Application of platelet products in dental regenerative treatments

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Introduction

Regenerative therapy in dentistry has been defined as the replacement and/or regeneration of oral tissues lost as a result of disease

Brief history of platelet concentrates

❖**First generation**

1) **PRP (Marx. 1998)**

2) **PRGF (Anitua. 1999)**

❖**Second generation**

1) **L-PRP (Choukroun. 2001)**

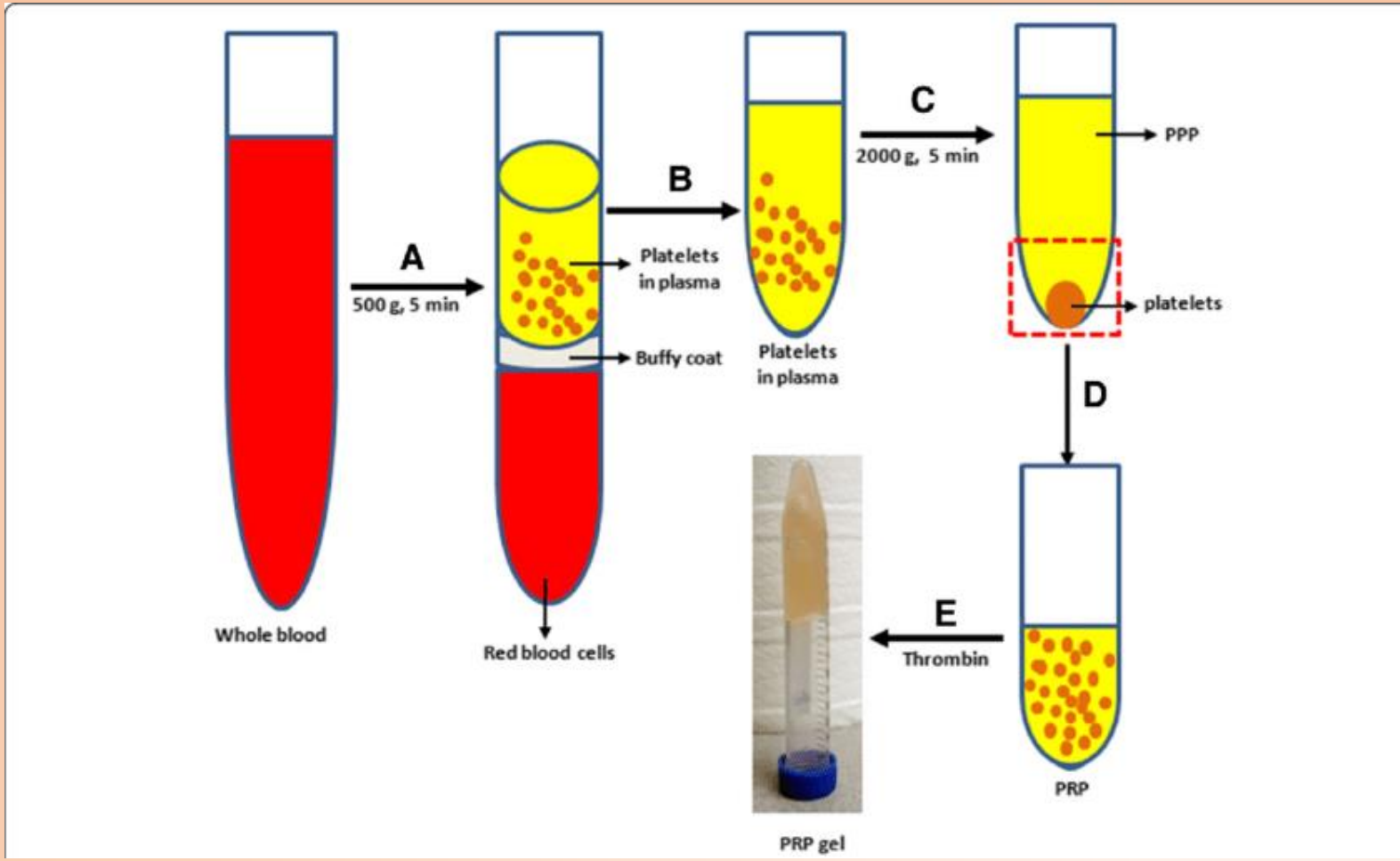
2) **A-PRF and A-PRF + (Ghannati. 2014)**

3) **I-PRF**

4) **CGF**

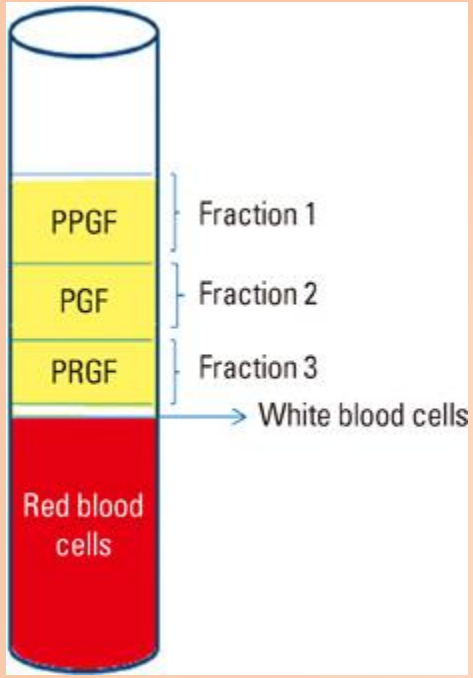
Preparation of platelet-rich plasma (PRP) from the whole blood by five steps. (Marx. 1998)

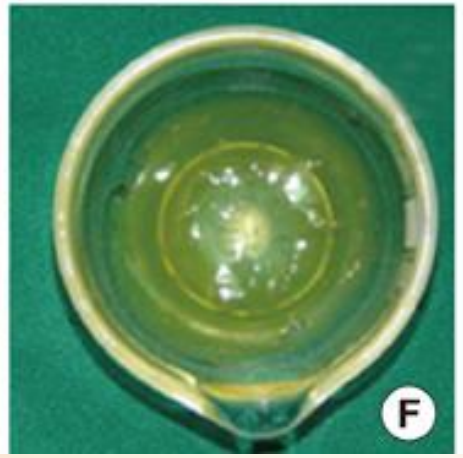
(a) Whole blood was separated into three layers by a centrifuge at 500g for 5 min. (b) The platelet-containing plasma (top layer) was transferred into a new centrifuge tube. (c) The platelet pallet was obtained by a centrifuge at 2000g for 5 min. (d) The PRP was prepared by suspending platelets with PPP. (e) The PRP gel was obtained by adding thrombin into PRP solution



Stages of the plasma rich growth factors (PRGF) preparation. (A, B) The withdrawal of the patient's peripheral blood. (C) The centrifuged product. (D) The collected PRGF from the two sample tubes combined into one tube. (E) The addition of calcium chloride for clotting. (F) The PRGF gel. (Anitua. 1999)

Three layers of plasma rich in growth factors (PRGF). PPGF: Plasma poor in growth factor, PGF: Plasma with growth factor.





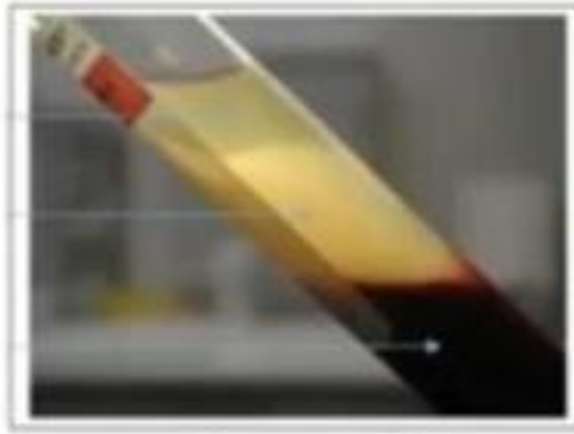
Limitations of PRP and PRGF

Antiquagulants

Platelet Rich Fibrin (PRF)

