

Research 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. Part 2: macroscopic, photonic microscopy and Scanning Electron Microscopy analysis of 4 kinds of L-PRF clots and membranes

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Submitted May 19th, 2014; accepted after minor corrections on June 4th, 2014.

Abstract

Background and Objectives. Platelet concentrates for surgical use (Platelet-Rich Plasma PRP or Platelet-rich fibrin PRF) are surgical adjuvants to improve healing and promote tissue regeneration. L-PRF (Leukocyte- and Platelet-Rich Fibrin) is one of the 4 families of platelet concentrates for surgical use and is widely used in oral and maxillofacial regenerative therapies. The objective of this second article was to evaluate the impact of the centrifuge characteristics (vibration intensity) on the cell and fibrin architecture of a L-PRF clot and membrane.

Materials and Methods. Four different commercially available centrifuges were used to produce L-PRF, following the original L-PRF production method widely described in the literature (glass-coated plastic tubes, 400g 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 3 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). All clots and membranes were collected into a sterile adequate surgical box (Xpression kit). The exact macroscopic (weights, sizes) and microscopic (photonic and scanning electron microscopy SEM) characteristics and the cell composition of the L-PRF clots and membranes produced with these 4 different machines with 4 different vibration intensity levels were evaluated.

Results. Intra-Spin showed the lowest temperature of the tubes. A-PRF and Salvin were both associated with a significant increase of temperature in the tube. Intra-Spin produced

by far the heaviest clot and quantity of exudate among the 4 techniques. For clot and membrane length and width, Intra-Spin and Salvin presented similar sizes. A-PRF and LW produced much lighter, shorter and narrower clots and membranes than the 2 other centrifuges. Light microscopy analysis showed relatively similar features for all L-PRF types (concentration of cell bodies in the first half of the fibrin mesh). 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 all the visible cell bodies appeared destroyed (squashed or shrunk).

Discussion and Conclusion. This study illustrated that the centrifuge characteristics (particularly the vibrations) are directly impacting the architecture and cell content of a L-PRF clot. The original L-PRF clot (Intra-Spin) used and validated since years 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 400g 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 at 400g. Further research would be interesting to evaluate how modifications of the protocol alone (for example reduction of the g forces) may influence the biological signature of the L-PRF clots and membranes, independently from the characteristics of the centrifuge.

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

1. Introduction

Leukocyte- and Platelet-Rich Fibrin (L-PRF) is one of the 4 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 9ml 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]. 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 tailored 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 was produced using this adequate material since more than 10 years. The original open-access method and associated devices used since the early phases of the development of this technique are nowadays marketed with CE/FDA clearance as the Intra-Spin L-PRF system and kit (Intra-Lock, Boca-Raton, FL, USA). This inexpensive 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 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 (for example 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 objective of this series of 3 articles was to point out the impact of the centrifuge characteristics and centrifugation protocol on the cell, growth factors and fibrin architecture of a L-PRF clot and membrane. In the first article, the mechanical vibrations (both radial and vertical) appearing during centrifugation were evaluated in 4 models of commercially available table centrifuges frequently used to produce L-PRF. It was proven that the original L-PRF centrifuge (Intra-Spin) was by far the most stable machine. At the classical speed of production of L-PRF, the level of undesirable vibration on this centrifuge is between 4.5 and 6 times lower than with other centrifuges. Moreover, Intra-Spin always remains under the threshold of resonance, unlike the 3 other tested machines. In this second article, 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 4 different machines were evaluated. As a secondary objective, the impact of the vibration parameter on the architecture and cell content of the L-PRF clots was discussed.

2. Materials and methods

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.

2.1. Description of the tested centrifuges

In this study, 4 different centrifuges, found on the market and used to produce L-PRF, were tested. 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 4 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 3 other centrifuges are not CE/FDA cleared for L-PRF, but they can be found relatively frequently available on the market for this use (mostly because they are much cheaper): 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).

