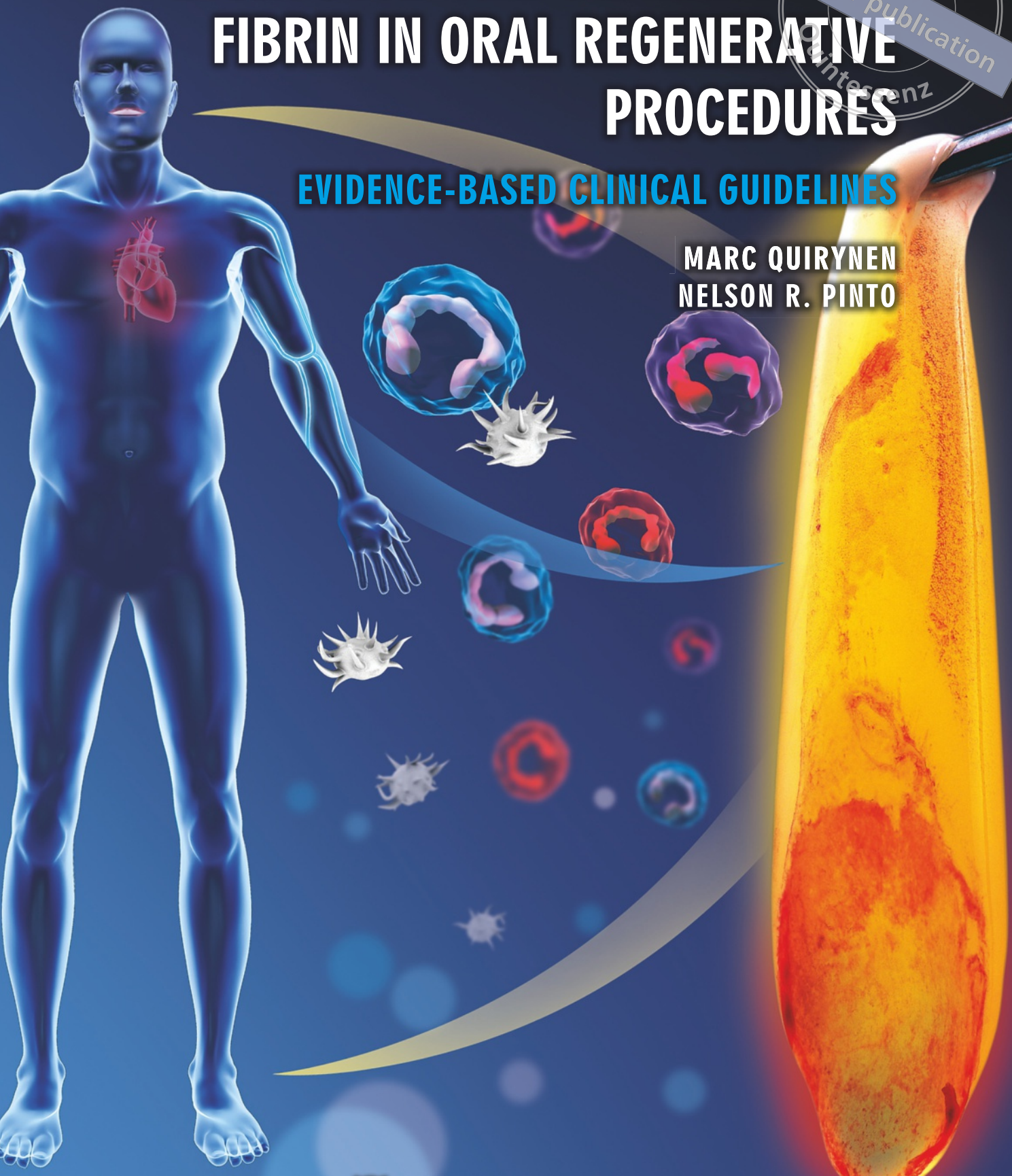
LEUKOCYTE- AND PLATELET-RICH FIBRIN IN ORAL REGENERATIVE PROCEDURES

EVIDENCE-BASED CLINICAL GUIDELINES

MARC QUIRYNEN
NELSON R. PINTO



QUINTESSENCE PUBLISHING

Leukocyte- and Platelet-Rich Fibrin in Oral Regenerative Procedures

Evidence-Based Clinical Guidelines

Edited by

Marc Quirynen, DDS, PhD

Periodontologist, Professor & Chairman
Periodontology Section
Department of Oral Health Sciences
Catholic University Leuven & University
Hospital Leuven
Leuven, Belgium

Nelson R. Pinto, DDS

Professor & Implantologist
Faculty of Dentistry
Graduate School of Periodontics and
Implant Dentistry
Universidad de los Andes
Santiago, Chile

In collaboration with:

Andy Temmerman, DDS, PhD
Ana Castro, DDS
Simone Cortellini, DDS
Catherine Andrade, DDS
Manoetjer Siawasch, DDS
Wim Teughels, DDS, PhD

QUINTESSENCE PUBLISHING

Berlin | Chicago | Tokyo
Barcelona | London | Milan | Mexico City | Paris | Prague | Seoul | Warsaw
Beijing | Istanbul | Sao Paulo | Zagreb

One book, one tree: In support of reforestation worldwide and to address the climate crisis, for every book sold Quintessence Publishing will plant a tree (https://onetreeplanted.org/).

A CIP record for this book is available from the British Library.
ISBN: 978-1-78698-105-9

QUINTESSENCE PUBLISHING DEUTSCHLAND

Quintessenz Verlags-GmbH
Ifenpfad 2–4
12107 Berlin
Germany
www.quintessence-publishing.com

Quintessence Publishing Co Ltd
Grafton Road, New Malden
Surrey KT3 3AB
United Kingdom
www.quintessence-publishing.com

Editing: Quintessence Publishing Co, Inc, Batavia, IL, USA
Layout, Production and Reproduction: Quintessenz Verlags-GmbH, Berlin, Germany

Printed and bound in Croatia by Grafički zavod Hrvatske d.o.o.

Contents

Authors

Catherine Andrade, DDS
Periodontologist
Department of Periodontology and Oral Implantology
Faculty of Dentistry
Universidad de los Andes
Santiago, Chile

Ana Castro, DDS
Periodontologist
Periodontology Section
Department of Oral Health Sciences
Catholic University Leuven & University Hospital Leuven
Leuven, Belgium

Simone Cortellini, DDS
Periodontologist
Periodontology Section
Department of Oral Health Sciences
Catholic University Leuven & University Hospital Leuven
Leuven, Belgium

Nelson R. Pinto, DDS
Periodontologist & Professor
Faculty of Dentistry
Graduate School of Periodontics and Implant Dentistry
Universidad de los Andes
Santiago, Chile

Chairman
Research Center for Regenerative Medicine and Tissue Engineering
Concepción, Chile

Marc Quirynen, DDS, PhD
Periodontologist, Professor & Chairman
Periodontology Section
Department of Oral Health Sciences
Catholic University Leuven & University Hospital Leuven
Leuven, Belgium

Manoetjer Siawasch, DDS
Periodontologist
Periodontology Section
Department of Oral Health Sciences
Catholic University Leuven & University Hospital Leuven
Leuven, Belgium

Andy Temmerman, DDS, PhD
Professor & Periodontologist
Periodontology Section
Department of Oral Health Sciences
Catholic University Leuven & University Hospital Leuven
Leuven, Belgium

Wim Teughels, DDS, PhD
Professor & Periodontologist
Periodontology Section
Department of Oral Health Sciences
Catholic University Leuven & University Hospital Leuven
Leuven, Belgium

Augmented Reality in this book

This book features augmented reality videos accessible free of charge from your smartphone or tablet. Easy, reliable, fast, and offline, discover the videos selected for you by the authors to enrich your reading experience.

Download

the “L-PRF & Tissue Regeneration“ application on the App Store (iOS).

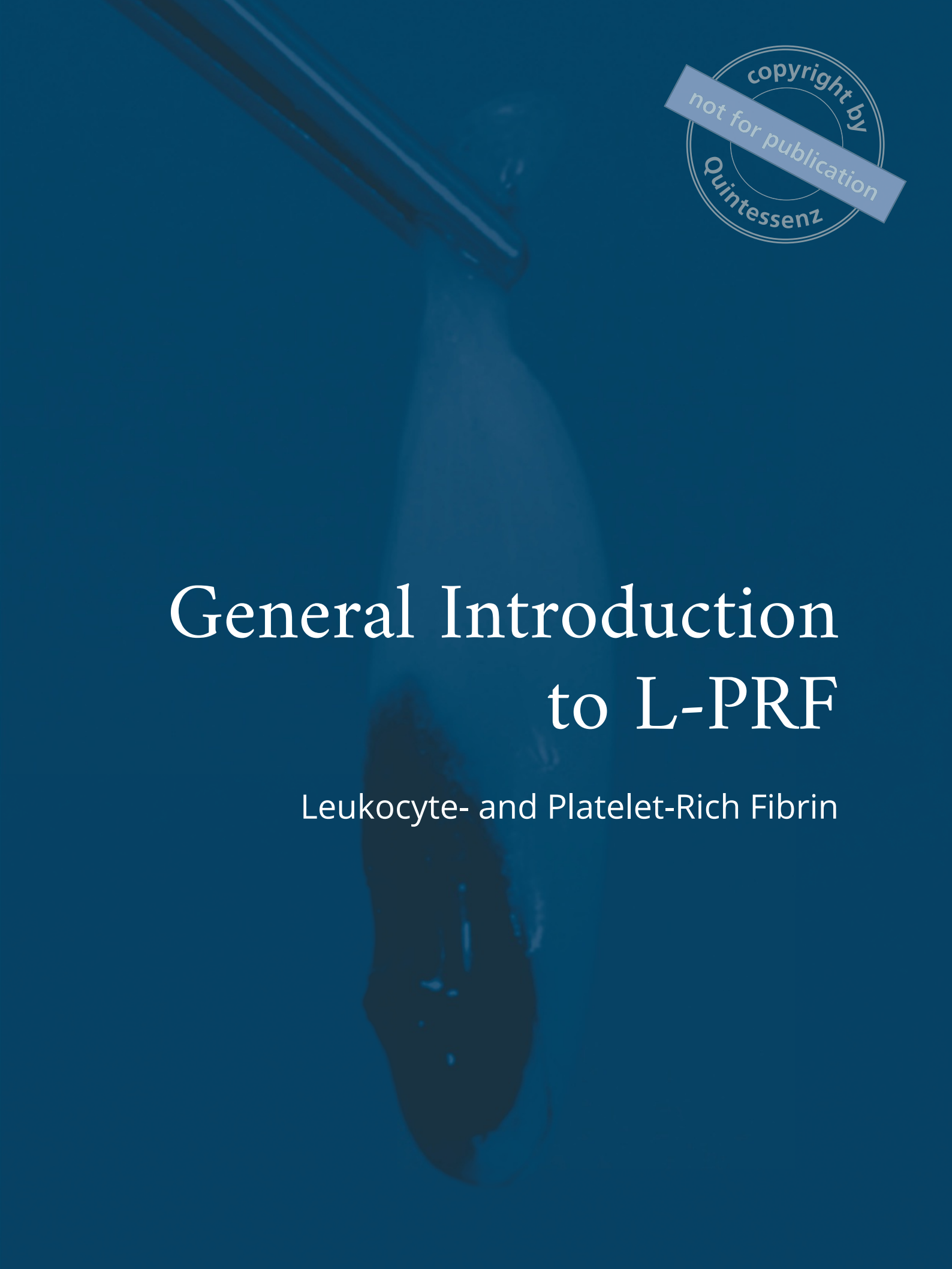
Locate

images in video boxes.