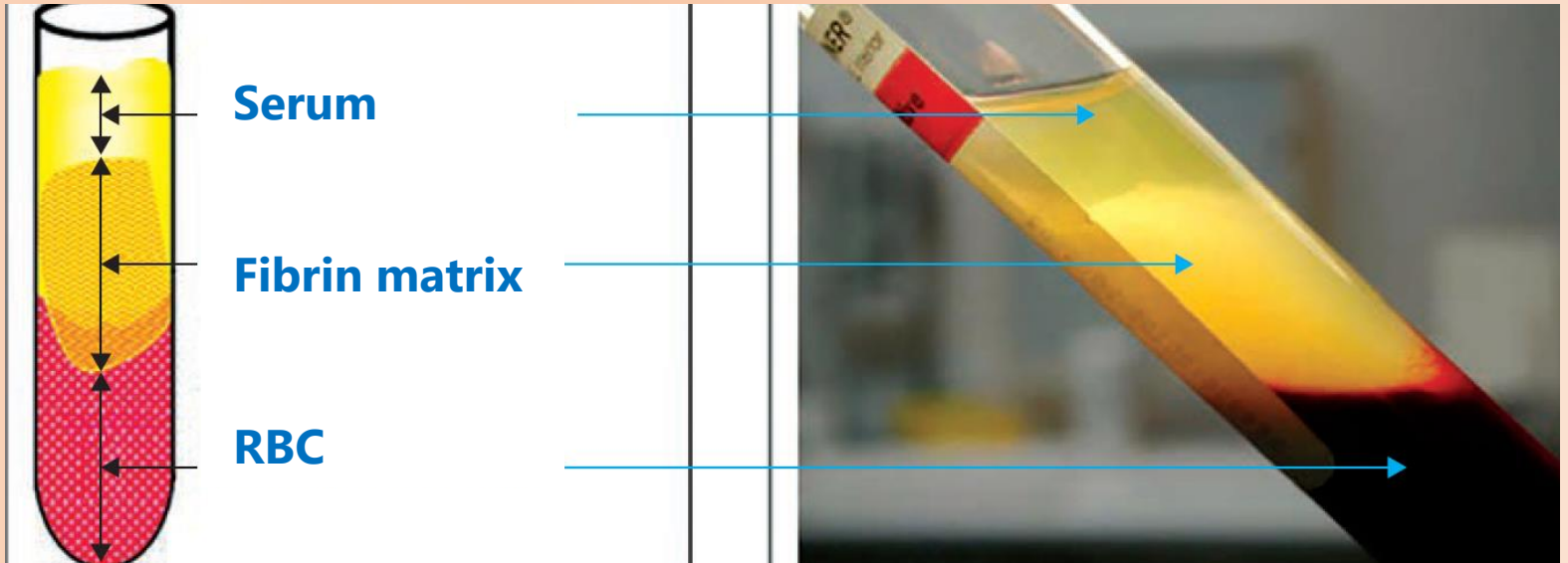
Step 1:
Blood Draw

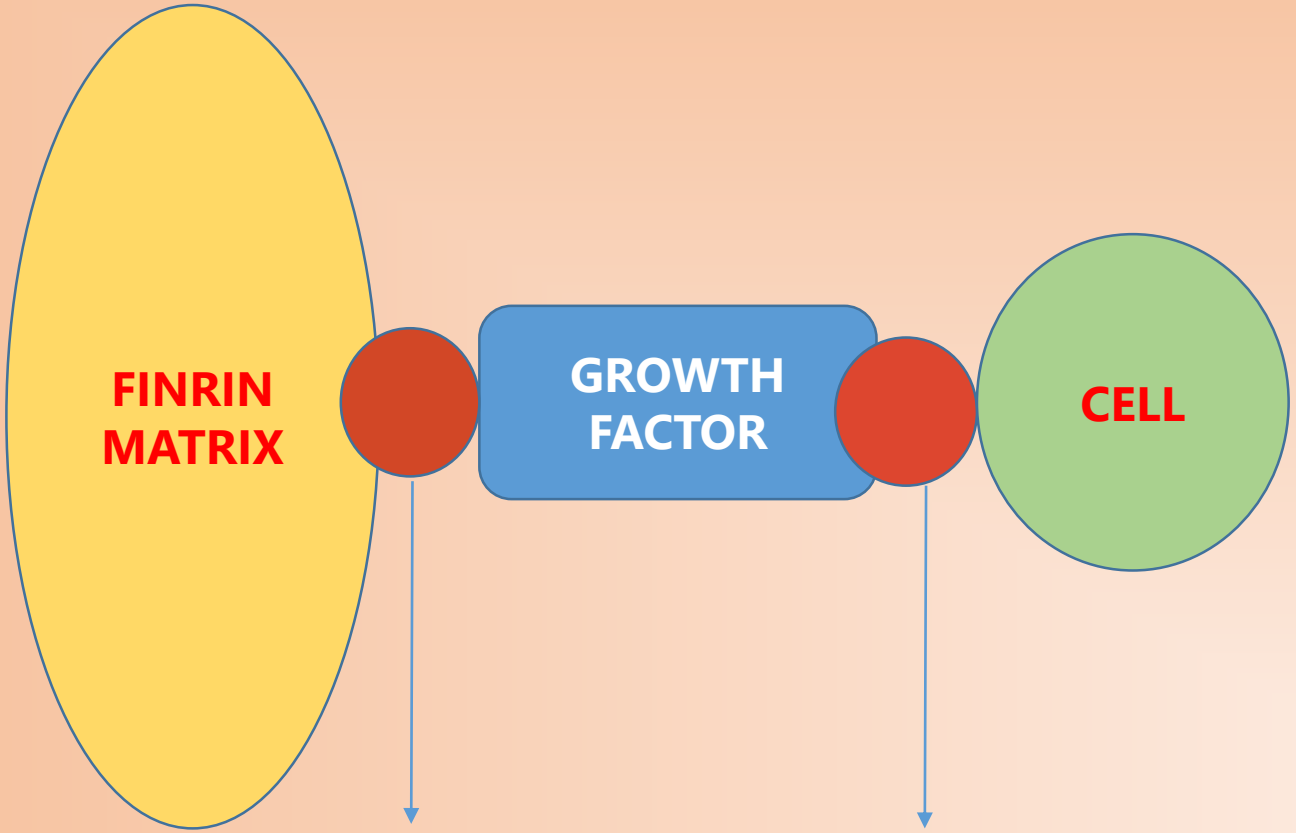
Step 2:
Centrifugation



Step 3:
PRF produced


Red Tube:
Made for membranes
Hydrophilic surfaces
Glass or silica-coated plastic
White Tube:
Made for liquid
Hydrophobic surfaces
PET Plastic


Serum
Fibrin matrix
RBC

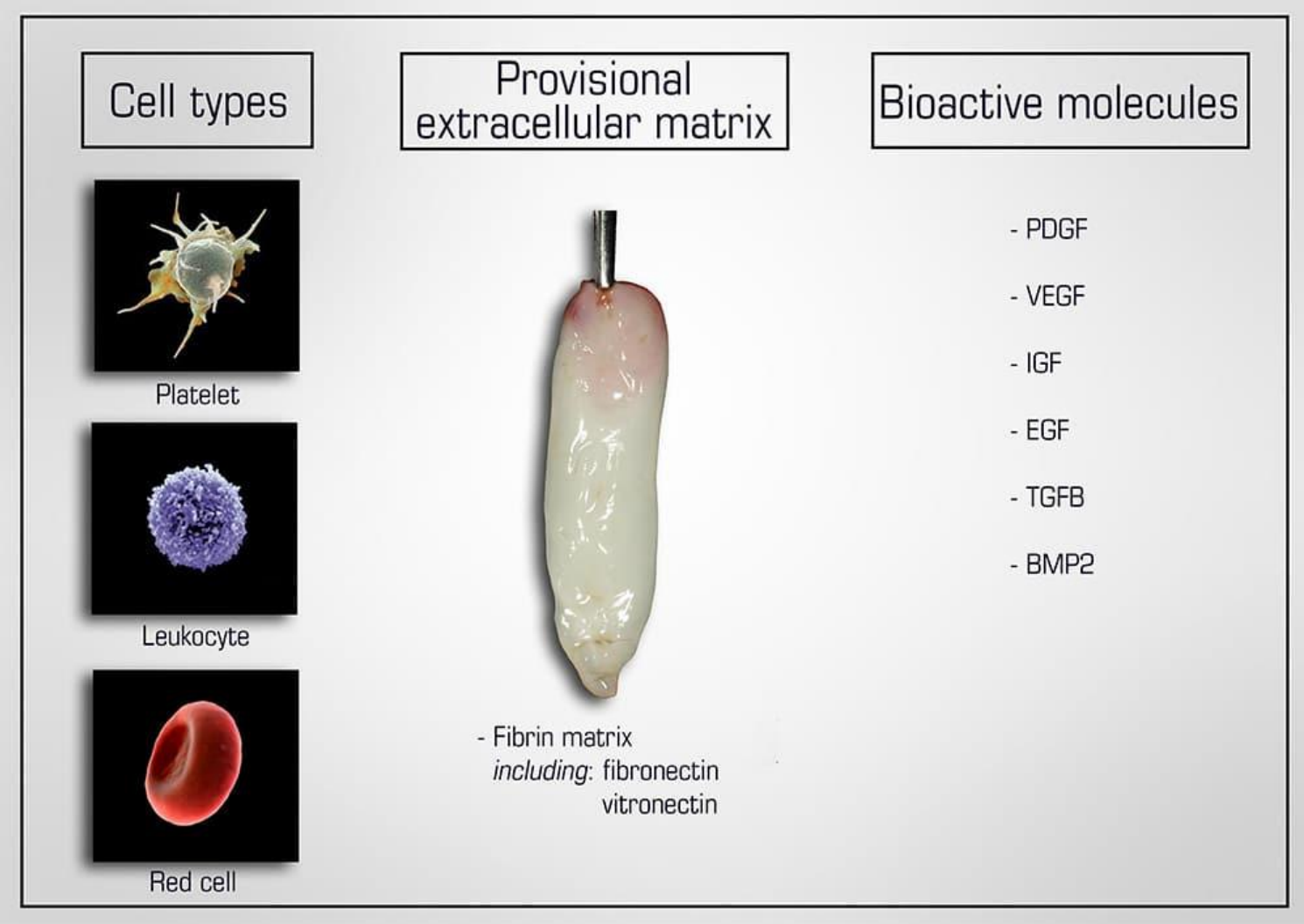

FINRIN
MATRIX
GROWTH
FACTOR
CELL
Heparin bonding domain
Functional domain

Natural components of PRF include:

1) Cell types (platelets, leukocytes and red blood cells)

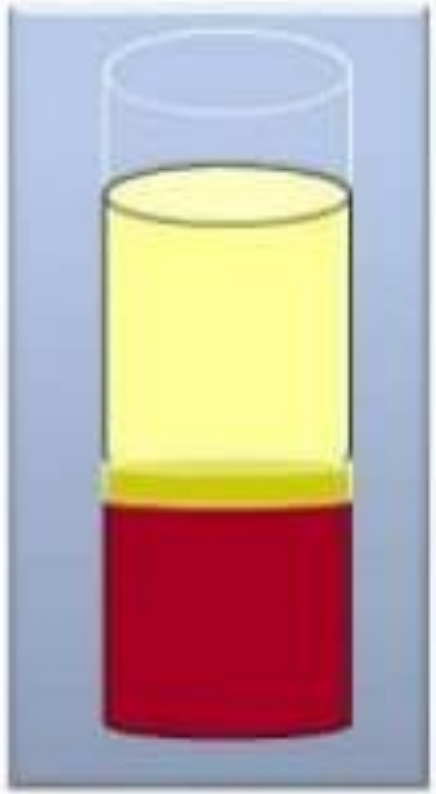
2) A provisional extracellular matrix 3-dimensional scaffold fabricated from autologous fibrin (including fibronectin and vitronectin) as well as

3) A wide array of over 100 bioactive molecules including most notably PDGF, VEGF, IGF, EGF, TGF-β and small quantities of BMP2



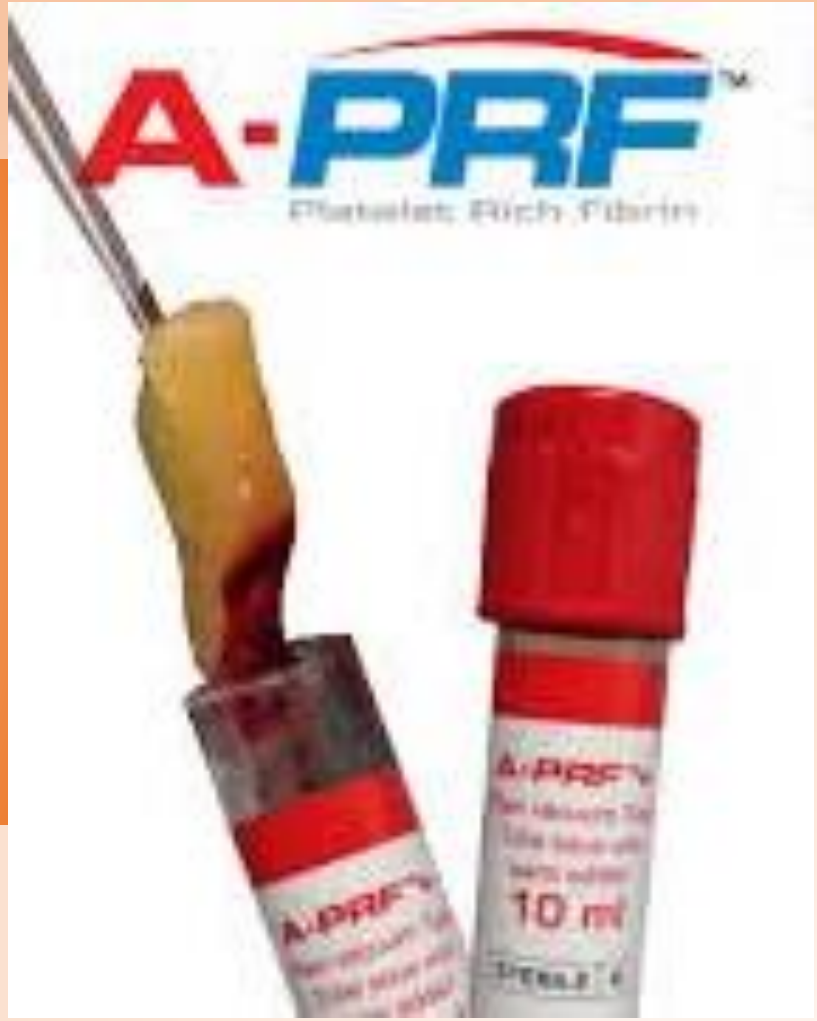
Buffy Coat

- A layer of mixed white cells and platelets created by high speed centrifugation of whole blood
- It is neutral or *buff* in colour
- Contains: most of the white cells and platelets and ~ 10 % of RBC