2.2. Preparation of L-PRF

Blood samples were collected at the San Bernardo University of the Andes Health Center from 8 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 2,5ml 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 10.0ml 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 400g 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 400g 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 were adjusted accordingly for each centrifuge, i.e. 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 400g. 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.

2.3. 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 1A**). The L-PRF fibrin clot obtained was placed on a sterile microscope slide (**Figures 1B, 1C**) placed in an individual tray for weight and size measurements (**Figure 2**). 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 1mm rubber stop (**Figure 1C**) 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 1mm-thick L-PRF membranes, which were weighted and measured individually (**Figure 3**).

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.

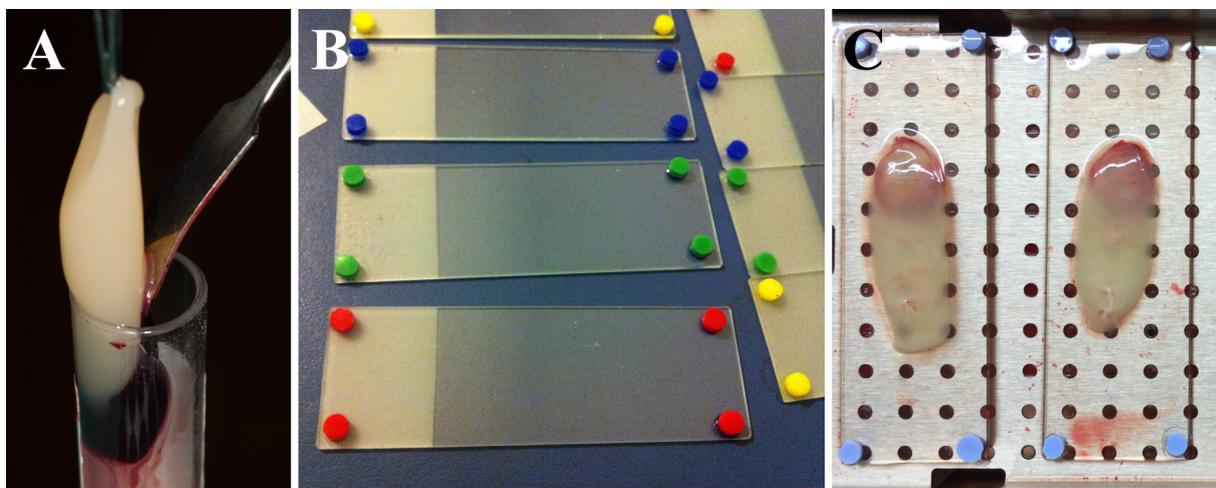


Figure 1. Material for PRF clot handling. L-PRF clots were collected in each tube, and the red blood cell part was gently removed with a smooth instrument and a light lateral pressure (**A**). Standard glass histological slides were used to support the clots during the macroscopic evaluation (**B**). Rubber stops were placed on each corner of the slides, in order to perform a standardized compression of all the samples into membranes between 2 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.

2.4. Light microscopy procedure

The membranes were fixed in 10% neutrally buffered formalin for 24 hours at room temperature for paraffin inclusion. Successive sections of 4 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).