Fly over

these images with your smartphone or tablet to play the videos.

The videos are also available by the QR code or at
https://video.qvnet.de/b23631/



General Introduction to L-PRF

Leukocyte- and Platelet-Rich Fibrin

What Is L-PRF?

Wound healing remains an extremely important topic in dentistry and other medical fields for healthy and, even more so for, medically compromised patients. Countless efforts have been made to find "bioactive" additives that promote and accelerate wound healing, regulate inflammation, and improve regeneration. Platelet concentrates (PC), prepared from patients' own blood, have achieved these goals by facilitating recruitment, proliferation, and maturation of cells participating in tissue healing and regeneration (Boswell et al[1]). Their use, during surgery or as an infiltration, is nowadays common in most fields of medicine, particularly in oral and maxillofacial surgery, but also orthopedics and sports medicine. The protocol for the preparation of leukocyte- and platelet-rich fibrin (L-PRF) as well as the protocols for each of its clinical applications have been improved significantly over the past 20 years, with evidence-based guidelines available for most procedures.

An L-PRF clot or membrane (Fig 1) can be defined as a solid fibrin or blood clot obtained via a "natural" coagulation process without any additives but rather via mechanical manipulation of the patient's own blood (ie, centrifugation). It consists of a polymerized fibrin network that comprises a specific population of cells (primarily leukocytes) and platelets. It deserves to be considered an *optimized blood clot* or a *human living tissue graft* because of:

- Its physical characteristics: The 3D fibrin network makes the membranes strong enough to be sutured and fixated (eg, with pins)
- The presence of cells and mediators trapped within the 3D fibrin mesh
- The long-term release of growth factors that favor tissue repair/regeneration
- Its antibacterial (bacteriostatic) capacity
- Its 100% autogenous nature

Because the cells in L-PRF must be activated but should not be damaged during the preparation, some specific guidelines have to be strictly followed. This includes: the correct centrifugation speed and duration, the correct blood tubes (glass tubes or silica-coated plastic tubes in order to promote natural coagulation), ideal timing, and optimal centrifuge (eg, device and stability).

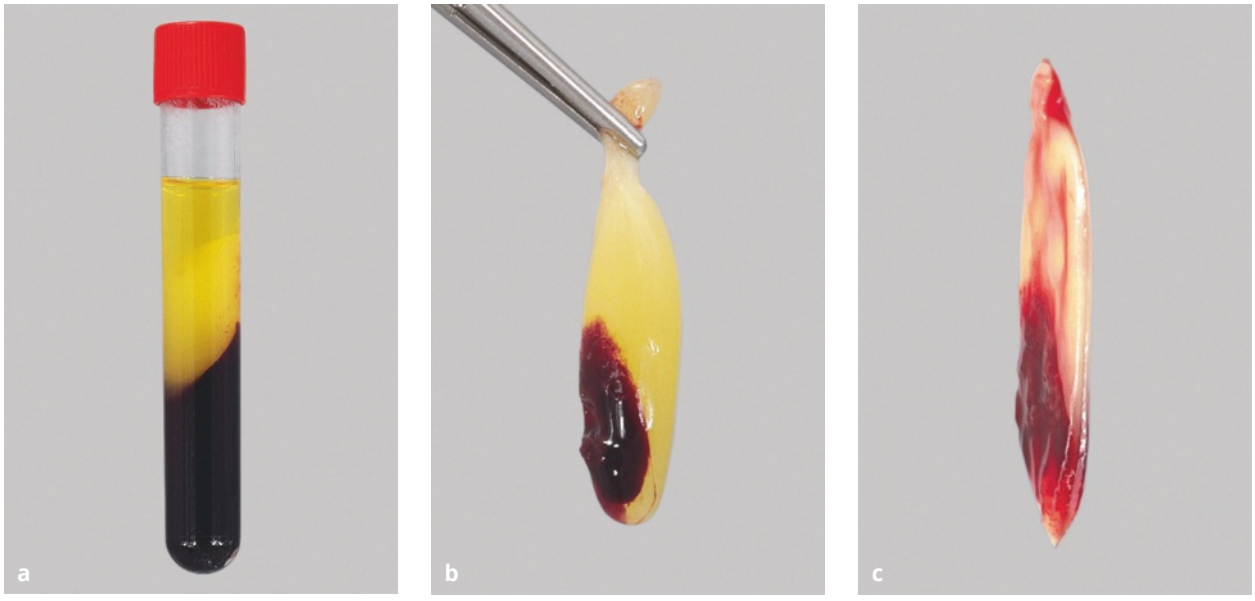


Fig 0-1 Three stages in the preparation of L-PRF. **(a)** The blood tube after centrifugation, with separation between red blood cells (at the bottom), the L-PRF clot (in the middle) and an acellular plasma (at the top). **(b)** The L-PRF clot collected with tweezers. **(c)** The L-PRF membrane obtained after gentle compression of the clot.

Evolution of PCs

Four variants of PCs have been introduced: two platelet-rich plasma (PRP) types, representing liquid platelet suspensions that can be transformed into a light fibrin gel after activation, and two platelet-rich fibrin (PRF) types, which represent solid platelet and fibrin clots (due to a strongly polymerized fibrin network). Based on the leukocyte content and fibrin structure, PCs can be classified into four main categories (Dohan Ehrenfest et al[2,3]):

- Pure platelet-rich plasma (P-PRP), without leukocytes and with a low-density fibrin network
- Leukocyte- and platelet-rich plasma (L-PRP), with leukocytes (the amount depends on the protocol) and a low-density fibrin network
- Pure platelet-rich fibrin (P-PRF), without leukocytes and with a high-density fibrin network
- Leukocyte- and platelet-rich fibrin (L-PRF), with leukocytes (the amount depends on the protocol) and a high-density fibrin network

The acronym PRF by itself should preferably not be used as it refers to a wide range of PRF-type products (including P-PRF) with variation in cell composition, growth factor release patterns, and biologic activities.

As shown in the following section, the current PCs are the result of a long period of development aimed at obtaining stronger and 100% autogenous bioactive compounds that can be prepared chairside via a simple protocol.

Important notice

In this book, the acronym L-PRF will be used for all L-PRF-like centrifugation protocols (including L-PRF, A-PRF, A-PRF+, etc), except when they are compared to each other. When clinical studies are analyzed in detail (eg, in tables), the acronym used in the paper will be maintained.

PRP: The first generation of PCs

The history of PCs starts with Matras's reports[4–6] in the 1970s on the beneficial impact of fibrin gels on the healing of skin wounds. In the following years (1975 to 1979), new forms of platelet-fibrin gel (*platelet-fibrinogen-thrombin mixture* or *platelet gelatine*) were introduced, showing some benefits in ophthalmology, general surgery, and neurosurgery. In the mid to late 1980s, Knighton and co-workers[7,8] developed a similar concept (termed *platelet-derived wound healing factors*), again to improve the healing of skin ulcers.

In the late 1990s, Whitman and co-workers[9] and Marx and co-workers[10] were the first to promote the use of PRPs in oral and maxillofacial surgery. These liquid platelet suspensions were obtained via a complex procedure including the use of additives. Patient's blood is treated first with anticoagulants and in a later stage with coagulation activators in two centrifugation steps. The main goal of PRP was to isolate the highest quantity of platelets and growth factors. Despite several modifications of the protocol over the years, to date a standardized protocol for its preparation and application is still lacking.

PRPs contain more than 95% of the platelets in the initial blood sample and have a direct impact on osteoblasts, connective tissue cells, periodontal ligament cells, and epithelial cells (Marx[11]). Unfortunately, PRPs have several limitations that prevented its intensive use, including:

- Complexity of preparation with significant interoperator variations
- Need for anticoagulant factors to prevent clotting at the beginning of the process
- Need for coagulation activators (primarily bovine thrombin and calcium chloride) afterward, both known inhibitors of wound healing
- Lengthy preparation time (30 to 60 minutes or more)
- Its liquid nature, requiring a combination with other often synthetic biomaterials
- Its fast "burst" release of growth factors (Kobayashi et al[12])
- High cost

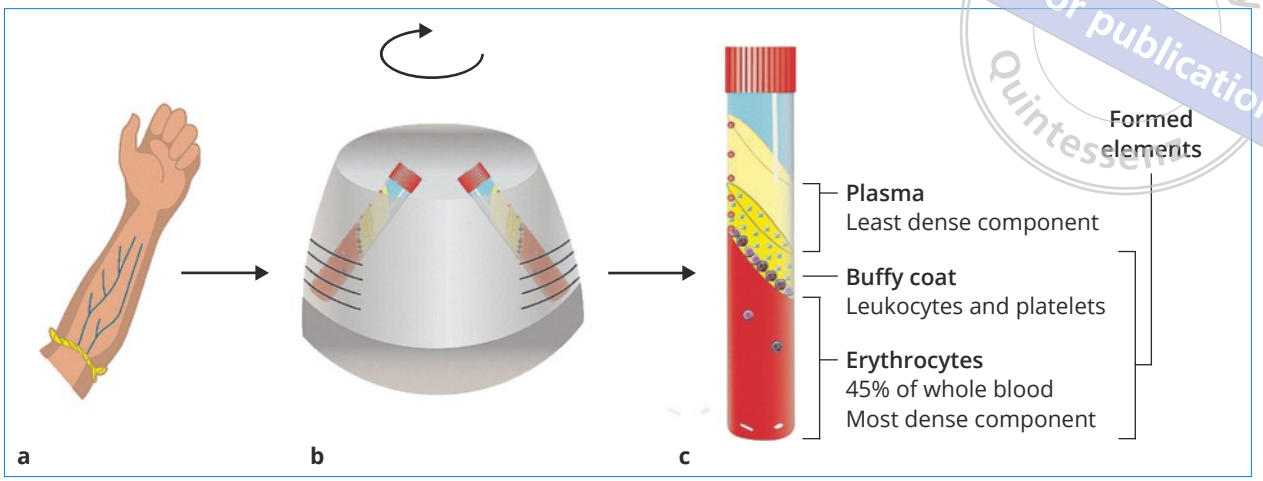


Fig 0-2 Full procedure to prepare L-PRF: **(a)** venipuncture, **(b)** one centrifugation cycle, **(c)** blood separation. By using a specific centrifugation protocol and special tubes (either glass tubes or plastic tubes with a silica coating) without additives, an optimal separation is obtained with the formation of a clot in the middle of the tube (surrounded by plasma and touching the red blood cells at the bottom).

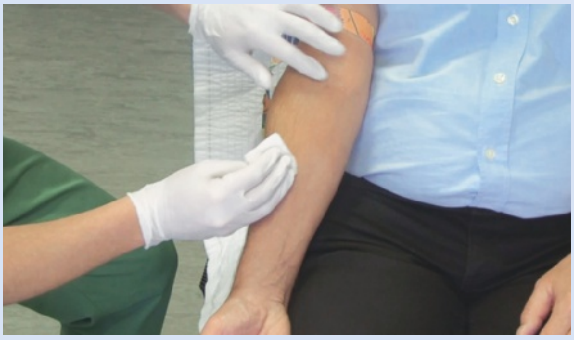
A well-known example of PRP is plasma rich in growth factors (PRGF), which was first introduced by Anitua and co-workers.[13,14] Similar to PRP, this PC requires external additives for processing and has several limitations, including its liquid form and complex preparation.