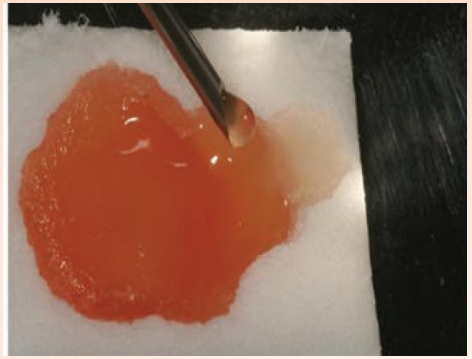


Advanced Platelet Rich Fibrin

- **Even cell distribution pattern**
- **Increased number of inflammatory cells and platelets**
- **Better permeability and vascularization due to higher porosity**



Injectable Platelet Rich Fibrin

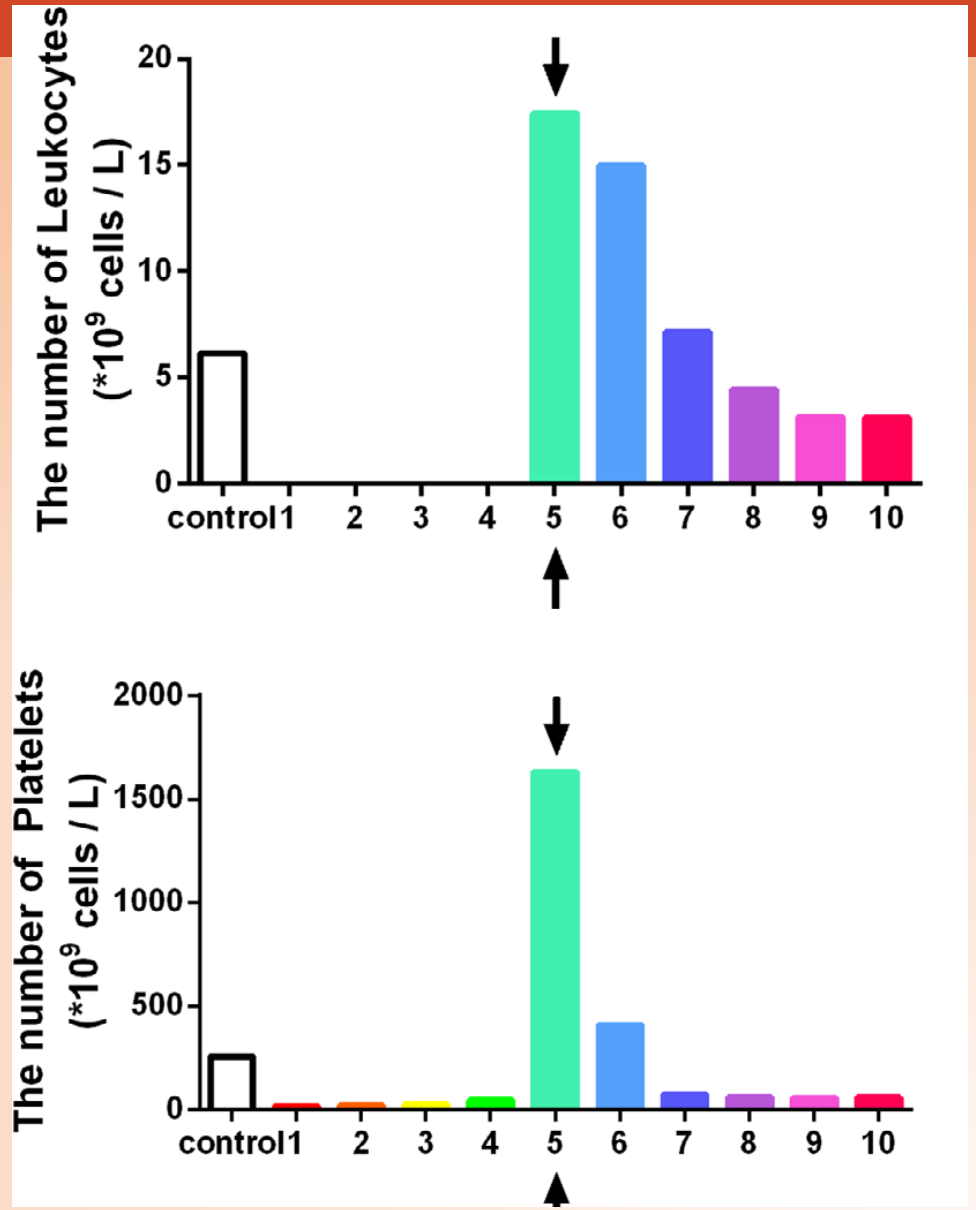


Advanced platelet-rich fibrin (A-PRF+)

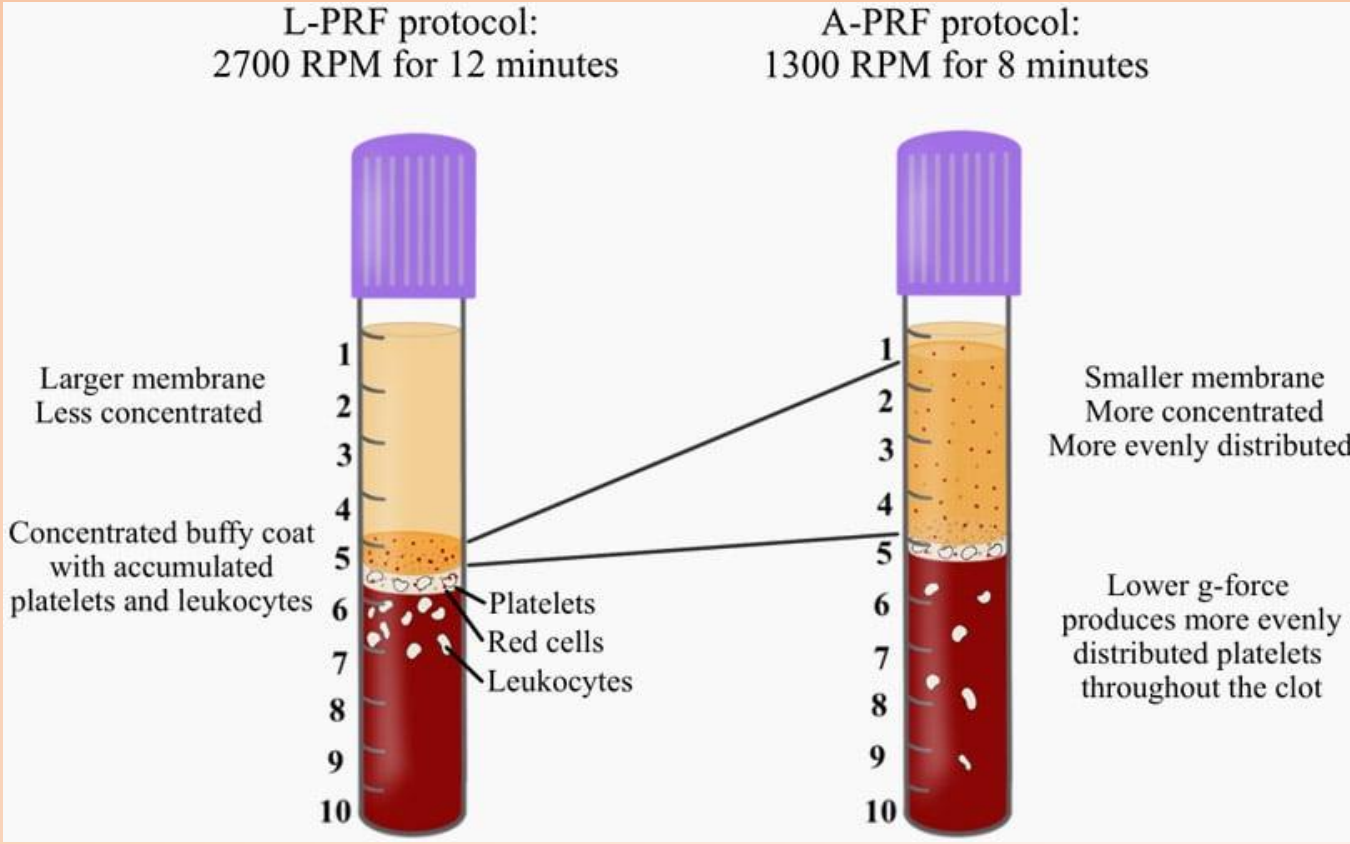
Comparison of A-PRF and A-PRF+:

- G-force is similar but time is reduced
- There is no difference in the number of cells
- Similar cell distribution
- Improved release of growth factors especially VGF

Majority of platelets accumulated directly within the fifth layer in the buffy coat. Furthermore, the highest concentration of leukocytes was also noted in this layer. The first four layers of this plasma layer, was typically devoid of all cells



The effects of both L-PRF and A-PRF protocols on the final outcomes. Since PRF is obtained from the upper layer of PRF tubes, it becomes obvious that the L-PRF protocols generate PRF membranes that are mainly devoid of cells with the largest accumulation observed directly within the buffy coat. In contrast, the A-PRF protocol is able to more evenly distribute cells. This is one of the advantages of utilizing this novel quantification method proposed in a recent article (Miron et al. 2019).



