots and membranes (produced with 10 mL blood). The A-PRF membranes dissolved in vitro after less than 3 days, while the L-PRF membrane remained in good shape during at least 7 days. The same centrifuge was used for both products in this study; only the protocol (particularly the centrifugation forces) was different between the original L-PRF and the A-PRF. Therefore, the two protocols produce two very different kinds of materials, independently from the characteristics of the centrifuge, and the clinical results are expected to be significantly different between the two products.

As a conclusion, it was clearly proven that the centrifuge characteristics and centrifugation protocols have a very significant impact on the cell, growth factors and fibrin architecture of a L-PRF clot and membrane and that any modification of the original L-PRF material and method shall be clearly investigated and identified separately from the original methods, in order to avoid to create confusion and inaccurate results in the literature.

Declaration of interests

The authors have no conflict of interest to report.

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