Today there is still a clear shortage in scientific data on the beneficial effects of PRPs (Roselló-Camps et al,[15] Pocaterra et al,[16] Stähli et al,[17] Franchini et al,[18] Donos et al[19]), primarily due to a great variability in study designs (small groups of patients, no control groups), but also in preparation protocols. Moreover, the need for animal thrombin as a coagulant (or other additives) raises legal issues in some countries. A recent systematic review, presented during the Fifth European Association for Osseointegration (EAO) Consensus Conference, reported a lack of consistent evidence supporting the clinical benefit of PRP in healthy patients and suggested that PRP "might" have a positive effect on wound healing and bone regeneration in compromised patients (Stähli et al[17]).

L-PRF: The second generation of PCs

In 2001, Choukroun introduced a new blood concentrate concept, originally called *PRF,* but since 2009 it has been referred to as *L-PRF*. He aimed for a simplified preparation (fewer steps, faster execution) and a product without the abovementioned disadvantages of PRPs (Choukroun et al,[20–22] Dohan et al[23–25]). With this new concept, the need for anticoagulants was excluded, so that the end product remains 100% autogenous. Moreover, the elimination of anticoagulants allowed the maintenance of physiologic cell functions without inhibition after centrifugation. As such, the approach became more suitable for clinical use. In 2003, the first clinical experience with L-PRF was published in French in a French journal (Gaultier et al,[26] Dohan et al[27,28]).

For this technique, peripheral blood is collected from the patient in specific tubes and immediately processed by one-step centrifugation (Fig 2). The contact of blood with the silica of the silica-coated plastic tube or with the glass of the glass tube activates the coagulation cascade, forming a strong fibrin clot during the centrifugation process. After centrifugation, the blood is separated into: a red cell fraction (bottom), plasma (top), and the L-PRF clot (in the middle), which can be removed from the tube with tweezers. After gentle removal of the remaining red blood cells from the clot, it can be compressed into a membrane. Both the clot and membrane consist of a dense 3D fibrin network enriched with platelets and a variety of leukocytes. (See Video 0-1.)



Video 0-1 Sequential steps in preparation L-PRF components:

Blood draw with butterfly needle, verify complete tube fill, rotate tubes to start spontaneous coagulation, place tubes in centrifuge within 1 minute (position tubes correctly for stability during centrifugation), centrifuge at 408 g (last tubes ≥ 12 minutes), retrieve L-PRF clots (carefully remove RBCs), compress clots into L-PRF plugs and membranes, collect L-PRF exudate.

L-PRF is thus a 100% autogenous biomaterial rich in fibrin, platelets (> 80% of what is present in the initial blood sample), white blood cells (> 75% of what is present in the initial blood sample), growth factors, cytokines, and other components conducive to tissue repair (Dohan Erhenfest et al,[29] Anitua et al,[30] Li et al,[31] Castro et al[32]). L-PRF releases interleukin-1, -4, and -6 and other growth factors, including transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), platelet-derived growth factor-AB (PDGF-AB), and bone morphogenetic protein (BMP)-1, -2, and -9 (Anitua et al,[30] Li et al,[31] Castro et al[32]). These components are effective in regulating the proliferation, differentiation, and apoptosis of repair-related cells and thus regulate and promote tissue healing and repair. Leukocytes are able to release a large number of immune regulation–related cytokines in the process of fibrinolysis, and this process is persistent and progressive (Anilkumar et al,[33] He et al[34]). As such, L-PRF promotes wound healing and accelerates regeneration.

Two systematic reviews by Castro and coworkers[35,36] confirmed the clinical significance of the benefits L-PRF offers in periodontal therapy (eg, pockets, soft tissue management, ridge preservation, sinus floor augmentation, osseointegration). A recent systematic review, presented during the Fifth EAO Consensus Conference (2018), reported moderate evidence supporting the clinical benefit of L-PRF on ridge preservation and in the early phase of osseointegration and suggested that more clinical support was necessary to comment on the role of L-PRF for other applications (Strauss et al[37]). In the meantime, several additional studies have been published resolving this lack of evidence, as will be discussed later.

It is also important to realize that the preparation of L-PRF for its clinical application is simple, inexpensive, 100% autogenous, and thus very safe. L-PRF is the result of a pure mechanical manipulation of the blood, creating an "optimized" or "super" blood clot.

Summary of the Characteristics and Evolution of PCs

Table 1 summarizes the most important differences between PRP, PRGF, and L-PRF (Giannini et al[38]). Table 2 summarizes the historical evolution of platelet concentrates over the past 50 years. As outlined above, a large number of modifications have been introduced, and while several of them really are milestones, unfortunately others are without scientific evidence for a significant clinical benefit.

Table 0-1 Major differences between first-generation (PRP, PRGF) and second-generation (L-PRF) PCs[a]

	PRP (1998)	PRGF (2001)	L-PRF (2004)
Protocol	Very complex	Complex	Easy
Speed	Slow	Very Slow	Fast
Reproducibility	Possible bias	Possible bias	No bias
Use of anticoagulants	Yes	Yes	No
100% autogenous	No	No	Yes
Amount obtainable	Sufficient	Poor	Good
Fibrin morphology	Tetramolecular	Tetramolecular	Trimolecular
Amount of leukocytes	0% to 50%	0%	≥ 65%
Immunomodulatory properties	Poor	No	Yes
Neo-angiogenic potential	Sufficient	Good	Very good
Osteoconductive potential (scaffolding)	Poor	Poor	High
Mechanical properties (sol-gel-membrane)	Sufficient	Poor	Good
Presence of mesenchymal stem cells	Yes	Yes	Yes
Costs of the protocol	High	High	Low

[a]Data based on Giannini et al.[38]

Table 0-2 Overview of the evolution of PCs[a]

Year	Event
1954	Kingsley[40] first used the term *PRP* to earmark a thrombocyte concentrate during experiments related to blood coagulation.
1970	"Fibrin glue," introduced by Matras,[41] improved healing of skin wounds in rat models. Fibrin glue was made by polymerizing fibrinogen with thrombin and calcium. However, due to a low concentration of fibrinogen in donor plasma, the quality and stability of fibrin glue was suboptimal.
1975–1978	Rosenthal and co-workers[42] introduced an enhanced blood extract called *platelet-fibrinogen-thrombin mixtures* to seal corneal wounds.
1979	Afterward, this substance was called *gelatin platelet-gel foam*. This new proposition asserted the performance of platelets and demonstrated exquisite preliminary results in general surgery, neurosurgery, and ophthalmology. However, to this point all these products were used primarily for their "gluey" effect, without consideration of the effects of growth factors or their healing properties.
1986	Knighton et al[7] first demonstrated that platelet concentrates successfully promote healing and used the term *platelet-derived wound healing factors (PDWHF)*. It was successfully tested for the management of skin ulcers.
1988	Knighton et al[8] introduced a slightly different term: *platelet-derived wound healing formula (PDWHF)*.
1997	Whitman et al[9] named their product *PRP* during preparation, but when the end product had a consistency of a fibrin gel, they labeled it as *platelet gel*.
1998	The development of these techniques continued slowly until an article by Marx et al[10] was published, which started the craze for these techniques. However, all of these products were designated as *PRP* without deliberation regarding their content or architecture, and this paucity of terminology continued for many years. Some commercial companies started labeling their products with distinct commercial names.

▶

Year	Event
1999	One of the popular methods to prepare pure PRP that was commercialized and advertised on a large scale (Anitua[13]) was *plasma rich in growth factors (PRGF;* also called *preparation rich in growth factors*; Endoret, BTI Biotechnology Institute). However, because of lack of specific pipetting steps and also lack of ergonomics, there were significant issues with this technique (Dohan Ehrenfest et al[2]). Another widely promoted technique for P-PRP was commercialized under the name Vivostat PRF. Despite the name, however, it is not a PRF but rather a PRP product.
2000	Simultaneously, Choukroun et al[20] developed another form of PC in France, which was labeled as PRF, based on the strong fibrin gel polymerization. It was stamped as a "second-generation" PC because it was obviously different from other PRPs. This proved an important milestone in the evolution of terminology.
2006	Bielecki et al[43] and Cieslik-Bielecka et al[44,45] proposed to define PRP as an inactive substance, while platelet-rich gel (PRG) was a more biologically activated fibrin matrix rich in platelets, leukocytes, and relative active molecules. Sacco[46] introduced a new concept of concentrated growth factors (CGF). For the preparation of CGF from venous blood, an rpm in the range of 2,400–2,700 was used. The fibrin-rich clots that were obtained were much larger, richer, and denser.
2008	Everts et al[47,48] focused on the leukocyte component of the PC and the two forms, ie, non-activated and activated. The non-activated product was called *platelet-leukocyte-rich plasma (P-LRP),* and the activated gel was labeled *platelet-leukocyte gel (PLG)*.
2009	The first classification of PCs was proposed by Dohan Ehrenfest et al.[2] This classification defined four main families based on separation of the products using two key parameters: the cellular content (primarily leukocytes) and the fibrin architecture: 1. Pure platelet-rich plasma (P-PRP) or leukocyte-poor platelet-rich plasma (LP-PRP) 2. Leukocyte- and platelet-rich plasma (L-PRP) 3. Pure PRF (P-PRF) or leukocyte-poor PRF 4. Leukocyte- and platelet-rich fibrin (L-PRF)
2010	The concept of sticky bone (autogenous fibrin glue mixed with bone graft) was introduced by Sohn.[49]
2012	Mishra et al[50] proposed another classification, which was limited to PRP and applicable to sports medicine only. They identified four types of PRP based on presence or absence of leukocytes and whether or not the PRP is activated: 1. L-PRP solution 2. L-PRP gel 3. P-PRP solution 4. P-PRP gel The term *solution* means non-activated PRP, and *gel* means activated PRP. At about the same time DeLong et al[51] introduced another classification system called PAW (*P*latelets quantity, *A*ctivation mode, *W*hite cells presence). However, it also was restricted to PRP families and was similar to the classification by Mishra et al above.
2012	Pinto[52] introduced the Natural Guided Regeneration Therapy concept for the management of chronic wounds with L-PRF.
2013	Tunalı et al[53] introduced a new product called *T-PRF*, an L-PRF prepared in titanium blood tubes.
2014	Ghanaati et al[54] introduced an advanced PRF called *A-PRF* (claimed to contain more monocytes).
2015	Mourão et al[55] wrote a detailed technical note on the preparation of injectable PRF (i-PRF).
2017	Fujioka-Kobayashi et al[56] introduced an advanced L-PRF, called *A-PRF+* (claimed to contain even more blood cells).
2018	Cortellini et al[57] introduced the L-PRF bone-block, a combination of chopped L-PRF membranes, bone substitute, and liquid fibrinogen. This combination resulted in significant advantages in bone regeneration.
2019	Miron et al[58] introduced C-PRF, a concentrated PRF in a liquid form.
2020	Fujioka-Kobayashi et al[59] introduced H-PRF, a PRF prepared with a horizontal centrifuge, which would give a better distribution of platelets and leukocytes over the entire membrane.