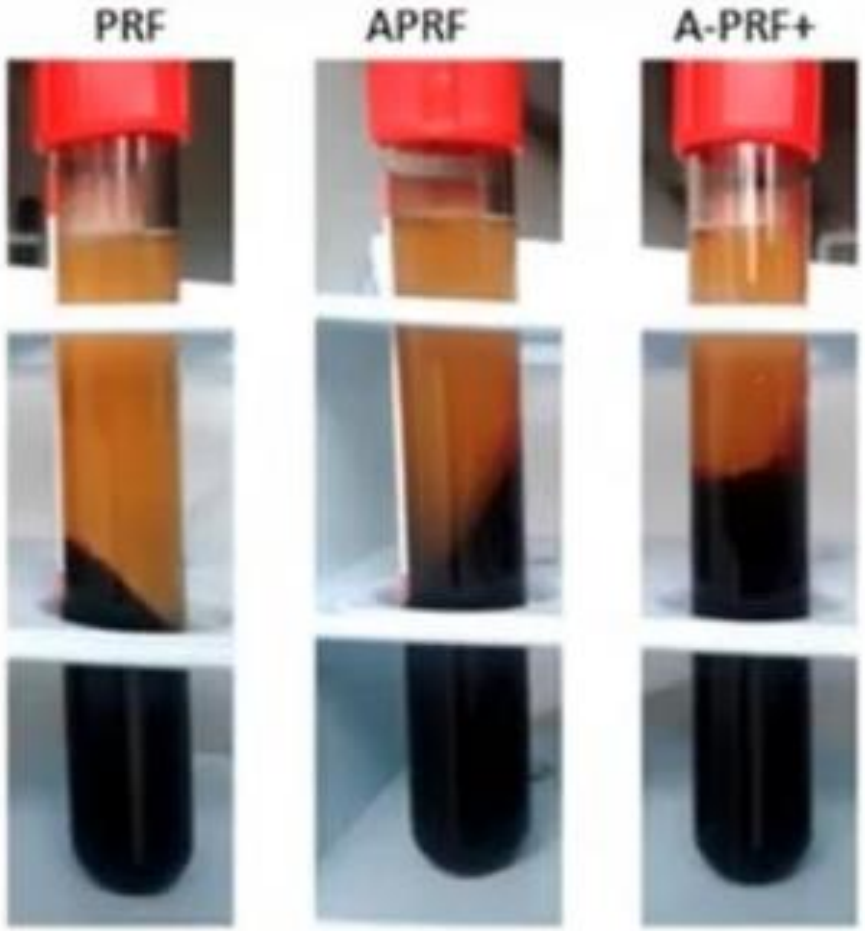
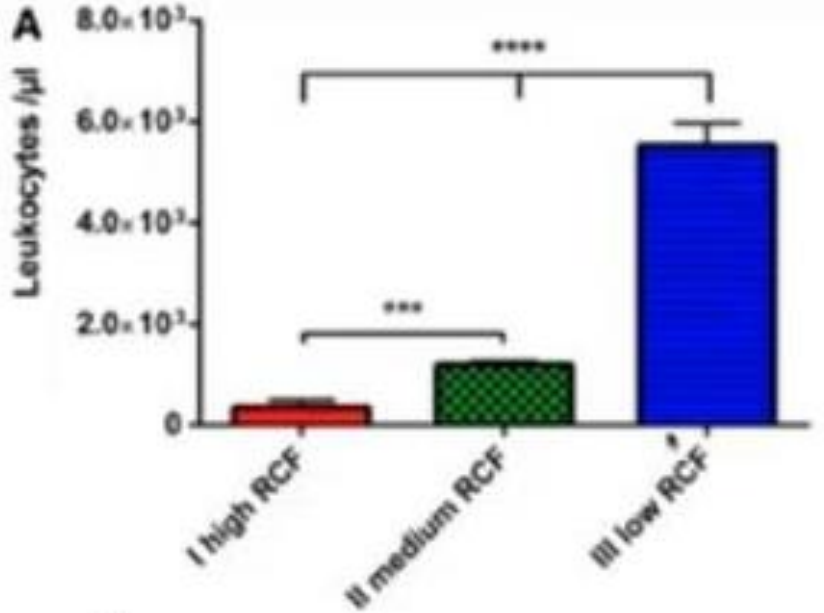
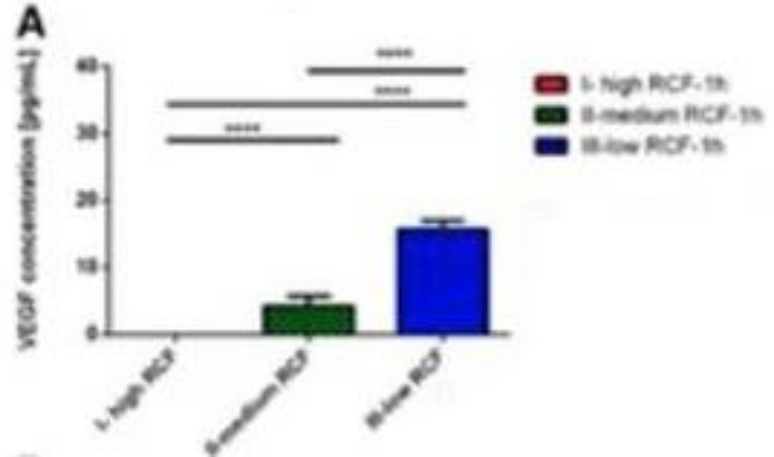


Fig. 1 The PRF-based matrices immediately following centrifugation

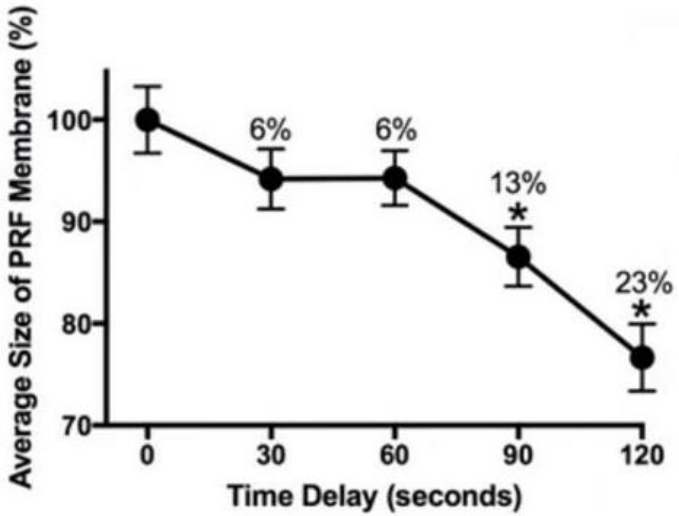




The effect of age, gender, and time between blood draw and start of centrifugation on the size outcomes of platelet-rich fibrin (PRF) membranes (Miron et al. 2018)

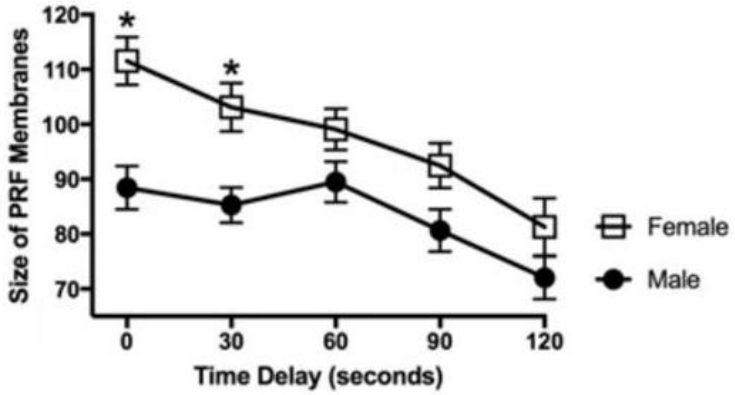
Effect of Time on PRF membranes

Blood draws should be within 90 seconds

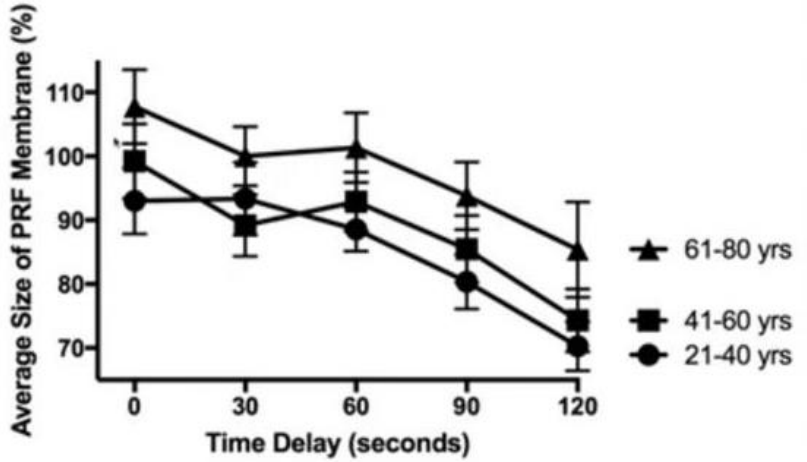


Bigger Membranes? Male or Females?

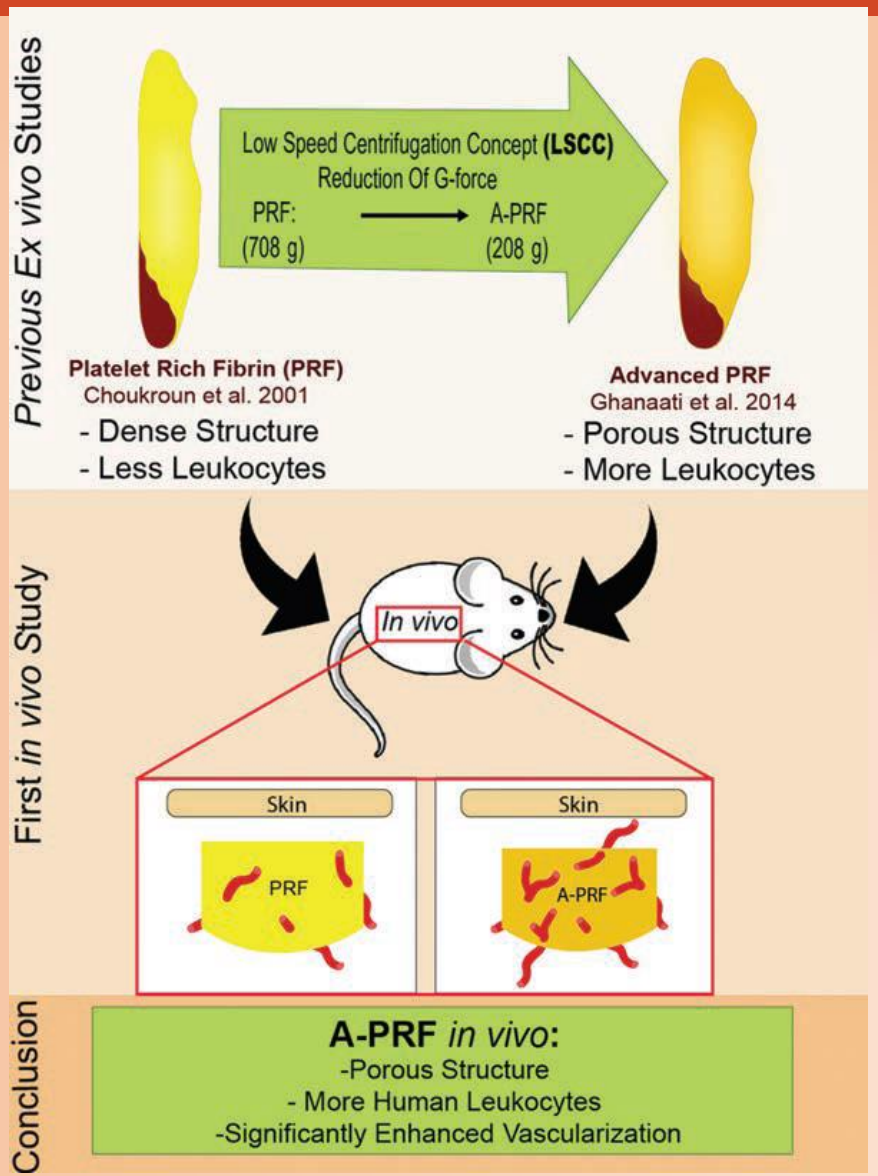
The normal hematocrit for men is 40 to 54%; for women it is 36 to 48%.