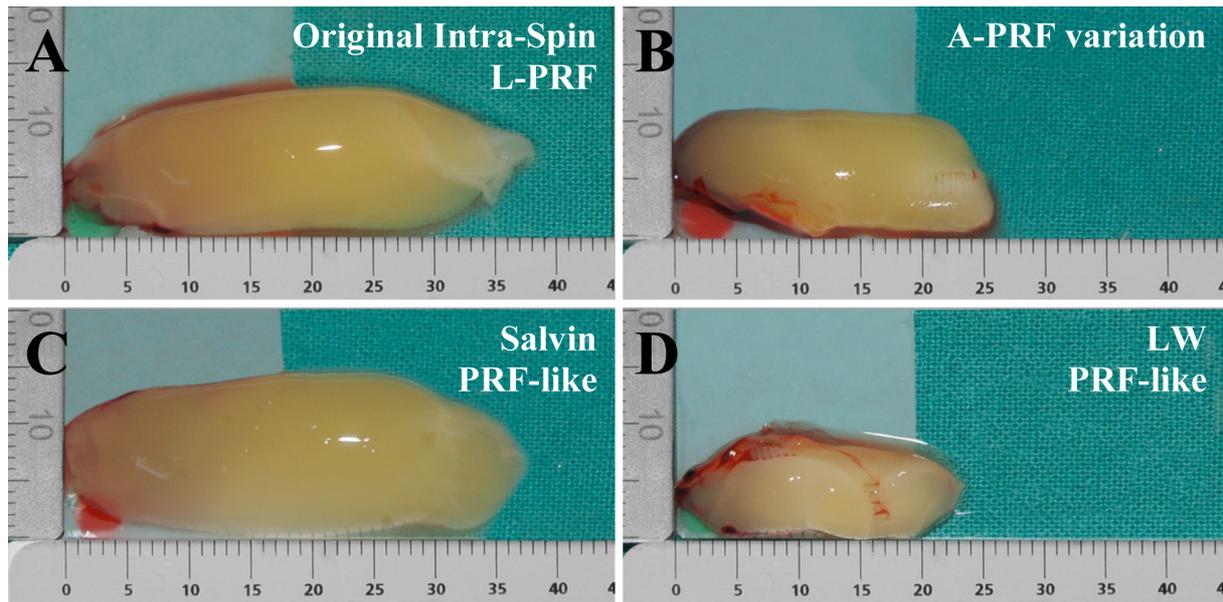


Figure 2. Macroscopic evaluation of the PRF clots produced with the 4 different centrifuges: original Intra-Spin L-PRF system (A), A-PRF system (B), Salvin centrifuge (C) and LW centrifuge (D). Obvious differences can be observed in terms of size and aspect, the original L-PRF (A) being always denser and heavier (and in most cases larger) than the others.