[a]Adapted and updated from Agrawal.[39]

References

1. Boswell SG, Cole BJ, Sundman EA, Karas V, Fortier LA. Platelet-rich plasma: A milieu of bioactive factors. Arthroscopy 2012;28:429–439. PMID: 22284405.
2. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: From pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol 2009;27:158–167. PMID: 19187989.
3. Dohan Ehrenfest DM, Andia I, Zumstein MA, Zhang CQ, Pinto NR, Bielecki T. Classification of platelet concentrates (platelet-rich plasma-PRP, platelet-rich fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: Current consensus, clinical implications and perspectives. Muscles Ligaments Tendons J 2014;4(1):3–9. PMID: 24932440.
4. Matras H. The use of fibrin sealant in oral and maxillofacial surgery. J Oral Maxillofac Surg 1982;40:617–622. PMID: 6981693.
5. Matras H. Fibrin sealant in maxillofacial surgery. Development and indications. A review of the past 12 years. Facial Plast Surg 1985;2:297–313. PMID: 3877660.
6. Matras H. Fibrin seal: The state of the art. J Oral Maxillofac Surg 1985;43:605–611. PMID: 3891930.
7. Knighton DR, Ciresi KF, Fiegel VD, Austin LL, Butler EL. Classification and treatment of chronic nonhealing wounds. Successful treatment with autologous platelet-derived wound healing factors (PDWHF). Ann Surg 1986;204:322–330. PMID: 3753059.
8. Knighton DR, Doucette M, Fiegel VD, Ciresi K, Butler E, Austin L. The use of platelet derived wound healing formula in human clinical trials. Prog Clin Biol Res 1988;266:319–329. PMID: 3289047.
9. Whitman DH, Berry RL, Green DM. Platelet gel: An autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg. 1997;55:1294–1299. PMID: 9371122.
10. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:638–646. PMID: 9638695.
11. Marx RE. Platelet-rich plasma: Evidence to support its use. J Oral Maxillofac Surg 2004;62:489–496. PMID: 15085519.
12. Kobayashi E, Flückiger L, Fujioka-Kobayashi M, et al. Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clin Oral Investig 2016;20:2353–2360. PMID: 26809431.
13. Anitua E. Plasma rich in growth factors: Preliminary results of use in the preparation of future sites for implants. Int J Oral Maxillofac Implants 1999;14:529–535. PMID: 10453668.
14. Anitua E, Sánchez M, Orive G, Andía I. The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. Biomaterials 2007;28:4551–4560. PMID: 17659771.
15. Roselló-Camps À, Monje A, Lin GH, et al. Platelet-rich plasma for periodontal regeneration in the treatment of intrabony defects: A meta-analysis on prospective clinical trials. Oral Surg Oral Med Oral Pathol Oral Radiol 2015;120:562–574. PMID: 26453383.
16. Pocaterra A, Caruso S, Bernardi S, Scagnoli L, Continenza MA, Gatto R. Effectiveness of platelet-rich plasma as an adjunctive material to bone graft: A systematic review and meta-analysis of randomized controlled clinical trials. Int J Oral Maxillofac Surg 2016;45:1027–1034. PMID: 26987695.
17. Stähli A, Strauss FJ, Gruber R. The use of platelet-rich plasma to enhance the outcomes of implant therapy: A systematic review. Clin Oral Implants Res 2018;29 (suppl 18):20–36. PMID: 30306686.
18. Franchini M, Cruciani M, Mengoli C, et al. The use of platelet-rich plasma in oral surgery: A systematic review and meta-analysis. Blood Transfus 2019;17:357–367. PMID: 31577533.
19. Donos N, Dereka X, Calciolari E. The use of bioactive factors to enhance bone regeneration: A narrative review. J Clin Periodontol 2019;46(suppl 21):124–161. PMID: 30623464.
20. Choukroun J. Une opportunité en paro-implantologie: Le PRF. Implantodontie 2001;42:55–62.
21. Choukroun J, Diss A, Simonpieri A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101(3):e56–e60. PMID: 16504852.
22. Choukroun J, Diss A, Simonpieri A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part V: Histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:299–303. PMID: 16504861.
23. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101(3):e37–e44. PMID: 16504849.
24. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101(3):e45–e50. PMID: 16504850.
25. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part III: Leucocyte activation: A new feature for platelet concentrates? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101(3):e51–e55. PMID: 16504851.
26. Gaultier F, Navarro G, Donsimoni JM, Dohan D. Concentrés plaquettaires: Technologies, biologie associée, applications cliniques, analyses histologiques 3e partie: Applications cliniques. Implantodontie 2003;13:3–11.
27. Dohan D, Donsimoni JM, Navarro G & Gaultier F. Concentrés plaquettaires: Technologies, biologie associée, applications cliniques, analyses histologiques, première partie: Technologies. Implantodontie 2003;12:5–16.
28. Dohan D, Donsimoni JM, Navarro G, Gaultier F. Concentrés plaquettaires: Technologies, biologie associée, applications cliniques, analyses histologiques, deuxième partie: Biologie associée. Implantodontie 2003;12:17–25.
29. Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier JB. Three- dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. J Periodontol 2010;81:546–555. PMID: 20373539.

30. Anitua E, Alkhraisat MH, Orive G. Perspectives and challenges in regenerative medicine using plasma rich in growth factors. J Control Release 2012;157:29–38. PMID: 21763737.
31. Li Q, Pan S, Dangaria SJ, et al. Platelet-rich fibrin promotes periodontal regeneration and enhances alveolar bone augmentation. Biomed Res Int 2013;2013:638043 [erratum 2020;2020:1794206]. PMID: 23586051.
32. Castro AB, Cortellini S, Temmerman A, et al. Characterization of the leukocyte- and platelet-rich fibrin block: Release of growth factors, cellular content, and structure. Int J Oral Maxillofac Implants 2019;34:855–864. PMID: 30742137.
33. Anilkumar K, Geetha A, Umasudhakar, Ramakrishnan T, Vijayalakshmi R, Pameela E. Platelet-rich-fibrin: A novel root coverage approach. J Indian Soc Periodontol 2009;13:50–54. PMID: 20376243.
34. He L, Lin Y, Hu X, Zhang Y, Wu H. A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:707–713. PMID: 19836723.
35. Castro AB, Meschi N, Temmerman A, et al. Regenerative potential of leucocyte- and platelet-rich fibrin. Part A: Intra-bony defects, furcation defects and periodontal plastic surgery. A systematic review and meta-analysis. J Clin Periodontol 2017;44:67–82. PMID: 27783851.
36. Castro AB, Meschi N, Temmerman A, et al. Regenerative potential of leucocyte- and platelet-rich fibrin. Part B: Sinus floor elevation, alveolar ridge preservation and implant therapy. A systematic review. J Clin Periodontol 2017;44:225–234. PMID: 27891638.
37. Strauss FJ, Stähli A, Gruber R. The use of platelet-rich fibrin to enhance the outcomes of implant therapy: A systematic review. Clin Oral Implants Res 2018;29(suppl 18):6–19. PMID: 30306698.
38. Giannini S, Cielo A, Bonanome L, et al. Comparison between PRP, PRGF and PRF: Lights and shadows in three similar but different protocols. Eur Rev Med Pharmacol Sci 2015;19:927–930. PMID: 25855914.
39. Agrawal AA. Evolution, current status and advances in application of platelet concentrate in periodontics and implantology. World J Clin Cases 2017;5(5):159–171. PMID: 28560233.
40. Kingsley CS. Blood coagulation; evidence of an antagonist to factor VI in platelet-rich human plasma. Nature 1954;173(4407):723–724. PMID: 13165629.
41. Matras H. Effect of various fibrin preparations on reimplantations in the rat skin [in German]. Osterr Z Stomatol 1970;67(9):338–359. PMID: 4917644.
42. Rosenthal AR, Egbert PR, Harbury C, Hopkins JL, Rubenstein E. Use of platelet-fibrinogen-thrombin mixture to seal experimental penetrating corneal wounds. Albrecht Von Graefes Arch Klin Exp Ophthalmol 1978;207(2):111–115. PMID: 308778.
43. Bielecki T, Gazdzik TS, Szczepanski T. Re: "The effects of local platelet rich plasma delivery on diabetic fracture healing." What do we use: Platelet-rich plasma or platelet-rich gel? Bone 2006;39:1388; author reply 1389. PMID: 16890506.
44. Cieslik-Bielecka A, Gazdzik TS, Bielecki TM, Cieslik T. Why the platelet-rich gel has antimicrobial activity? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103: 303–305; author reply 305–306. PMID: 17197209.
45. Cieslik-Bielecka A, Bielecki T, Gazdzik TS, Arendt J, Król W, Szczepanski T. Autologous platelets and leukocytes can improve healing of infected high-energy soft tissue injury. Transfus Apher Sci 2009;41(1):9–12. PMID: 19524487.
46. Sacco L. Lecture. Presented at the International Academy of Implant Prosthesis and Osteoconnection, 12 April 2006.
47. Everts PAM, Hoffmann J, Weibrich G, et al. Differences in platelet growth factor release and leucocyte kinetics during autologous platelet gel formation. Transfus Med 2006;16:363–368. PMID: 16999760.
48. Everts PAM, van Zundert A, Schönberger JPAM, Devilee RJJ, Knape JTA. What do we use: Platelet-rich plasma or platelet-leukocyte gel? J Biomed Mater Res A 2008;85:1135–1136. PMID: 17907242.
49. Sohn DS. Lecture on sinus and ridge augmentation with CGF and AFG. Presented at the Symposium on CGF and AFG, Tokyo, 6 June 2010.
50. Mishra A, Harmon K, Woodall J, Vieira A. Sports medicine applications of platelet rich plasma. Curr Pharm Biotechnol 2012;13:1185–1195. PMID: 21740373.
51. DeLong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: The PAW classification system. Arthroscopy 2012;28: 998–1009. PMID: 22738751.
52. Pinto N. Natural Guided Regeneration Therapy for the Management of Chronic Wounds with L-PRF. Presented at the 4th Congress of the World Union of Wound Healing Societies, Yokohama, Japan, 5 Sept 2012.
53. Tunalı M, Özdemir H, Küçükodacı Z, Akman S, Fıratlı E. In vivo evaluation of titanium-prepared platelet-rich fibrin (T-PRF): A new platelet concentrate. Br J Oral Maxillofac Surg 2013;51:438–443. PMID: 22951383.
54. Ghanaati S, Booms P, Orlowska A, et al. Advanced platelet-rich fibrin: A new concept for cell-based tissue engineering by means of inflammatory cells. J Oral Implantol 2014;40:679–689. PMID: 24945603.
55. Mourão CF, Valiense H, Melo ER, Mourão NB, Maia MD. Obtention of injectable platelets rich-fibrin (i-PRF) and its polymerization with bone graft: Technical note. Rev Col Bras Cir 2015;42:421–423. PMID: 26814997.
56. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. Optimized platelet-rich fibrin with the low-speed concept: Growth factor release, biocompatibility, and cellular response. J Periodontol 2017;88:112–121. PMID: 27587367.
57. Cortellini S, Castro AB, Temmerman A, et al. Leucocyte- and platelet-rich fibrin block for bone augmentation procedure: A proof-of-concept study. J Clin Periodontol 2018;45:624–634. PMID: 29421855.
58. Miron RJ, Chai J, Zhang P, et al. A novel method for harvesting concentrated platelet-rich fibrin (C-PRF) with a 10-fold increase in platelet and leukocyte yields. Clin Oral Investig 2020;24:2819–2828. PMID: 31788748.
59. Fujioka-Kobayashi M, Kono M, Katagiri H, et al. Histological comparison of platelet rich fibrin clots prepared by fixed-angle versus horizontal centrifugation. Platelets 2021;32:413–419. PMID: 32306811.