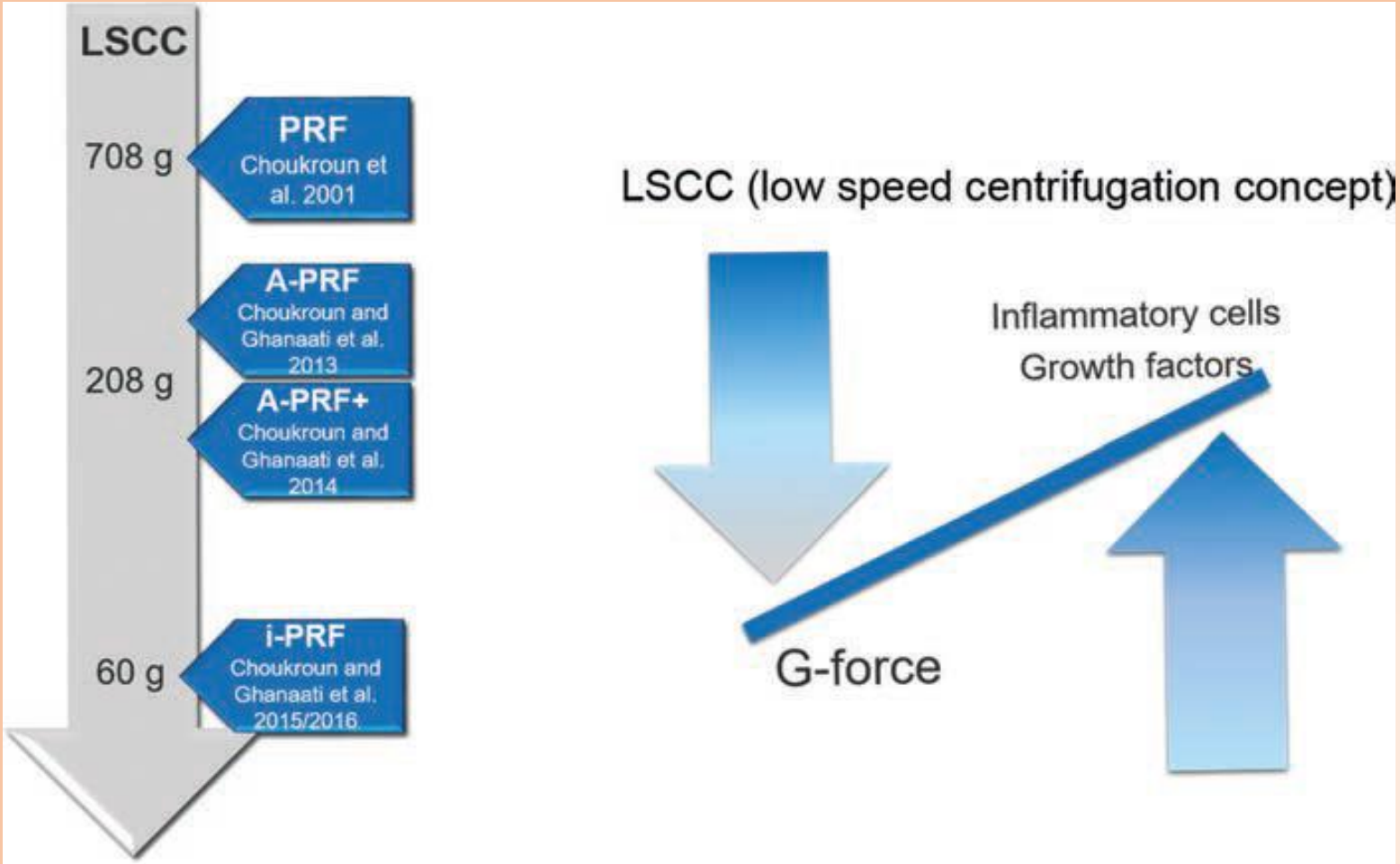
Bigger Membranes? Younger or Older?



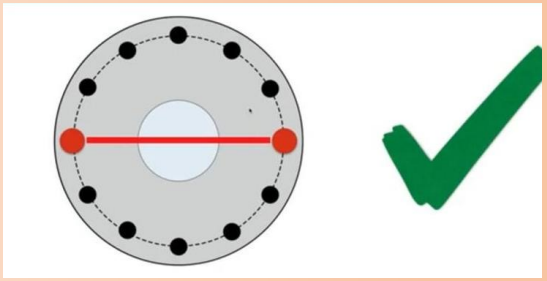
Comparative illustration of PRF and A-PRF. In vivo results highlight the enhanced regeneration capacity of A-PRF based on the LSCC.

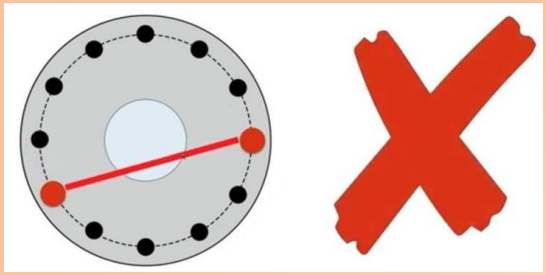


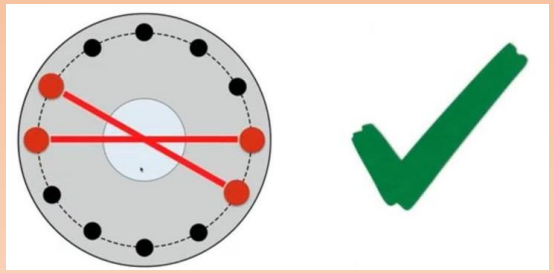
Development of solid and injectable PRFs following the low-speed centrifugation concept (LSCC).

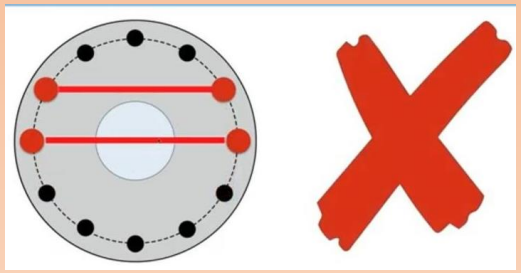


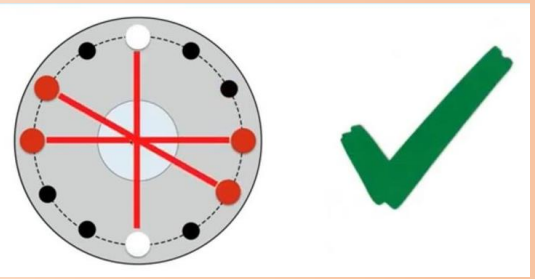
Placement of tubes in centrifuge

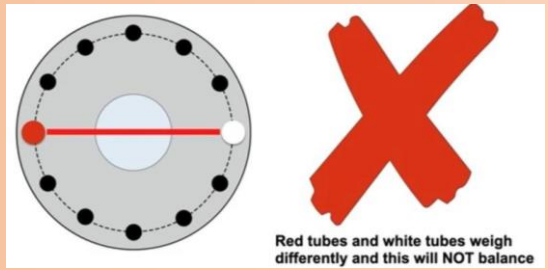


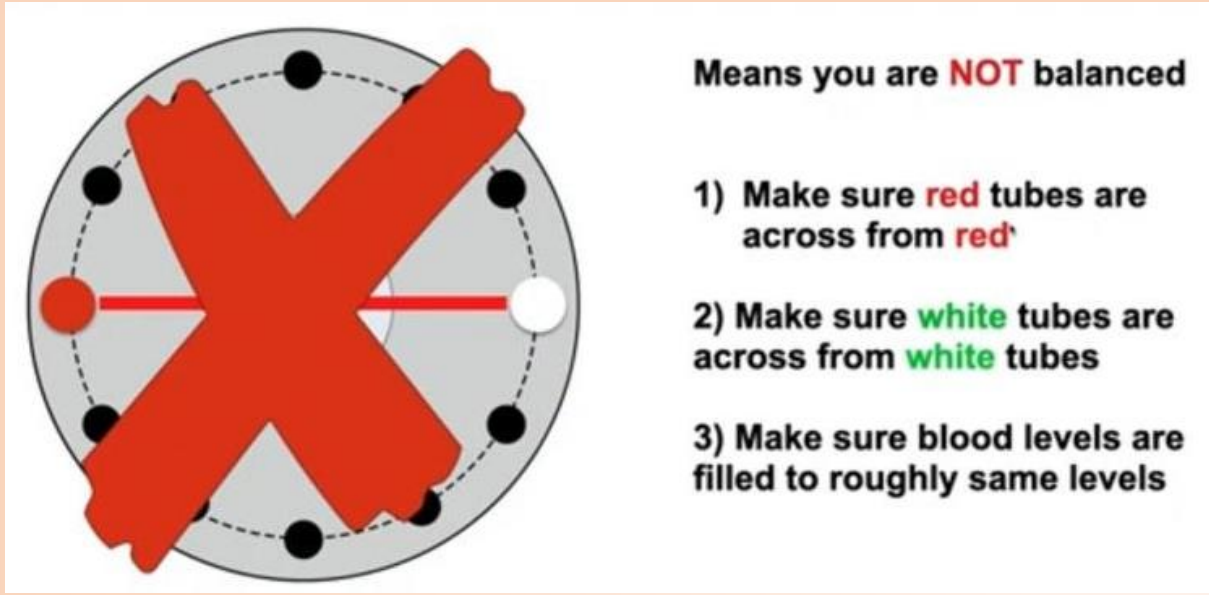






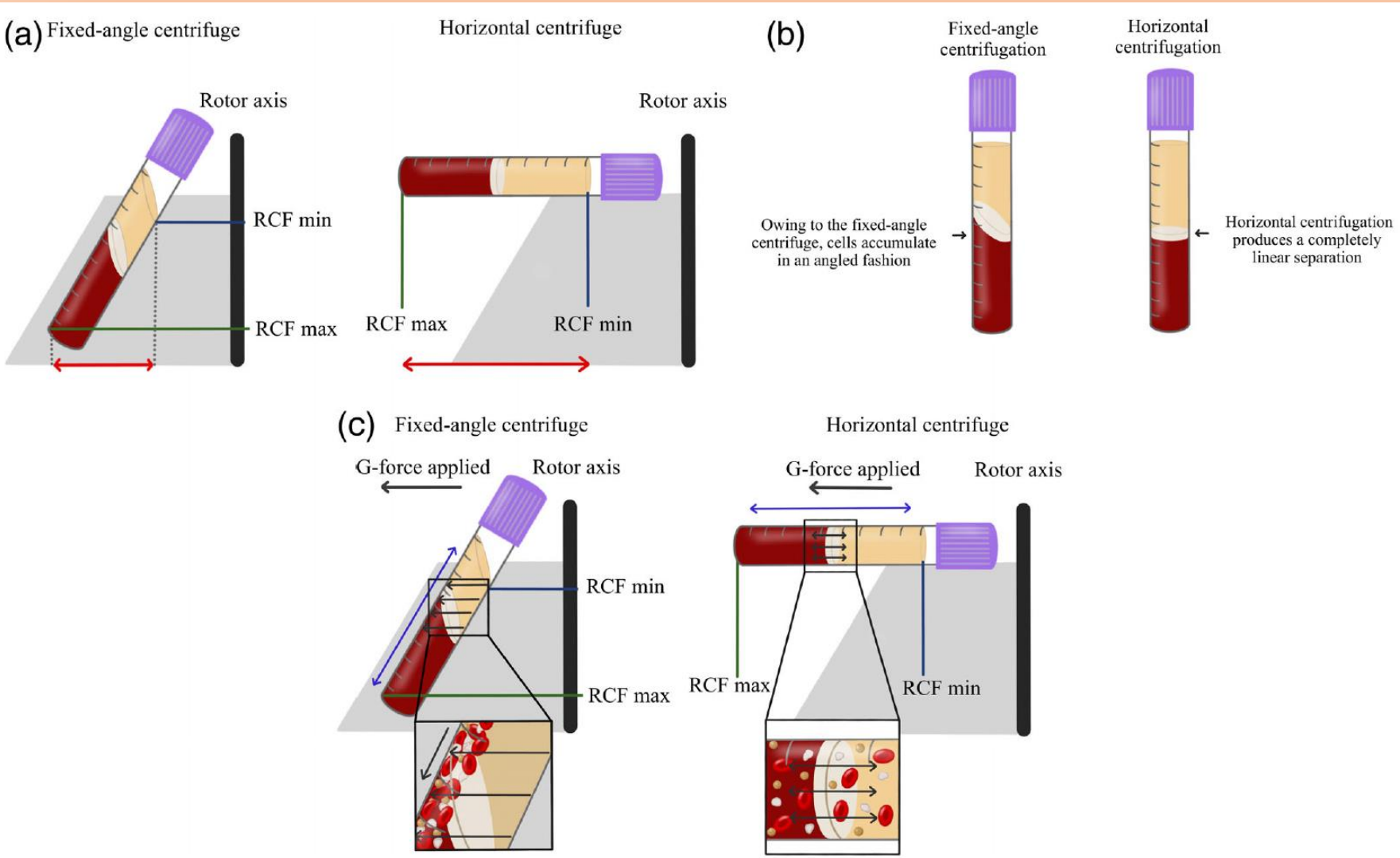






Overview table comparing the three blood products about their most important characteristics