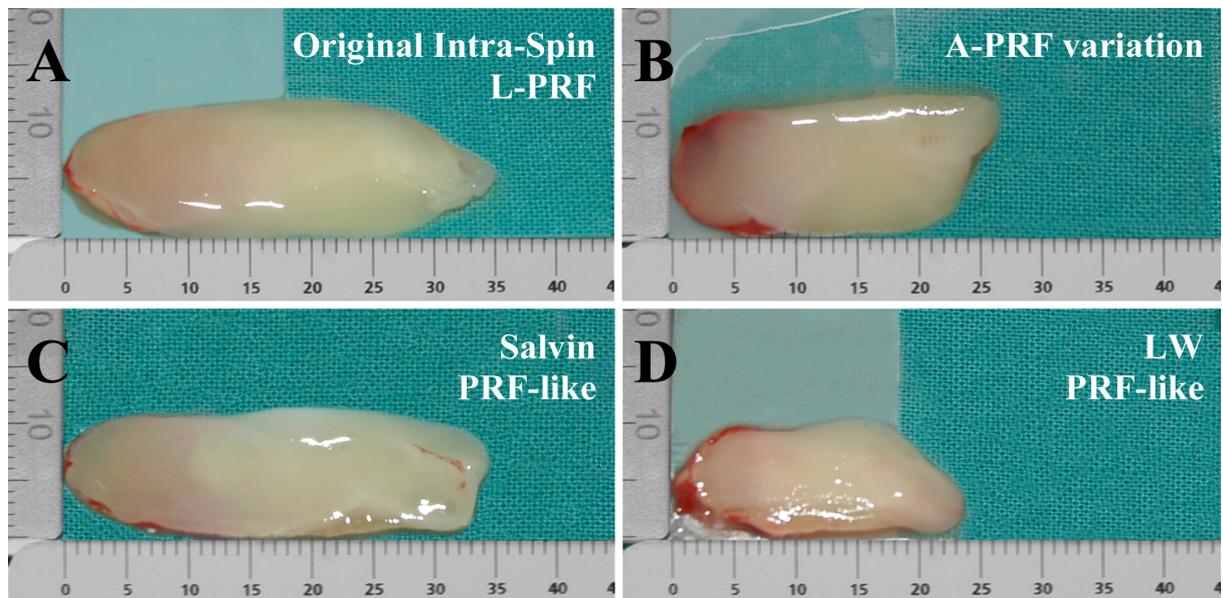


Figure 3. Macroscopic evaluation of the PRF membranes produced with the 4 different centrifuges: original Intra-Spin L-PRF system (A), A-PRF system (B), Salvin centrifuge (C) and LW centrifuge (D). Obvious differences can be observed in terms of size and aspect, the original L-PRF (A) being always denser and in most cases larger than the others.

2.5. Scanning Electron Microscopy (SEM) Procedure

A morphologic evaluation of the L-PRF membranes was done with a scanning electron microscope. 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 20nm 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 to 20kV using 2,000 to 5,000X magnifications. This study was mainly descriptive.

3. Results

3.1. Macroscopic analysis

All the macroscopic results are presented in the **Table**. The numeric values are clearly illustrated by the observation of the clots and membranes in the **Figures 2 and 3**.

For the temperature of the tubes, Intra-Spin allowed to keep the lowest temperature among the 4 tested machines. A-PRF and Salvin were both associated with a significant increase of temperature in the tube.

For the clot and exudate weights, Intra-Spin produced by far the heaviest clot and quantity of exudate among the 4 techniques. Salvin remains high but far behind. 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 more narrow.

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

	IntraSpin	A-PRF	Salvin	LW
Variable	Mean (SD)	Mean (SD)	Mean (SD)	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 10ml	20.94 (2.4)	13.98 (2.6)	17.42 (2.63)	7.41 (1.45)

Table. Results of the macroscopic analysis of the clots and membranes produced with the 4 tested centrifuges. Values expressed in Mean and Standard Deviation (SD).

3.2. Light microscopy analysis

In light microscopy (**Figure 4**), 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, 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 and 12% distal), as the clot and membranes were particularly small and shrunk. Light microscopy did not allow to observe in more details the exact state of these cell bodies.