Part A

BASIC SCIENCE AND PREPARATION OF L-PRF

1

Blood Composition and Cellular Characteristics

Blood Composition

Our blood is a mixture of about 55% plasma and 45% blood cells. It accounts for about 7% to 8% of total body weight and represents approximately 5 L. A healthy adult can lose almost 20% of blood volume (1 L) before the first symptoms (eg, restlessness) appear and approximately 40% of volume (2 L) before hypovolemic shock sets in.

Important notice

This indicates that it should not really be a problem for a patient to donate up to 24 tubes of blood (about 240 mL) for the preparation of leukocyte- and platelet-rich fibrin (L-PRF) membranes. This amount is sometimes needed for a large guided bone regeneration procedure or for the treatment of a large chronic extraoral wound.

Red blood cells (RBCs) account for about 40% to 45% of blood volume, while white blood cells (WBCs) account for less than 1%. Platelets (< 1%) are, in contrast to RBCs and WBCs, not cells but rather small fragments of cells (also called *thrombocytes*).

Even though RBCs are smaller than WBCs, they are pushed to the bottom of blood tubes during centrifugation because they are denser (higher specific weight). The relative proportion of RBCs, measured via the hematocrit test, differs between men and women. It is normally 40.7% to 50.3% for men (4.7 to 6.1 million cells per µL) and 36.1% to 44.3% for women (4.2 to 5.4 million cells per µL). On an individual level, the hematocrit value decreases in men in their sixth decade and in women in their seventh decade, and the change becomes more prominent with advancing age, especially in men (Woodman et al,[1] Zeng et al[2]).

Important notice

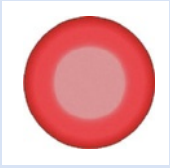
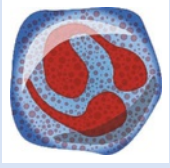
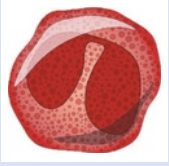
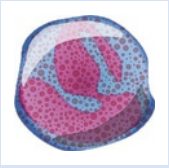
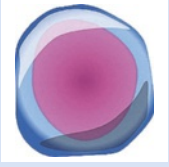
Individual variations, as well as sex- and age-related differences, in hematocrit will have an impact on the final size of L-PRF membranes: the lower the hematocrit, the larger the L-PRF membranes.

Table 1-1 summarizes important information about the constituents of blood.

References

1. Woodman R, Ferrucci L, Guralnik J. Anemia in older adults. Curr Opin Hematol 2005;12:123–128. PMID: 15725902.
2. Zeng SM, Yankowitz J, Widness JA, Strauss RG. Etiology of differences in hematocrit between males and females: Sequence-based polymorphisms in erythropoietin and its receptor. J Gend Specif Med 2001;4:35–40. PMID: 11324238.
3. Mader S. Lab Manual for Human Biology, ed 14. New York: McGraw-Hill, 2016.

Table 1-1 Description of various blood constituents[a]

Type	Form[b]	Description[b]	Cells/µL	Development	Life span	Function
Erythrocytes (RBCs): biconcave, anucleate discs						
		Salmon-colored; Ø 7-8 µm	4–6 million	±15 days	100–120 days	Transport oxygen and carbon dioxide
Leukocytes (WBCs): spherical, nucleated cells; in total: 4,800–10,800 cells/mm³						
Granulocytes						
Neutrophil		Multi-lobed nucleus; inconspicuous cytoplasmic granules; Ø 10–12 µm	3,000–7,000	±14 days	6 hours to a few days	Phagocytize bacteria
Eosinophil		Bi-lobed nucleus; red cytoplasmic granules; Ø 10–14 µm	100–400	±14 days	±5 days	Kill parasitic worms; complex role in allergy and asthma
Basophil		Lobed nucleus; large blue-purple cytoplasmic granules; Ø 10–14 µm	20–50	1–7 days	Hours to a few days	Release histamine and other mediators of inflammation; contain heparin
Agranulocytes						
Lymphocytes		Spherical/ indented nucleus; pale blue cytoplasm; Ø 5–17 µm	1,500–3,000	Days to a week	Hours to years	Mount immune response by direct cell attack or via antibodies
Monocytes		U- or kidney-shaped nucleus; gray-blue cytoplasm; Ø 14–24 µm	100–700	2–3 days	Months	Phagocytosis; develop into macrophages in tissues
Platelets: discoid cytoplasmic fragments						
		Granules; stain deep purple; Ø 2–4 µm	150,000–400,000	4–5 days	5–10 days	Seal small tears in blood vessels; crucial in blood clotting

[a]Adapted from Mader.[3]
[b]When stained with Wright stain.

2

Wound Healing

Phases of Wound Healing

The major advantage of the use of leukocyte- and platelet-rich plasma (L-PRF) is its significant beneficial effect on wound healing and tissue repair. In order to help the reader understand these benefits, this section provides a simplified summary of the wound-healing process.

In undamaged skin, the epidermis (surface layer) and dermis (deeper layer) form a protective barrier against the external environment. When this barrier is broken, a regulated sequence of biochemical events is set into motion to repair the damage. This process is divided into predictable phases (Gurtner et al[1] and Guo and Dipietro[2]; for more details see Kumar et al[3]): blood clotting (hemostasis), inflammation, tissue growth (proliferation), and tissue remodeling (maturation), as shown in Fig 2-1. The first stage, blood clotting, is sometimes considered to be part of the inflammation stage instead of a separate stage.

Phase 1: Hemostasis

Hemostasis starts immediately at the onset of an injury with the objective to stop the bleeding. The blood clotting system acts as a biologic emergency kit, forming a temporary dam (thrombus) to block the drainage. In this process blood platelets play a key role. In this process, their contact with collagen activates them, and they begin aggregating. The enzyme thrombin initiates the formation of a fibrin mesh, which strengthens the platelet clumps into a stable clot to plug the break in the blood vessel, slowing or preventing further bleeding. This process will be discussed in greater detail in the "Hemostasis" section later in this chapter.

Phase 2: Inflammation

This phase focuses on destroying the bacteria and removing debris from the wound. In essence, it prepares the wound bed for the growth of new tissue and begins immediately after phase 1. First, polymorphonuclear neutrophils enter the wound to eliminate bacteria and debris. They often reach their peak population between 24 and 48 hours after injury and reduce greatly in number after 3 days. As they leave, monocytes infiltrate the wound and differentiate into macrophages that continue clearing the debris. These cells also secrete growth factors and proteins that attract immune system cells into the wound to facilitate tissue repair. This phase takes approximately 4 to 6 days and is often associated

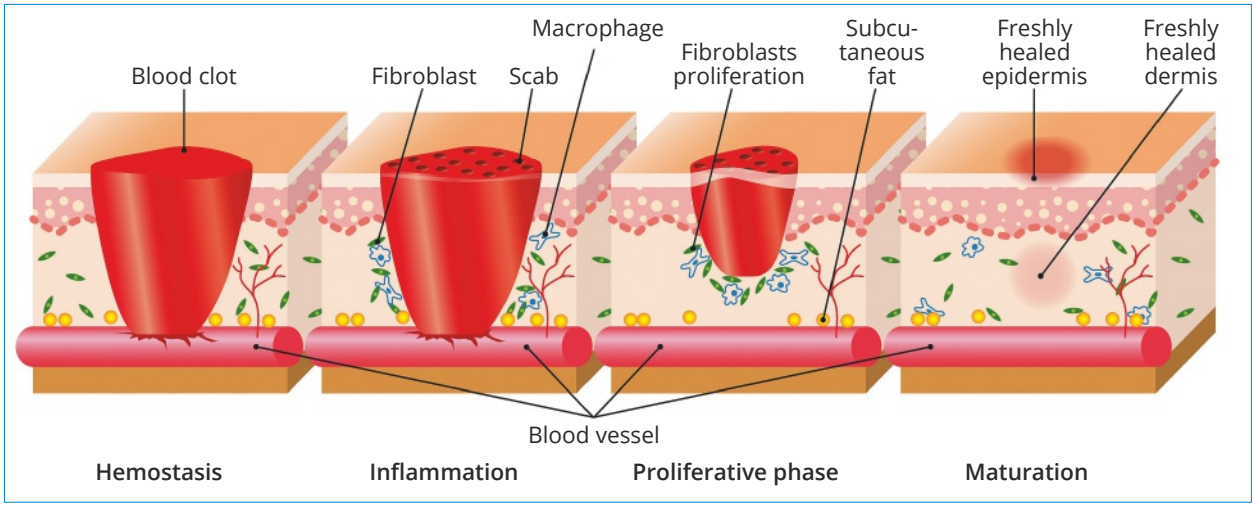


Fig 2-1 The four stages of wound healing: hemostasis (blood clotting), inflammation, tissue growth (proliferation), and tissue remodeling (maturation).

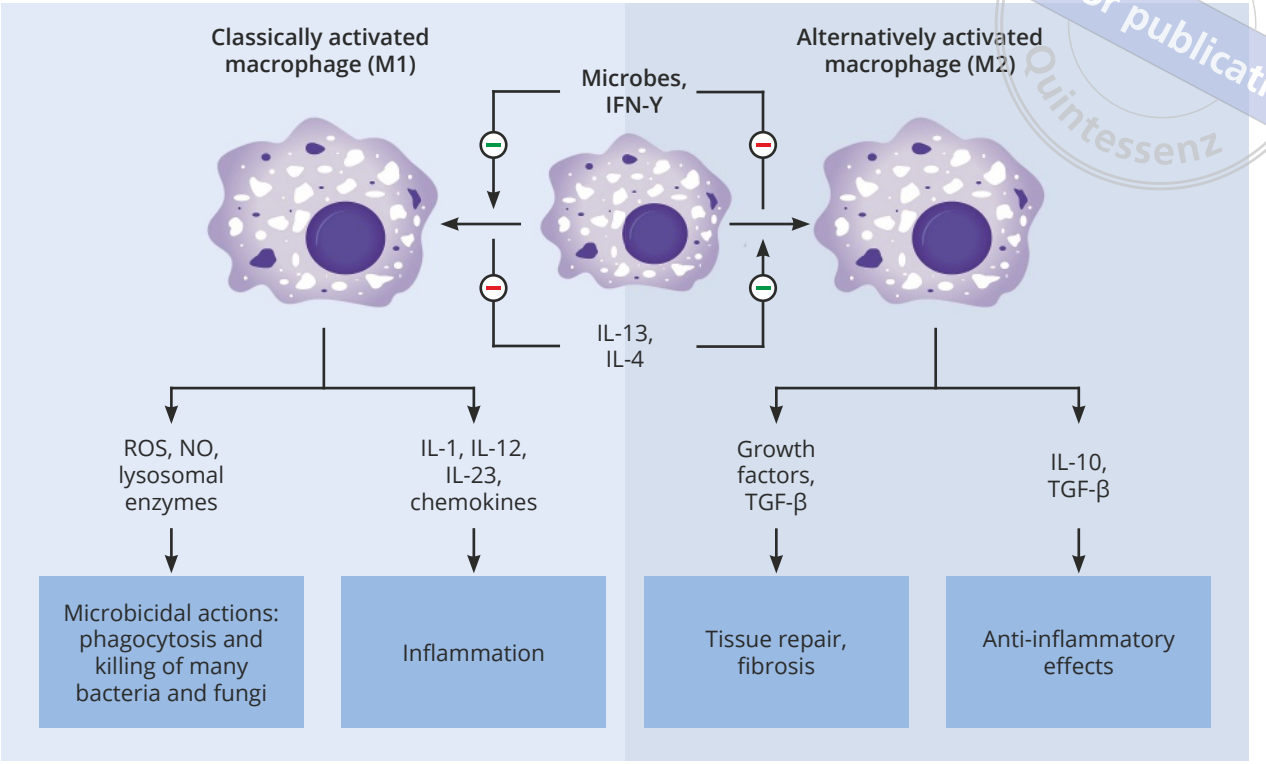


Fig 2-2 Macrophage activation into type M1 and M2 and further development. ROS, reactive oxygen species; NO, nitric oxide; IL, interleukin; IFN-Y, interferon-Y; TGF-β, transforming growth factor-β. (Adapted from a slide by Jerry Aldrin, 2015.)

with edema, erythema, heat, and pain. During these days, platelet-derived growth factors are released into the wound, causing the migration and division of cells in the proliferative phase.