Blood products	PRF (2004)	PRGF (2001)	PRP (1998)
Protocol	Easy	Complex	Very complex
Speed-rate	Fast	Very Slow	Slow
Reproducibility	No Bias	Possible Bias	Possible Bias
Use of anticoagulants	No	Yes	Yes
Amount obtainable	Good	Poor	Enough
Costs of the protocol	Low	High	Moderate
Amount of fibrin obtainable	High	Low	Low
Speed of fibrin formation	Physiological	High	High
Fibrin morphology	Trimolecular	Tetramolecular	Tetramolecular
Leukocytes amount	65%	0%	0-50%
Immunomodulatory properties	Yes	No	Poor
Neo-angiogenic potential	+++++	++	+
Osteoconductive potential (scaffolding)	High	Poor	Poor
Mechanical properties (sol-gel-membrane)	Good	Poor	Enough
Presence of MSCs	Yes	Yes	Yes


(a) Fixed-angle centrifuge
Rotor axis
RCF min
RCF max
Horizontal centrifuge
Rotor axis
RCF max
RCF min
(b)
Fixed-angle centrifugation
Owing to the fixed-angle centrifuge, cells accumulate in an angled fashion
Horizontal centrifugation
Horizontal centrifugation produces a completely linear separation
(c) Fixed-angle centrifuge
G-force applied
Rotor axis
RCF min
RCF max
Horizontal centrifuge
G-force applied
Rotor axis
RCF max
RCF min

Group	Protocol	Total volume of plasma (mL)	[leukocytes] 10^9/L	%control - [leukocytes] (6.125 × 10^9/L)	[platelets] 10^9/L	%control - [platelets] (257 × 10^9/L)	[monocytes] 10^9/L	%control - [monocytes] (0.28 × 10^9/L)
Solid-PRF (IntraSpin)	2700 rpm × 12 min (~700g)	5	4.13	67.4	415	161.5	0.34	121.4
Solid-PRF (process)	1300 rpm × 8 min (~200g)	4.5	2.07	33.8	505	196.5	0.14	50.0
Solid-PRF (horizontal)	700g × 8	4.2	7.78	127.0	610	237.4	0.58	207.1
Liquid-PRF (IntraSpin)	2700 rpm × 3 min (~700g)	3.7	3.33	54.4	546	212.5	0.29	103.6
Liquid-PRF (process)	800 rpm × 3 min (~60g)	1.5	7.53	122.9	531	206.6	0.68	242.9
Liquid-PRF (horizontal)	200g × 8	2	10.92	178.3	641	249.4	0.96	342.9

Abbreviation: PRF, platelet rich fibrin.

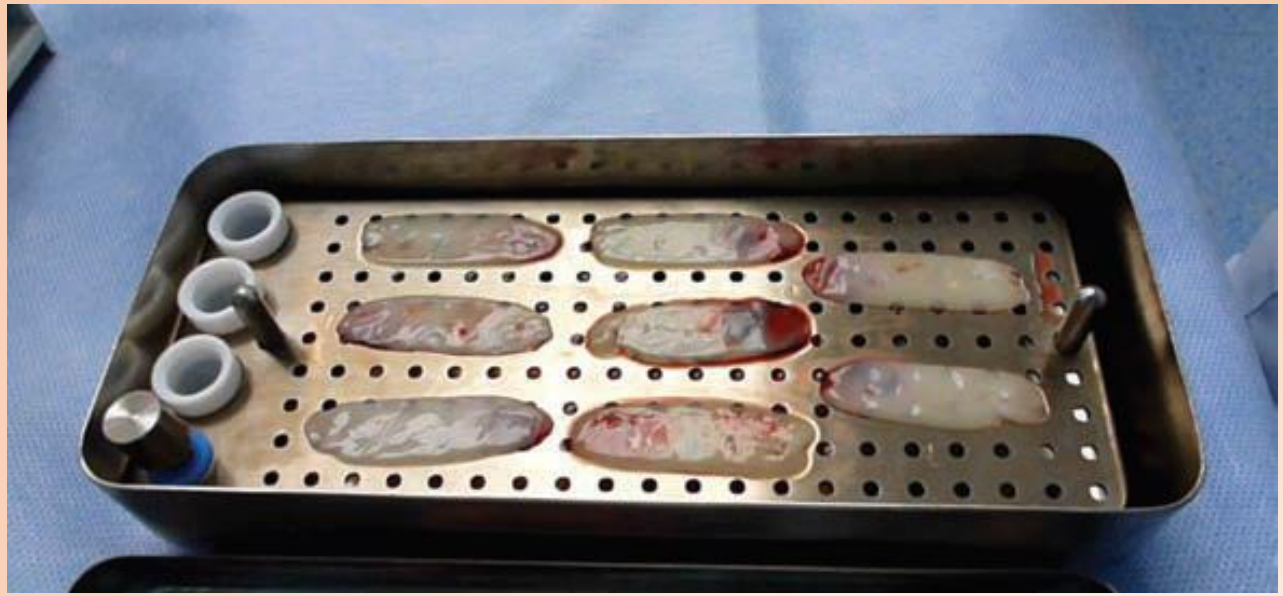
TABLE 2 Total volume of plasma (mL), leukocyte number, percentage of leukocytes number when compared to whole blood, platelet number, percentage of platelet numbers, monocyte number, and percentage of total monocyte numbers

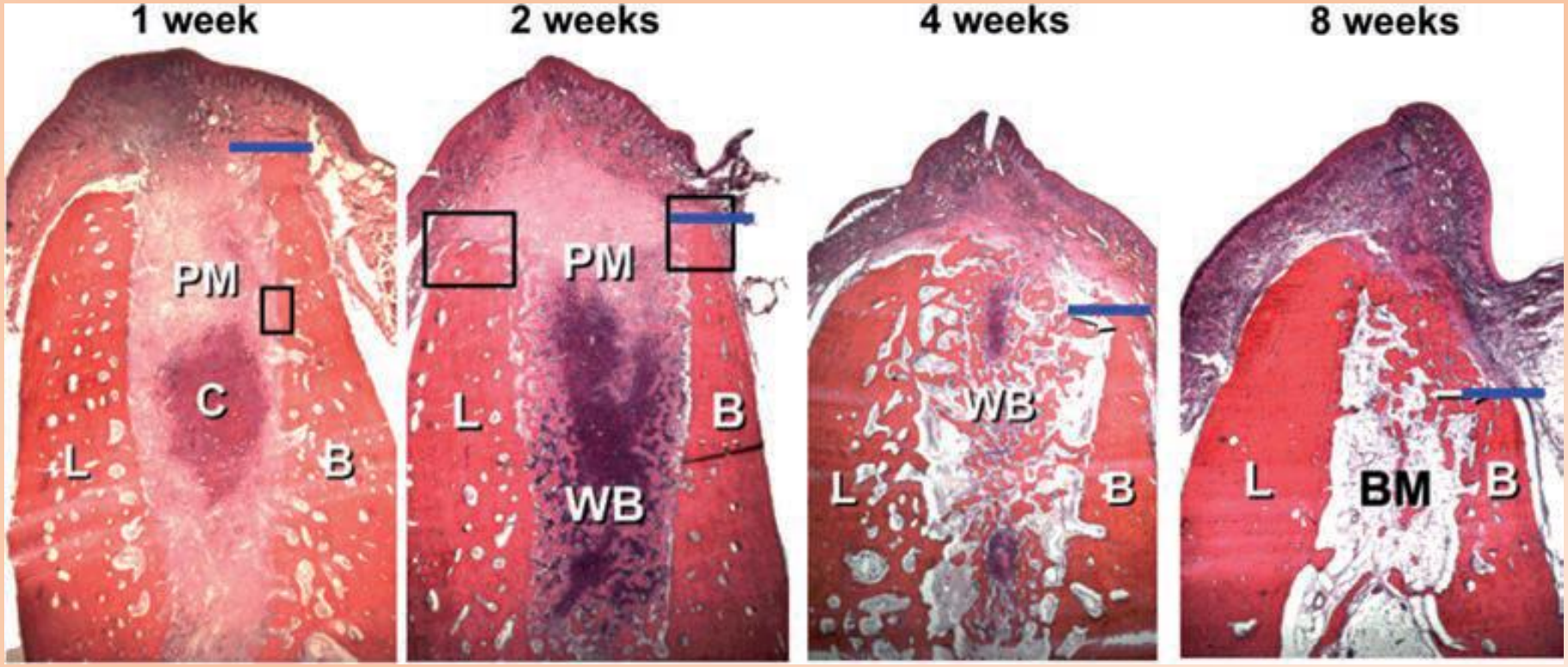
Group	Protocol	Total volume of plasma (mL)	Total number of leukocytes (10^6)	%Total leukocytes (61.25 × 10^6)	Total number of platelets (10^6)	%Total platelets (2570 × 10^6)	Total number of monocytes (10^6)	%Total monocytes (2.8 × 10^6)
Solid-PRF (IntraSpin)	2700 rpm × 12 min (~700g)	5	20.7	33.7	2075	80.7	1.7	60.7
Solid-PRF (process)	1300 rpm × 8 min (~200g)	4.5	9.3	15.2	2,272.5	88.4	0.63	22.5
Solid-PRF (horizontal)	700g × 8	4.2	32.7	53.3	2,562	99.7	2.436	87.0

Uses of Platelet Rich Fibrin in Regenerative Dentistry: An Overview

PRF as a barrier membrane in GBR procedures

Platelet rich fibrin (PRF) scaffolds that have been flattened to be later utilized as barrier membranes during GBR procedures.





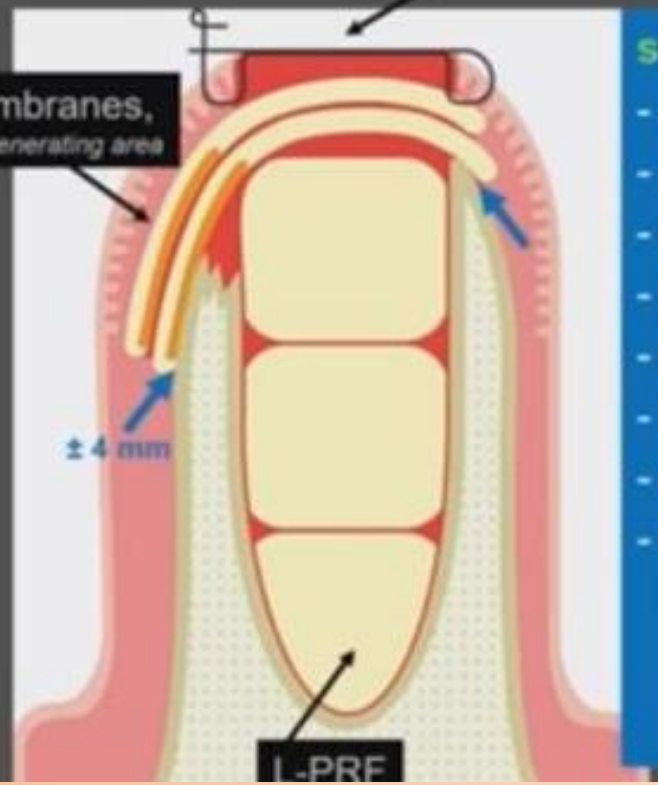
It was found that within 8 weeks post-extraction, a marked loss of bone was observed, most notably on the thin buccal surface

ridge preservation: L-PRF alone as a scaffold

clot compressed in cylinder

healing by secondary intention,
wound closure not necessary!!!!