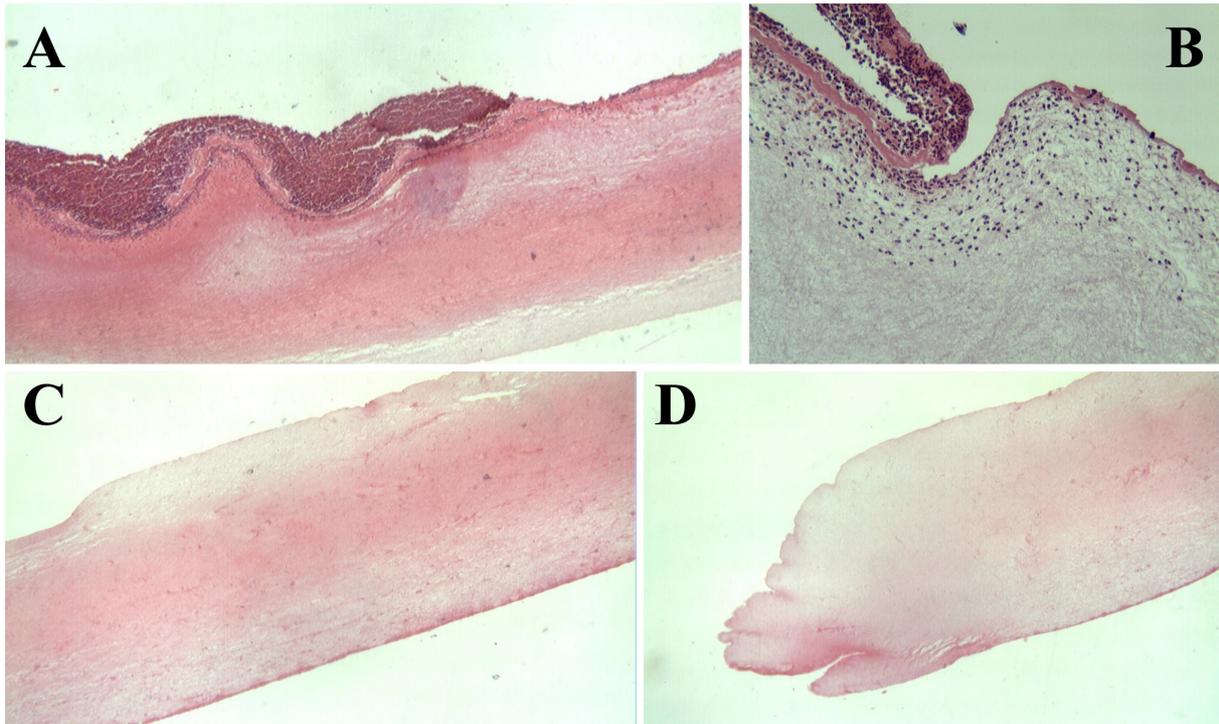


Figure 4. Microscopic evaluation of the PRF membranes produced with the 4 different centrifuges in light microscopy (hematoxylin eosin). The different membranes showed similar organization in light microscopy, with a concentration of most visible cell bodies (75%) in the first 1/3 proximal part of the 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.

3.3. SEM analysis

The SEM analysis allowed to evaluate in details the aspect of the fibrin network and of the cell content of each membrane (**Figures 5 and 6**).

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 3 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 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.