In inflammatory reactions, progenitors in the bone marrow produce monocytes that enter the blood and various tissues, where they differentiate into macrophages (Fig 2-2). There are two major pathways for macrophage activation (Winkler et al[4]): the classical activation (M1), which may be induced by an external danger, and the alternative activation (M2), which is orchestrated by T-lymphocytes. The latter macrophages are not actively microbicidal; instead, the function of alternatively activated macrophages (M2) is tissue repair (Champagne et al[5]). They secrete growth factors that have been shown to promote angiogenesis, activate fibroblasts, stimulate collagen synthesis, and promote osteogenic mineralization during in vitro studies. The concept of M1 and M2 macrophages provides a useful framework for understanding macrophage heterogeneity; however, numerous other subpopulations have also been described.

Although the products of activated macrophages eliminate damaging agents and initiate the repair process, they are also partly responsible for tissue injury in chronic inflammation. Epithelial and stromal cells should also be considered as agents in this process as they can produce some of the same growth factors.

Phase 3: Proliferation

Once the wound is cleaned, the proliferative phase can begin, with a focus on filling, contracting, and covering the wound. In this phase, angiogenesis, collagen deposition, granulation tissue formation, wound contraction, and epithelialization occur. In angiogenesis, vascular endothelial cells form new blood vessels. In fibroplasia and granulation tissue

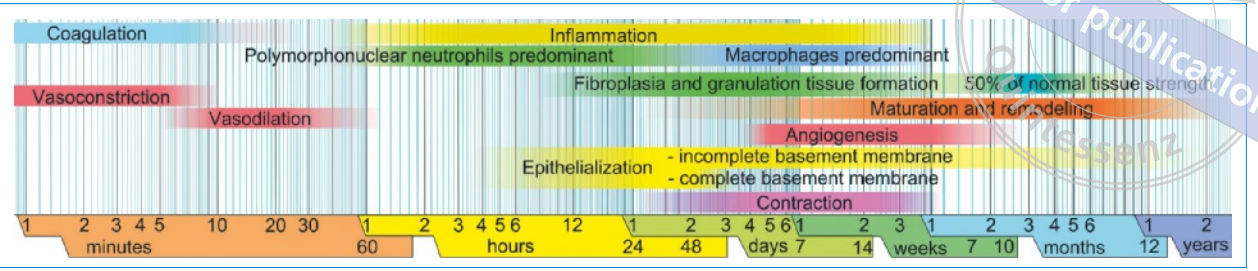


Fig 2-3 Approximate durations of the different phases of wound healing (logarithmic scale), with faded intervals marking substantial variation, depending mainly on wound size and healing conditions. (Courtesy of Mikael Häggström, 2010.)

formation, fibroblasts grow and form a new provisional extracellular matrix (ECM) by excreting collagen and fibronectin. A shiny, deep red granulation tissue fills the wound. During wound contraction, myofibroblasts decrease the wound's size by gripping the wound edges and contracting, using a mechanism that resembles that of smooth muscle cells. Concurrently, re-epithelialization of the epidermis occurs. Epithelial cells proliferate and "crawl" from the wound bed or margins over the wound surface, providing a cover for the new tissue. When the cells' roles are close to complete, unneeded cells undergo apoptosis. The proliferative phase often lasts anywhere from 4 to 24 days.

Phase 4: Maturation/Remodeling

During this phase, the newly formed tissue slowly gains strength and flexibility through the process of remodeling. Here, collagen fibers reorganize, and the tissue remodels and matures with an overall increase in tensile strength, with the initial maximum strength limited to 80% of the pre-injury strength. The maturation phase varies greatly from wound to wound, often lasting anywhere from 21 days to 2 years.

This healing process is very complex (summarized in Fig 2-3). In addition, it is susceptible to interruption due to many local factors, including moisture, infection, and maceration, as well as systemic factors, such as age, nutritional status, medication, and body type. Factors contributing to nonhealing chronic wounds are diabetes, venous or arterial disease, infection, and metabolic deficiencies of old age.

The survival of an organism relies on its ability to repair the damage caused by toxic agents and/or associated inflammation. Tissue repair occurs by two types of reactions: *(1)* regeneration by proliferation of remaining cells and maturation of the tissue or *(2)* the deposition of connective tissue to form a scar. The regeneration involving cell proliferation and differentiation is driven by growth factors and is critically dependent on the organization of an ECM. Several cell types proliferate during tissue repair. These include: *(1)* the remnants of the injured tissue that attempt to restore the normal structure, *(2)* vascular endothelial cells necessary to create new vessels to provide the nutrients required for the repair process, and *(3)* fibroblasts that are the source of the fibrous tissue that will fill the defects that cannot be improved by regeneration (Kumar et al[3]).

Angiogenesis, ie, the formation of new blood vessels from existing vessels, is critical in tissue repair. The process of angiogenesis involves several signaling pathways, cell-to-cell communication, interaction between proteins from the ECM, and tissue enzymes. Vascular endothelial growth factor (VEGF) plays an important role in the initiation of this process. It stimulates both migration and proliferation of endothelial cells and thus initiates the process of capillary sprouting. Other growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β), also participate in this process by recruiting smooth muscle cells and enhancing the production of ECM proteins (Morgan and Nigam[6]).

Cell proliferation is determined by signals provided by the ECM and growth factors. Growth factors are normally produced by cells near the injured area. The most important sources of growth factors are macrophages that are activated after tissue injury. Macrophages derive from hematopoietic stem cells that are known as monocytes when circulating in blood. Macrophages are specialists in phagocytosis of microbes and senescent cells, but they also serve many other roles in inflammation and repair.

There is a spectrum of scar formation, with scarless regeneration on one end, "normal" scar formation in the center, and pathologic scar formation, including hypertrophic and keloid scarring, on the other end (Marshall et al[7]). Keloid and hypertrophic scarring contribute to much of the morbidity of scarring after surgery. A *hypertrophic* scar can be defined as a scar forming after injury, that is larger or more raised than usual, or that results in contracture. A hypertrophic scar is more likely to occur after infection of the wound, closure of the wound with excessive tension, or with position of the wound in areas of skin with high natural tension (such as the shoulders, neck, and sternum). *Keloid* scars, on the contrary, represent an abnormally exuberant scarring response that extends beyond the borders of the original injury. Keloids cause symptoms of pruritus and hyperesthesia and tend to recur after excision, as opposed to hypertrophic scars, which may not recur if the scar is revised appropriately. While hypertrophic scars often flatten over several years, keloid scars typically do not regress.

Even in the adult human, the oral mucosa is able to heal after injury with little scarring, resembling the regenerative healing of fetal skin (Marshall et al[7]). Even though the oral mucosal wound progresses through the same stages of wound healing as a skin wound, there is lower inflammatory response at the beginning, and the overall rate of healing is higher. The presence of saliva accelerates wound healing in mouse skin, and mice that underwent sialoadenectomy and were allowed to lick their wounds healed more slowly than controls, suggesting that the absence of normal saliva inhibited healing (Bodner et al[8]). Extraoral tissue transplanted into the oral cavity remains histologically distinct from mucosal tissue and produces scar tissue. In addition, recent experiments showed that dermal fibroblasts possessing a "scarring phenotype" transplanted into the oral mucosa produce more scar-like connective tissue compared with oral mucosal fibroblasts transplanted into the dermis (Rinkevich et al[9]). Together, these results strongly suggest that factors intrinsic to cells residing in the oral mucosa account for much of the reduced scarring seen in that tissue.

Important notice

It is important to realize that platelets (present in high concentrations in L-PRF membranes) are key in the early phases of tissue regeneration (during hemostasis and fibrin clot formation). Moreover, they secrete a number of important growth factors including PDGF, VEGF, coagulation factors, adhesion molecules, cytokines/chemokines, and a variety of other angiogenic factors capable of stimulating the proliferation and activation of cells involved in the wound-healing process, including fibroblasts, neutrophils, macrophages, and mesenchymal stem cells (Nurden[10]). As such, it should not be a surprise that L-PRF membranes can positively stimulate/facilitate wound healing and/or tissue repair.

Hemostasis

Hemostasis (cessation of bleeding after injury) is the first stage of wound healing and involves blood changing from a liquid to a gel (ie, blood coagulation). Under normal conditions endothelial cells prevent blood clotting via the secretion of inhibitors for coagulation and platelet aggregation. After endothelial injury (or rupture), however, the endothelial cells stop the secretion of these inhibitors and instead secrete von Willebrand factor (vWF), which initiates hemostasis. Hemostasis has four major