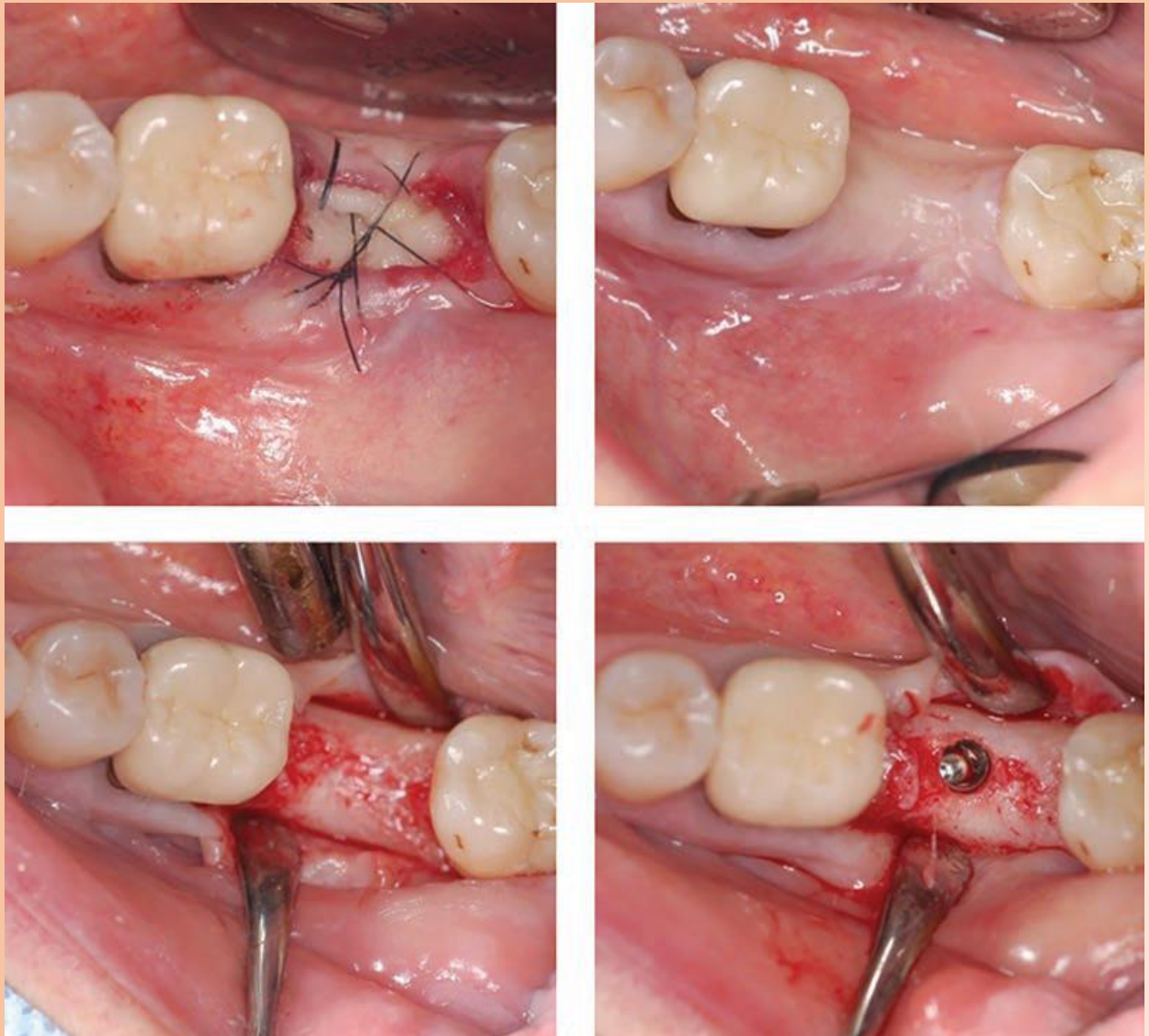
L-PRF membranes,
face towards regenerating area



step by step:

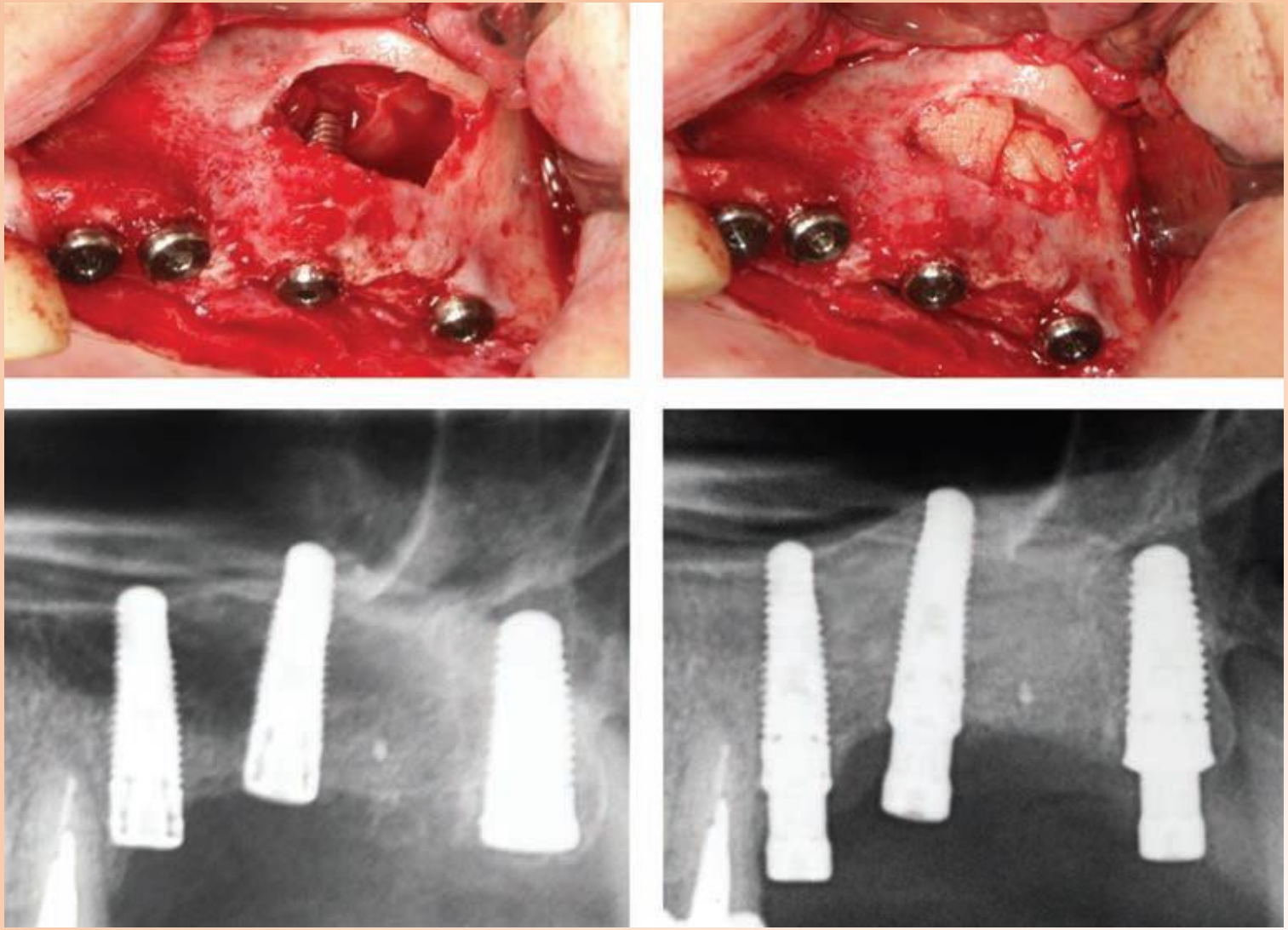
- a-traumatic extraction *(root separation)*,
- remove pocket epithelium,
- remove granulation tissue *(ball bur)*,
- prepare envelope *(bone ↔ periosteum)*,
- rinse socket with L-PRF exudate *(disinfection)*,
- insert ≥ 3-4 L-PRF *membranes or plugs* + condense,
- seal socket with 2 L-PRF *membranes (in envelope)*,
- modified horizontal matress suture

(complete closure not needed)

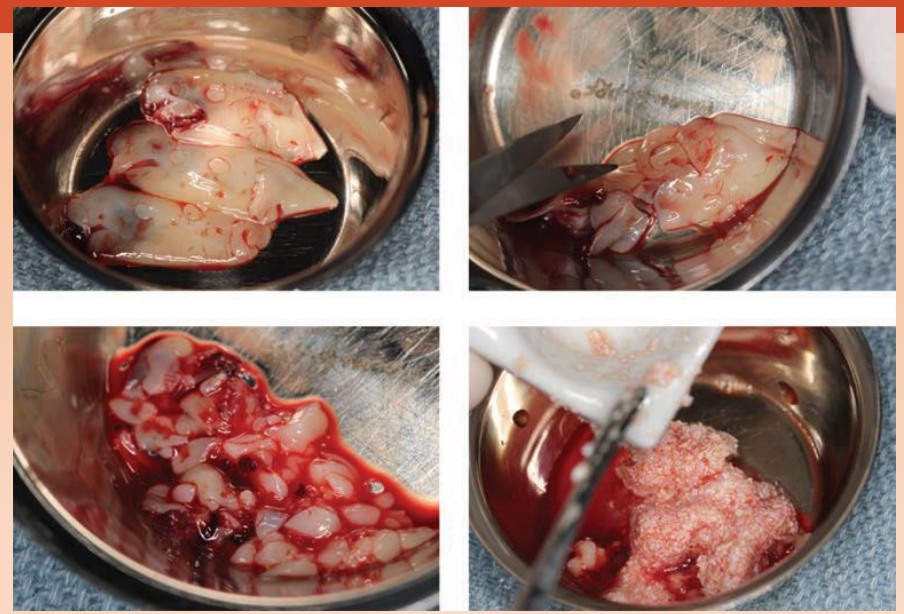


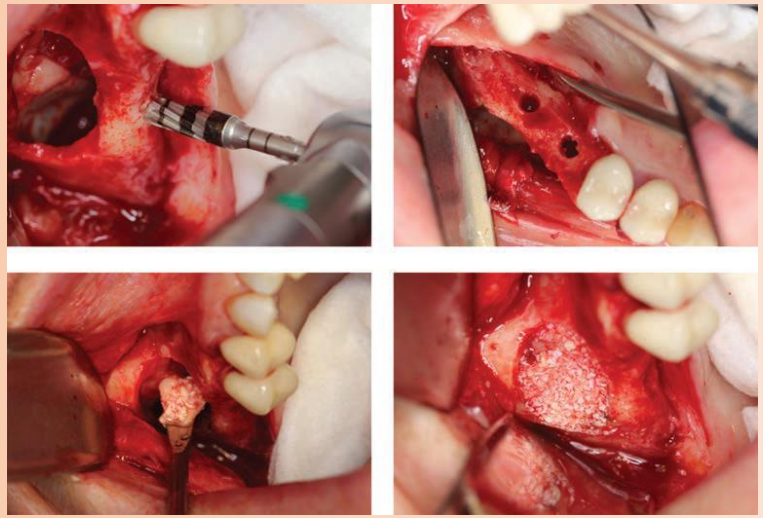
Maxillary Sinus Floor Elevation Procedures with Platelet Rich Fibrin:

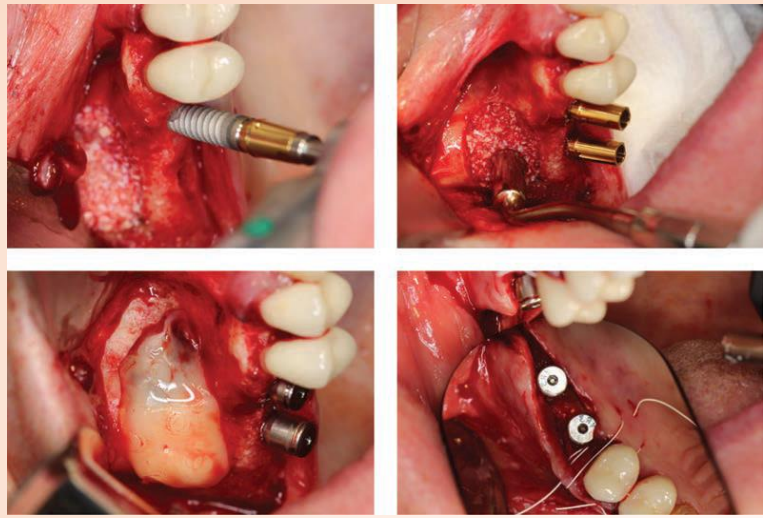
PRF as a sole grafting material during sinus lifting procedures



Three PRF membranes cut and mixed with a bone-grafting material. Implant bed preparation and filling of the sinus cavity with a bone grafting material combined with PRF. Implant placement followed by coverage of the lateral window with PRF membranes.

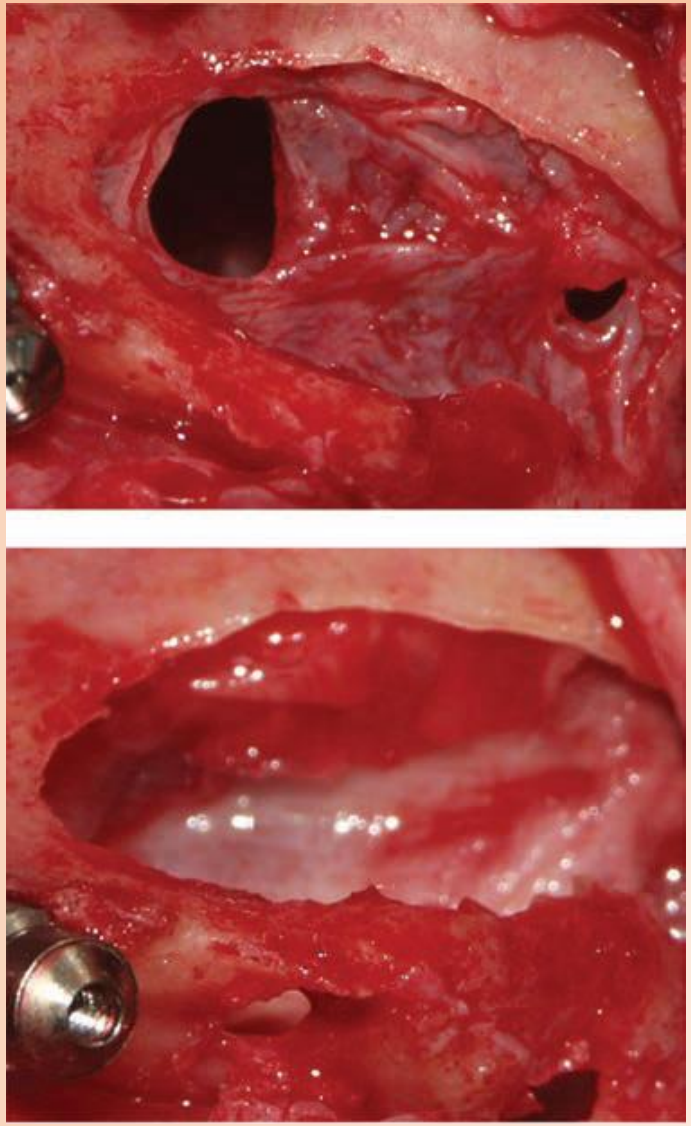






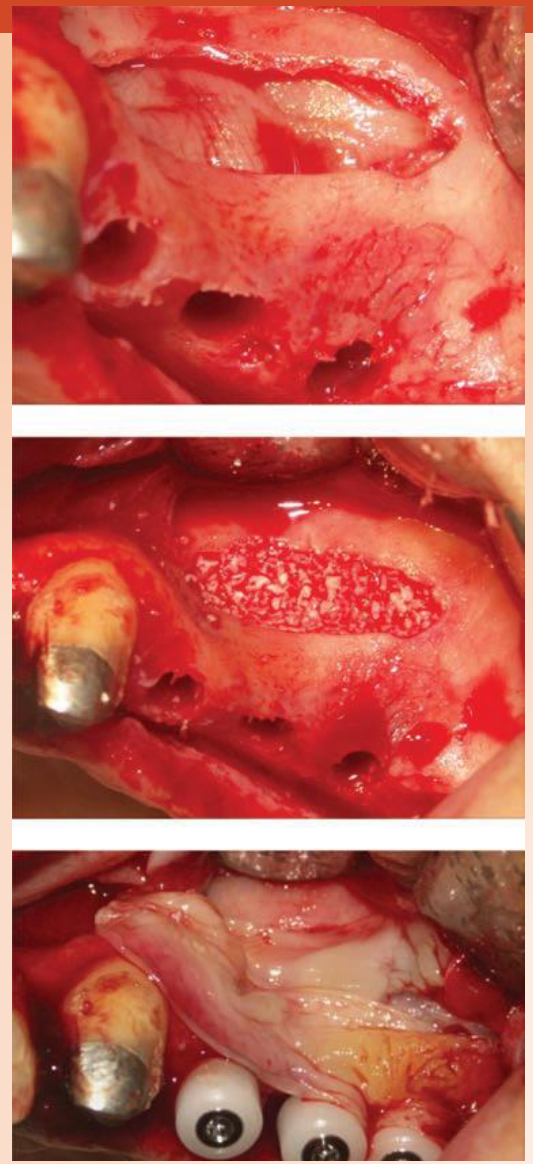
PRF for the repair of Schneiderian membranes

Large Schneiderian membrane perforation covered with a double layer of PRF membranes.



PRF for the closure of the lateral maxillary access window

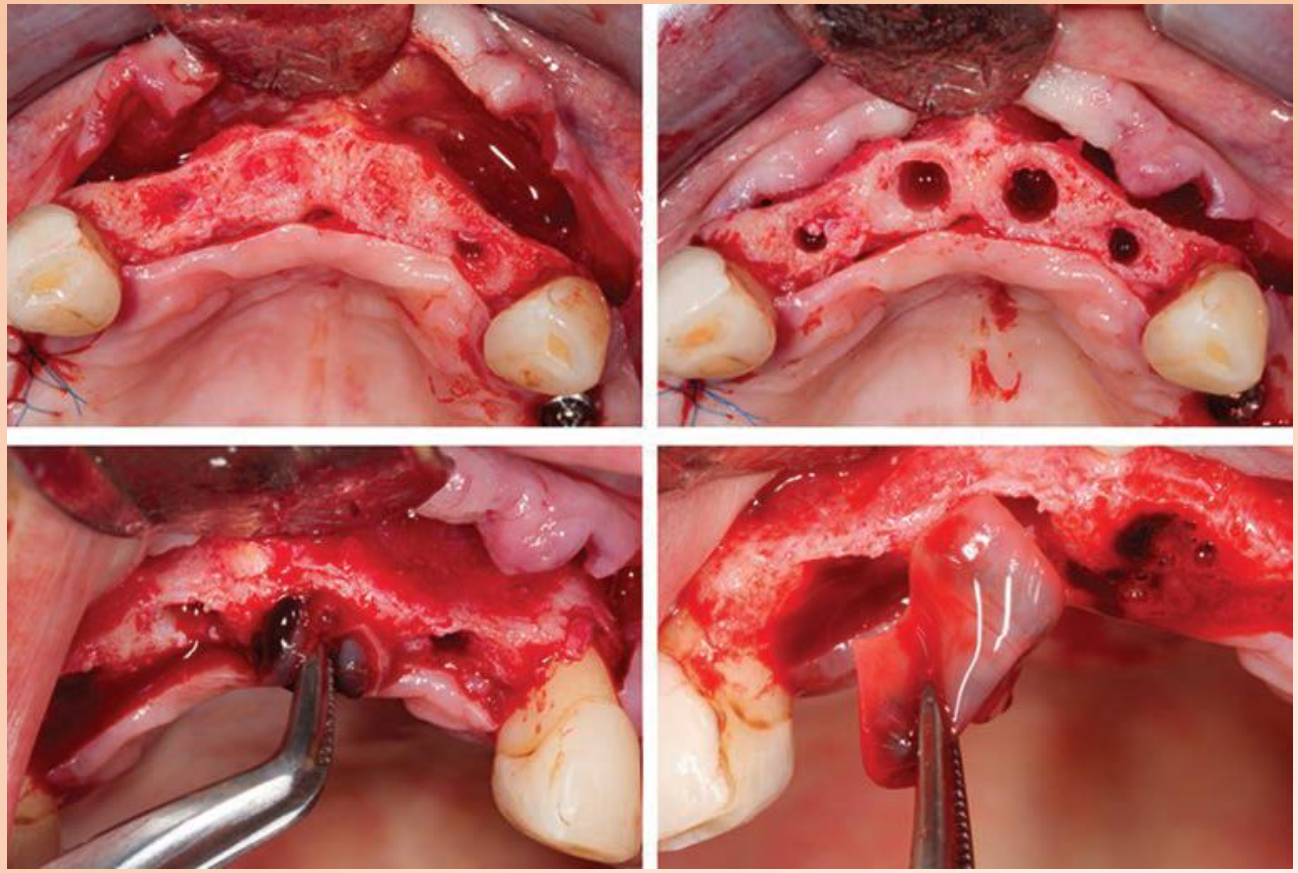
Lateral window sinus augmentation procedure performed with two PRF membranes utilized to protect the Schneiderian membrane, followed by allograft placement and lateral window closure with PRF.



PRF and buccal gap management

The use of PRF at osteotomy preparation

Implant bed preparation and insertion of PRF membranes into defect sites.



PRF in combination with bone-grafting materials

Two main strategies exist, whereby bone grafting materials may either be combined with:

1) osteogenic cells or mesenchymal stem cells

2) bioactive growth factors

Two methods to combine PRF with GBR procedures.

1) The first acts as a barrier membrane

2) The second aim is to supply bone-grafting particles with PRF by cutting PRF membranes into small “fragments” and thereafter mixing them with bone-grafting materials.

Example of two PRF membranes that are cut with surgical scissors and thereafter mixed with particulate bone grafts. Notice the sticky consistency and the better handling properties of bone grafts following this combination approach.


BIO
PRF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