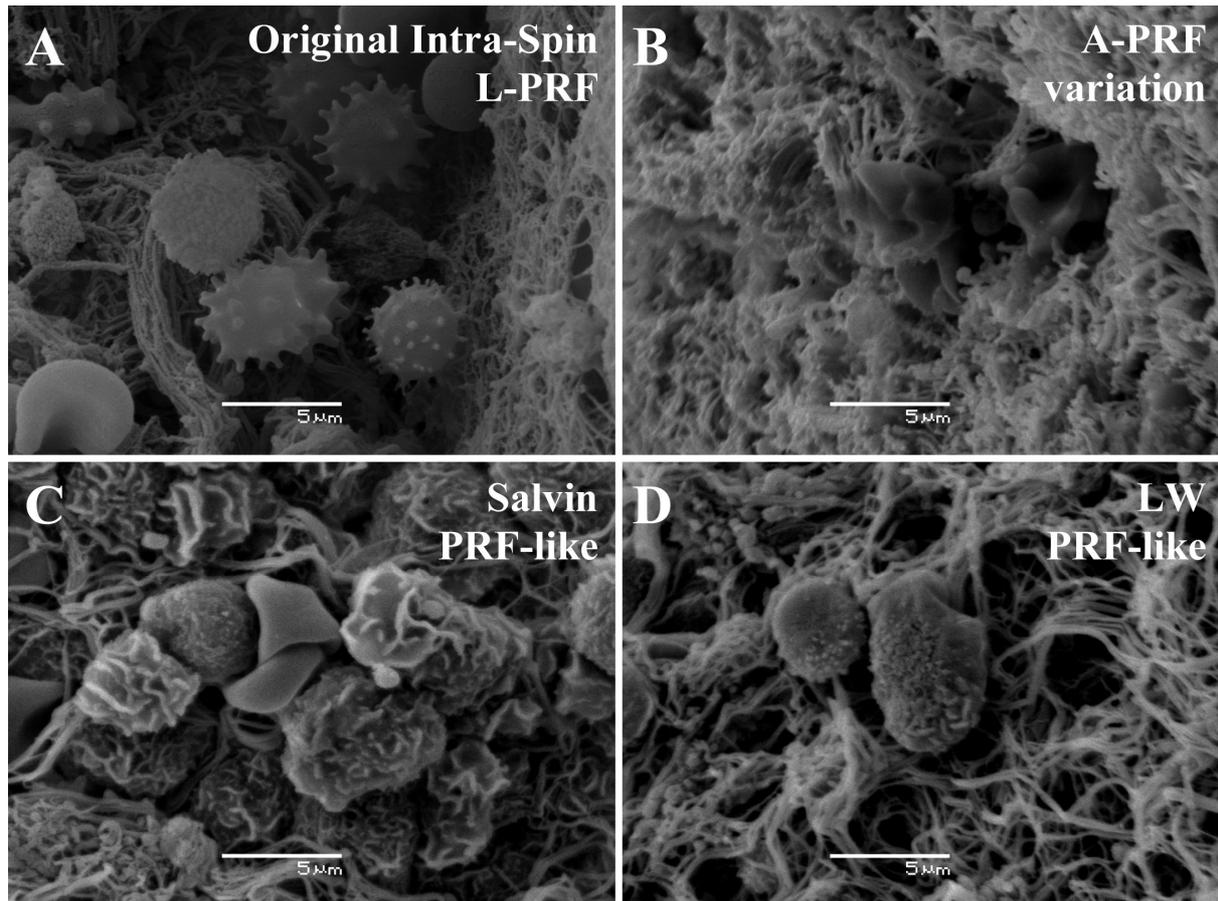


Figure 5. SEM Microscopic evaluation of the PRF membranes produced with the 4 different centrifuges. The different membranes showed very different aspects during SEM analysis. The original L-PRF membrane (Intra-Spin, A) presented a strongly polymerized fibrin network and the presence of a large living 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.