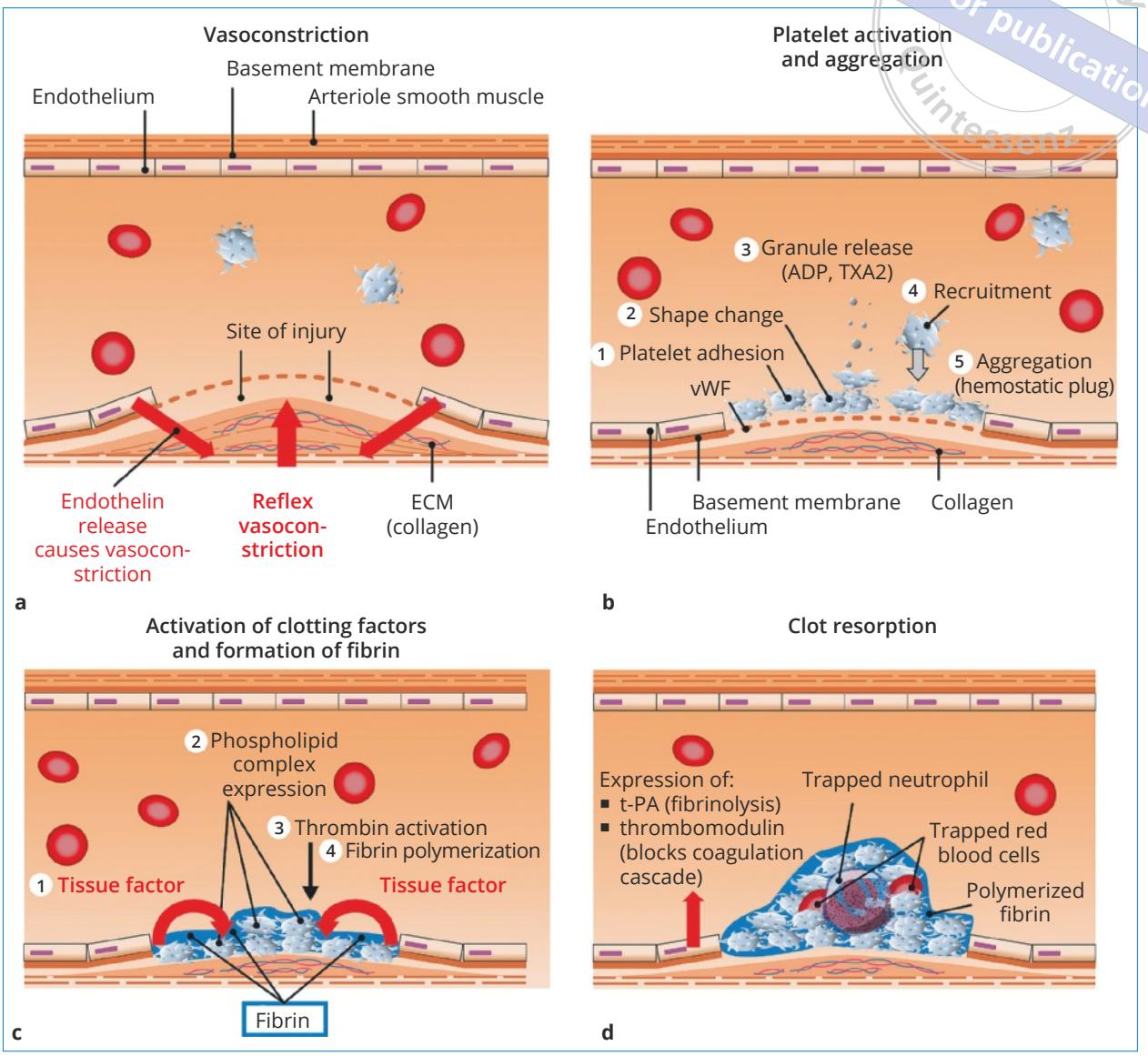


Fig 2-4 The four major steps in hemostasis. **(a)** Immediately after vascular injury, a transient vasoconstriction is induced via the contraction of the smooth muscle cells within the wall of the blood vessels. **(b)** Blood platelets *(gray)* bind to vWF from the exposed underlying basement membrane and are activated (ie, they change in shape, become sticky, and release granules). These granules (adenosine diphosphate [ADP], thromboxane A2 [TXA2], serotonin) will recruit more sticking platelets, forming a platelet plug (ie, primary hemostasis) and releasing more chemicals (creating a positive feedback loop). **(c)** A local coagulation cascade (described in more detail later in this chapter) is initiated, involving tissue factor and platelet phospholipids and resulting in fibrin *(blue)* polymerization "cementing" the platelets into a strong secondary hemostatic plug. **(d)** Formation of a solid plug (by contraction), activation of counter-regulatory mechanisms to limit clotting to the site of injury (eg, tissue plasminogen activator [t-PA] secreted by endothelial cells), and eventually clot resorption. (Adapted from Park and Koh.[11])

steps: *(1)* arteriolar vasoconstriction, *(2)* temporary blockage of a blood vessel break by a platelet plug (primary hemostasis), *(3)* formation of a fibrin clot, and *(4)* clot resorption (Fig 2-4). These processes seal the injury until tissues are repaired (for details see Park and Koh[11] and Kumar et al[12]).

Arteriolar vasoconstriction

As a first and immediate response after a vascular injury, the blood vessel will constrict in order to reduce the blood loss (ie, contraction of vascular smooth muscle cells within the wall of the blood ves-

sels). Their contracting properties are controlled by the vascular endothelium itself. This is mediated by reflex neurogenic mechanisms and augmented by the local secretion of factors such as endothelin, a potent endothelium-derived vasoconstrictor.

Platelet plug formation: Primary hemostasis (platelet aggregation)

Under normal conditions, blood platelets do not adhere, neither to each other nor to the blood vessel wall. Disruption of the endothelium exposes subendothelial collagen and vWF. They both promote the adherence and activation of platelets. The activated platelets change in shape (from rounded discs to flat plates with very sticky protrusions) and release cytoplasmic granules such as adenosine diphosphate (ADP, which attracts more platelets to the affected area), serotonin (a vasoconstrictor), and thromboxane A2 (TXA2, which assists in platelet aggregation, vasoconstriction, and degranulation). As more chemicals are released, more platelets stick and release their chemicals, creating a platelet plug and continuing the process in a positive feedback loop (chain reaction). Platelets alone are thus responsible for stopping the bleeding of unnoticed wear and tear of our skin on a daily basis. This is referred to as *primary hemostasis*.

Clot formation: Secondary hemostasis (formation of platelet clot)

Once the platelet plug is formed, clotting factors (a dozen proteins that travel along the blood plasma in an inactive state) are activated in a sequence of proteolytic events known as a *coagulation cascade*. These events are mainly localized on the surface of activated platelets. The activation is introduced by the tissue factor (TF), a membrane-bound glycoprotein on subendothelial cells in the blood vessel wall (eg, smooth muscle cells and fibroblasts). This will lead to the formation of a 3D fibrin network from inactive fibrinogen (a plasma protein). As such, a fibrin mesh is produced around the platelet plug to hold it in place (referred to as *secondary hemostasis*). During this process, red and white blood cells are also trapped, which makes the primary plug stronger. The resultant plug is called a *thrombus* or *clot*.

Clot stabilization and resorption (antithrombotic events)

The polymerized fibrin and platelet aggregates undergo contraction to form a solid, permanent plug to prevent further hemorrhage. At this stage, counter-regulatory mechanisms are set into motion to limit clotting to the site of injury (eg, tissue plasminogen activator [t-PA], which functions as a fibrinolytic product, is secreted by endothelial cells, and thrombomodulin, an integral membrane protein that serves as a cofactor for thrombin, is expressed on the surface of endothelial cells). This will finally lead to clot resorption and tissue repair.

Important notice

Endothelial cells are the central regulators of hemostasis (the balance between the antithrombic and prothrombotic activities of the endothelium determines whether thrombus formation, propagation, or dissolution occurs). Blood platelets play a critical role in the hemostasis by forming the primary plug that initially seals the vascular defect and by providing a surface that binds and concentrates activated clotting factors for the coagulation cascade.

Blood Coagulation

Because blood clotting is an essential step in the preparation of the second-generation platelet concentrates, this section summarizes the clotting procedure. Blood coagulation in vivo involves the activation, adhesion, and aggregation of platelets and a deposition and maturation of fibrin (ie, coagulation cascade).

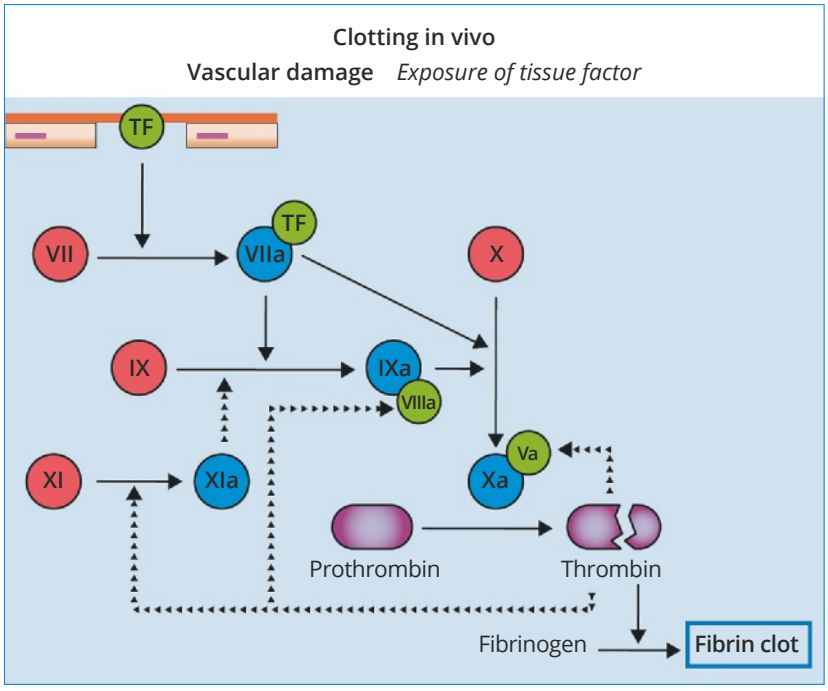


Fig 2-5 In vivo, TF is the major initiator of the coagulation cascade, which occurs on the surface of platelets. The cascade is further amplified by loops involving thrombin *(dotted lines)*. The *red* polypeptides are inactive factors, the *blue* are active factors, and the *green* are cofactors. The final step is the conversion of fibrinogen into fibrin.

The coagulation cascade can be seen as a dance in which coagulation factors are passed from one partner to the next, finally leading to the deposition of an insoluble fibrin clot. Each step involves an enzyme (an activated coagulation factor), a substrate (an inactive form of a coagulation factor), and a cofactor (a reaction accelerator). These components are assembled on a negatively charged phospholipid surface from activated platelets.

The coagulation cascade can be activated via the intrinsic pathway (also known as the *contact activation pathway*) and/or the extrinsic pathway (ie, the *tissue factor pathway*), both leading to the same reaction (common pathway) to produce fibrin. In the intrinsic pathway all components are already present in the blood, whereas in the extrinsic pathway a TF, secreted by damaged cells, is required.

The coagulation cascade is quite complex, having several feedback loops. In the following paragraphs, simplified schemes are presented (for more details see Park and Koh[11] and Kumar et al[12]).

Extrinsic (TF) pathway (Fig 2-5)

This pathway is called the *extrinsic pathway* because it requires that blood reaches the subendothelial space. After vascular damage, circulating factor VII comes into contact with TF (a cell-surface integral membrane protein present in subendothelial tissue and leukocytes), forming an activated complex (TF–factor VIIa). TF is expressed on TF-bearing cells (stromal fibroblasts, vascular smooth muscle cells, leukocytes). The TF–factor VIIa complex activates factor IX and factor X. From here, the common pathway begins.

Intrinsic (contact activation) pathway (Fig 2-6)

The intrinsic pathway is initiated by the activation of factor XII by certain negatively charged artificial surfaces, including glass (eg, glass blood tubes or silica-coated plastic blood tubes). High-molecular-weight kininogen and prekallikrein are two proteins in blood that facilitate this activation. The enzyme form of factor XII (factor XIIa) catalyzes the conversion of factor XI to its enzyme form (factor XIa). Factor XIa catalyzes the conversion of factor IX to the activated form, fac-

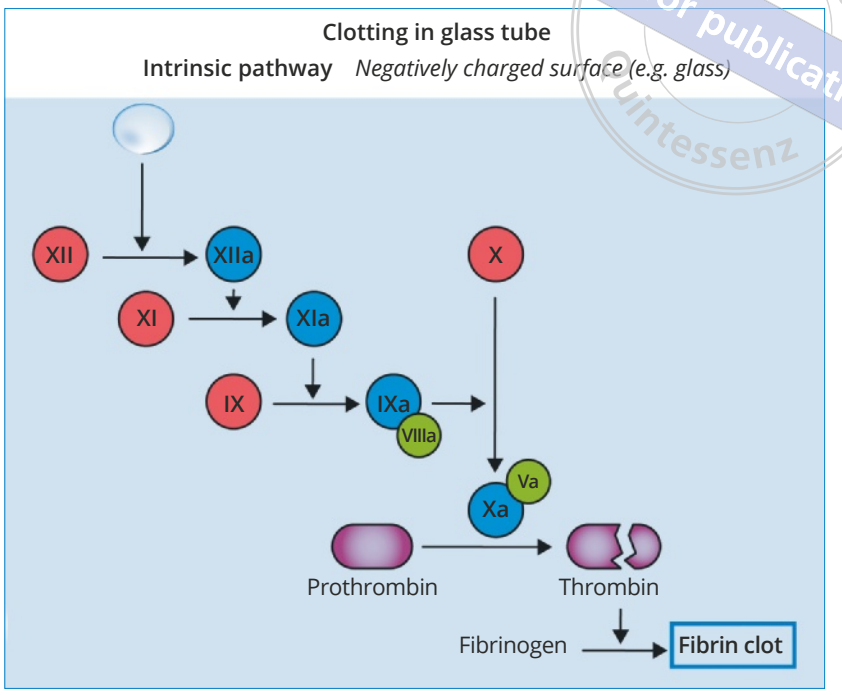


Fig 2-6 In vitro, blood clotting is initiated by either a negatively charged substance (such as a glass surface) or a source of TF (comparable to the extrinsic pathway). During the preparation of L-PRF, the glass surface or silica coating inside the blood tubes are responsible for initiating the coagulation process. The *red* polypeptides are inactive factors, the *blue* are active factors, and the *green* are cofactors. The final step is again the conversion of fibrinogen into fibrin.

tor IXa. Factor IXa assembles with factor VIII. The factor IXa–factor VIII complex binds to factor X, which is activated to factor Xa. From here, the common pathway begins.

Important notice

The intrinsic pathway occurs in the blood tubes (silica-coated plastic tubes or glass tubes) that are used for the preparation of L-PRF. Some plastic or silicone surfaces do not have this property (eg, the white-cap tubes in the Intra-Lock system). These are used if it is necessary to delay the coagulation cascade, such as during the preparation of liquid fibrinogen, needed for the preparation of an L-PRF bone-block.

Common pathway

Factor X is cleaved by VIIa to form factor Xa. Prothrombin, bound to glycoprotein IIb/IIIa on the activated platelet surface, is converted to thrombin by Xa (Va and calcium ions are cofactors for this reaction). However, minimal amounts of thrombin are formed by this pathway during the initiation phase of coagulation. The trace amounts of thrombin generated during the initiation phase provide further activation of platelets, factor V, and factor XI. Larger amounts will be generated during the amplification phase (Monković and Tracy,[13] Hoffman and Monroe[14]). The formation of a complex comprising factors VIIIa, IXa, and calcium ions on the platelet surface leads to the large-scale generation of factor Xa. Factor Xa, with factor Va and calcium ions, forms the prothrombinase complex that produces the burst of thrombin needed for the conversion of fibrinogen to fibrin. Furthermore, thrombin activates factor XIII, resulting in clot stabilization, and the thrombin-activatable fibrinolysis inhibitor, which modulates fibrinolysis (Green[15]).

Important notice

Blood platelets thus play a critical role in hemostasis by forming the primary plug that initially seals the vascular defect and by providing a surface that binds and concentrates activated coagulation factors.

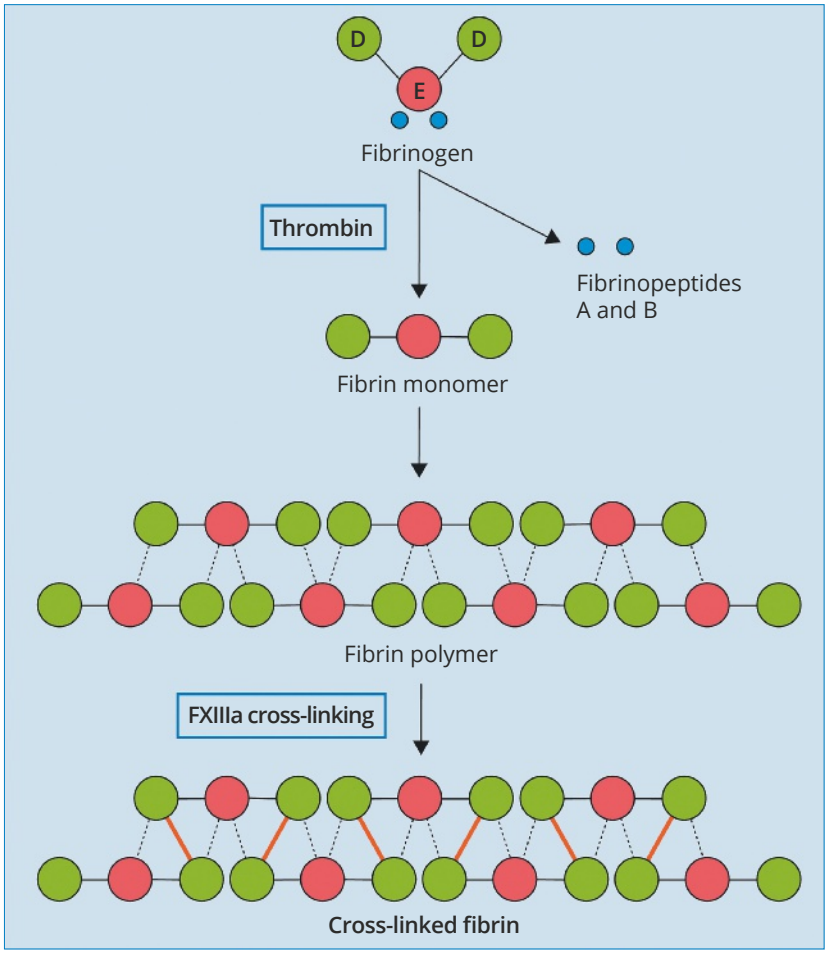


Fig 2-7 Step-by-step formation of an insoluble cross-linked fibrin polymer network. (Adapted from Riley et al.[16])

Formation of Fibrin

Fibrinogen (factor I) is a glycoprotein that circulates in the human blood. After tissue and vascular injury, it is converted enzymatically by thrombin to fibrin and then to a fibrin-based blood clot. Fibrin clots function primarily to occlude blood vessels to stop bleeding. Fibrin also binds and reduces the activity of thrombin. This activity, sometimes referred to as *antithrombin I*, limits blood clotting.

Fibrinogen is made and secreted into blood primarily by hepatocytes. Mature fibrinogen is a long flexible protein array of three nodules held together by a very thin thread (Fig 2-7). The two end nodules (termed D regions or domains) are alike in consisting of Bβ and γ chains, while the slightly smaller center nodule (termed the E region or domain) consists of two intertwined A alpha chains. The length of a dried fibrinogen molecule is 47.5 ± 2.5 nm. Stimulus of the coagulation cascade ultimately produces fibrin monomers that strengthen the initial platelet plug by spontaneously aggregating into end-to-end and side-to-side fibrils (Riley et al[16]; see Fig 2-7). Fibrinogen is transformed to fibrin monomers through the cleavage of two small fragments (fibrinopeptides A and B, *small blue circles* in Fig 2-7) from the molecule by thrombin. During this process, the negative charge of the E domain of fibrinogen (*red circle* in Fig 2-7) is converted to a positive charge, permitting spontaneous polymerization of the fibrin monomers into a polymer stabilized by hydrogen bonds. Thrombin also activates in blood-circulating transglutaminase enzyme, factor XIII, which stabilizes the initial fibrin polymer by catalyzing the formation of cross-linked covalent bonds between adjacent D domains (*orange lines* in Fig 2-7).

References

1. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature 2008;453(7193):314–321. PMID: 18480812.
2. Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res 2010;89:219–229. PMID: 20139336.
3. Kumar V, Abbas AK, Aster JC (eds). Inflammation and repair. In: Robbins Basic Pathology. Philadelphia: Elsevier, 2018:57–96.
4. Winkler IG, Sims NA, Pettit AR, et al. Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs. Blood 2010;116:4815–4828. PMID: 20713966.
5. Champagne CM, Takebe J, Offenbacher S, Cooper LF. Macrophage cell lines produce osteoinductive signals that include bone morphogenetic protein-2. Bone 2002;30: 26–31. PMID: 11792561.
6. Morgan C, Nigam Y. Naturally derived factors and their role in the promotion of angiogenesis for the healing of chronic wounds. Angiogenesis 2013;16:493–502. PMID: 23417553.
7. Marshall CD, Hu MS, Leavitt T, Barnes LA, Lorenz HP, Longaker MT. Cutaneous scarring: Basic science, current treatments, and future directions. Adv Wound Care (New Rochelle) 2018;7(2):29–45. PMID: 29392092.
8. Bodner L, Knyszynski A, Adler-Kunin S, Danon D. The effect of selective desalivation on wound healing in mice. Exp Gerontol 1991;26:357–363. PMID: 1936194.
9. Rinkevich Y, Walmsley GG, Hu MS, et al. Skin fibrosis. Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. Science 2015;348(6232):aaa2151. PMID: 25883361.
10. Nurden AT. Platelets, inflammation and tissue regeneration. Thromb Haemost 2011;105(suppl 1): S13–S33. PMID: 21479340.
11. Park J, Koh J-W. Era of bloodless surgery: Spotlights on hemostasic material and techniques. Hanyang Med Rev 2018;38(1):3–15.
12. Kumar V, Abbas AK, Aster JC (eds). Hemodynamic disorders, thromboembolism and shock. In: Robbins Basic Pathology. Philadelphia: Elsevier, 2018:97–120.
13. Monković DD, Tracy PB. Functional characterization of human platelet-released factor V and its activation by factor Xa and thrombin. J Biol Chem 1990;265(28): 17132–17140. PMID: 2211616.
14. Hoffman M, Monroe DM 3rd. A cell-based model of hemostasis. Thromb Haemost 2001;85:958–965.
15. Green D. Coagulation cascade. Hemodial Int 2006;10(suppl 2):S2–S4. PMID: 17022746.
16. Riley RS, Gilbert AR, Dalton JB, Pai S, McPherson RA. Widely used types and clinical applications of D-dimer assay. Lab Med 2016;47(2):90–102. PMID: 27016528.

This book will guide clinicians to achieve great results with L-PRF. It starts with basic science chapters on tissue regeneration, blood centrifugation, and an explanation of the biologic power of platelet concentrates. However, the true value of this book lies in an evidence-based evaluation of the benefits of L-PRF in periodontal and maxillofacial surgery, as well as in chronic wound therapy. For each treatment, a step-by-step approach has been created by experienced clinicians. The most crucial steps are explained in 18 videos via augmented reality.

Prof Marc Quirynen graduated as a dentist in 1980 from the Catholic University of Leuven, Belgium, where he went on to complete training in periodontology and obtain his PhD. In 1990, he was appointed as a professor at Catholic University and is currently Chairman of the Department of Periodontology. His research focuses on simplification and optimization of periodontal therapy, and over the past decade, he has explored platelet concentrates in soft and hard tissue regeneration. Prof Quirynen has published over 450 papers in international peer-reviewed journals and serves on several editorial boards, including for the *Journal of Clinical Periodontology* and *Clinical Oral implants Research*. His most inspiring lectures deal with the clinical benefits of L-PRF and the prevention of peri-implantitis.

Prof Nelson R. Pinto is Professor in the Department of Periodontology and Implantology at Universidad de Los Andes in Santiago, Chile, and Founder of the Research Center for Regenerative Medicine and Tissue Engineering. He is visiting faculty in the Department of Periodontology at Catholic University of Leuven, Belgium, and in the Advanced Education Program in Implant Dentistry at Loma Linda University in California. His main accomplishment is the development of Natural Guided Regeneration Therapy (NGRT), and his research in this area was recognized in 2012 by the World Union of Wound Healing Societies (WUWHS) and in 2016 with the *Journal of Wound Care* & WUWHS Award for Best Contribution to Clinical Research for 2012 to 2016. In 2018, he received the Punyaarjan Humanitarian Service Award for his professional dedication to chronic wound healing and helping change patients' lives through NGRT.

ISBN: 978-1-78698-105-9
9 781786 981059
www.quintessence-publishing.com