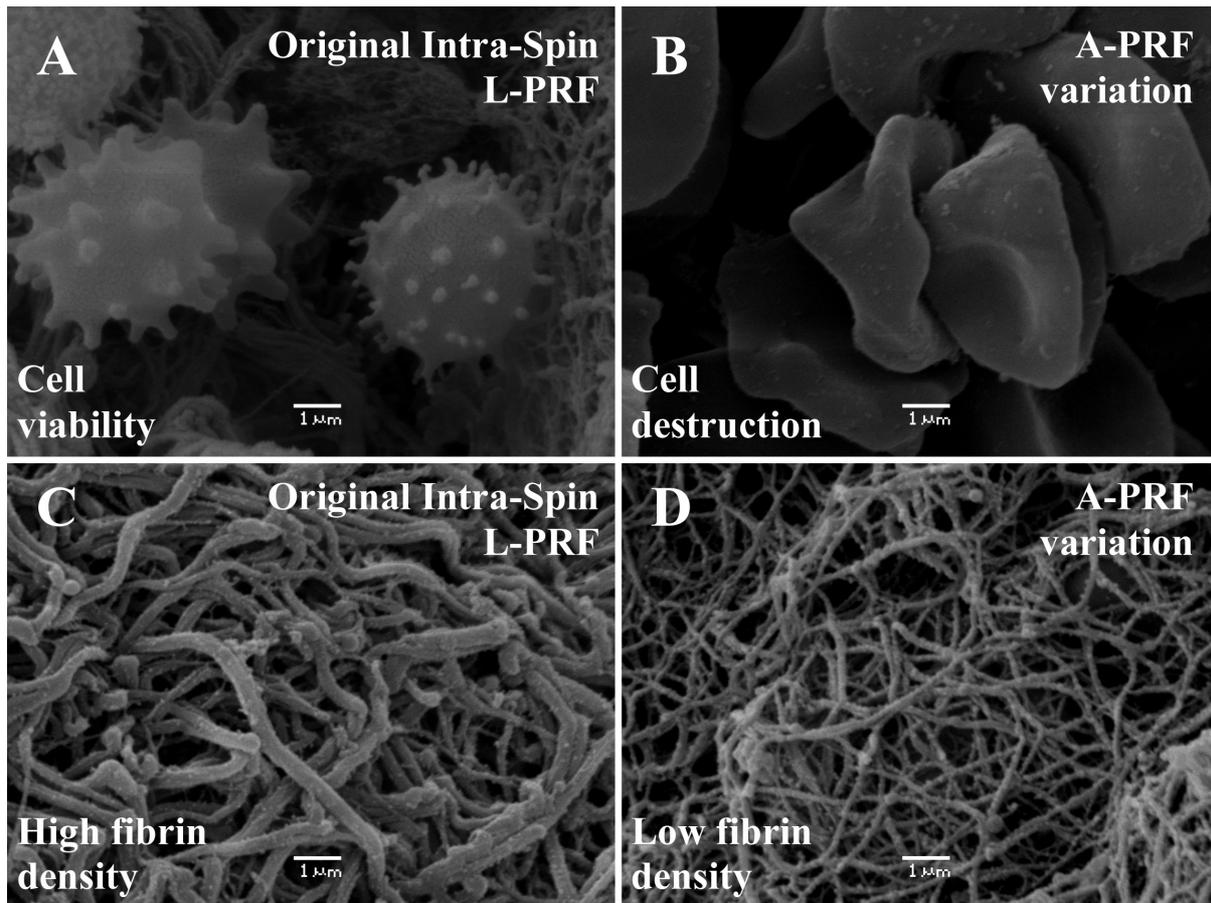


Figure 6. SEM Microscopic evaluation and comparison of the PRF membranes produced with 2 different centrifuges. The original Intra-Spin L-PRF membranes (**A**, **C**) presented a large cell population (**A**), and all observed cells appeared alive with a normal shape. Lymphocytes presented typical textured 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**).

4. Discussion

4.1. 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 4 products was the hardware (the centrifuge). After

blood centrifugation in the 4 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 4 centrifuges, the hypothesis was that mechanical vibrations may 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 series of articles 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 4 products, it is therefore possible to associate the differences of L-PRF weight, volume, fibrin architecture and cell content between the 4 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 400g 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} (see article 1), so far under the threshold of 1. For the 3 other centrifuges, the rpm speed used to stay in the 400g centrifugation forces were 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 3 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.

4.2. 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 can not 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 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].

5. Conclusion

This study definitively illustrated and clarified what is exactly a L-PRF clot or membrane, and that the centrifuge characteristics (particularly the vibrations) are directly impacting the architecture and cell content of a L-PRF clot. 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 400g 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 at 400g. To conclude this series of studies, it would be interesting to evaluate how the changes of the protocol (for example reduction of the g forces) alone may influence the biological signature of the L-PRF clots and membranes, independently from the characteristics of the centrifuge.

Disclosure of interests

The authors have no conflict of interest to report.

Acknowledgements

This research work on new biotechnologies and therapeutic strategies in regenerative medicine was supported by the PACT (Platelet & Advanced Cell Therapies) Forum Civitatis of the POSEIDO Academic Consortium (Periodontology, Oral Surgery, Esthetic & Implant Dentistry Organization), by a grant from the National Research Foundation of Korea (NRF) funded by the Korean government-MEST (No. 2011-0030121) and by the LoB5 Foundation for Research, France.

Author Contributions

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

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This article can be cited as:

Pinto NR, Pereda A, Jiménez P, Del Corso M, Kang BS, Wang HL, Quirynen M, Dohan Ehrenfest DM. *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. Part 2: macroscopic, photonic microscopy and Scanning Electron Microscopy analysis of 4 kinds of L-PRF clots and membranes.* **POSEIDO.** 2014;2(2):141-54.