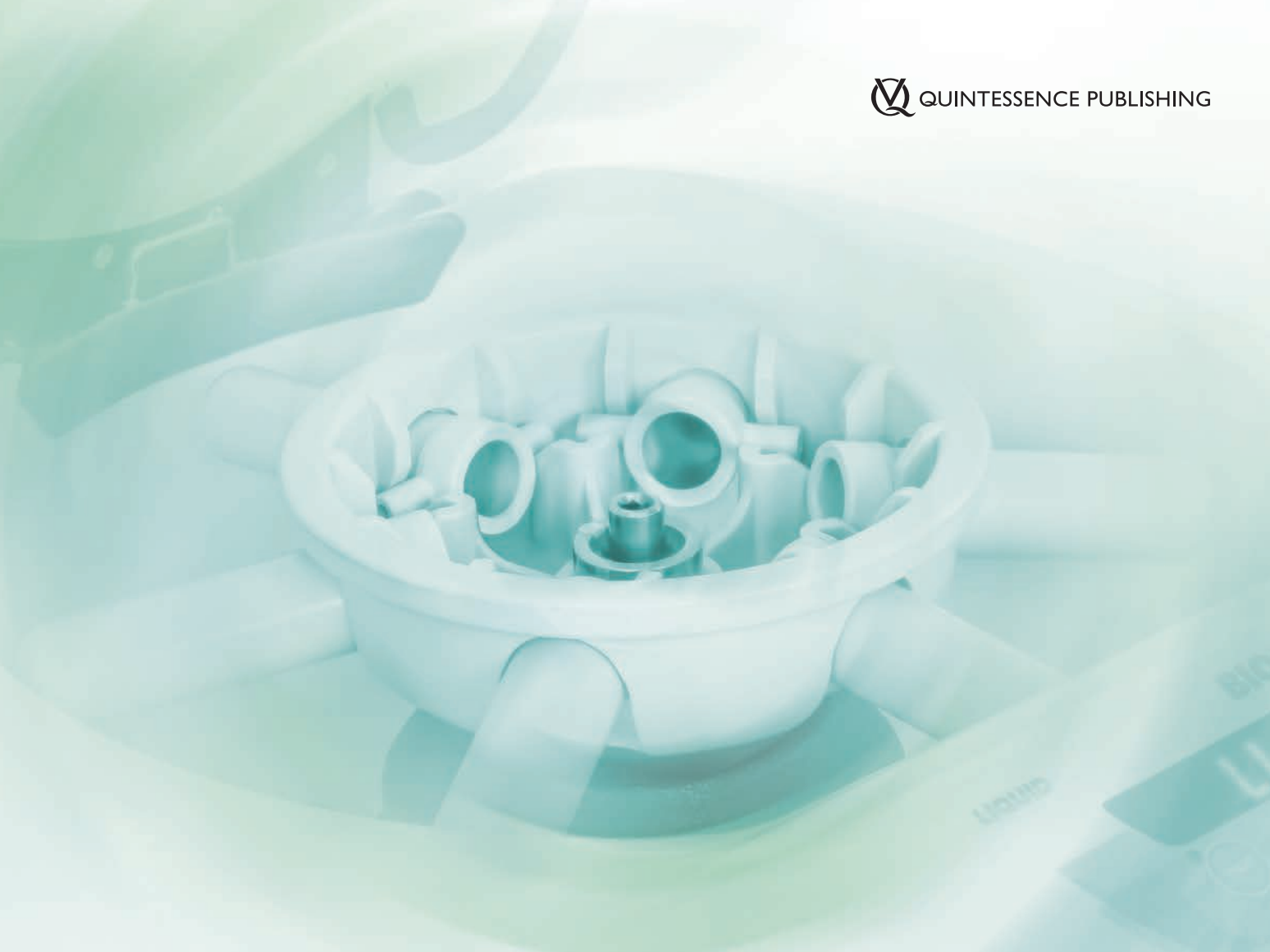
QUINTESSENCE PUBLISHING



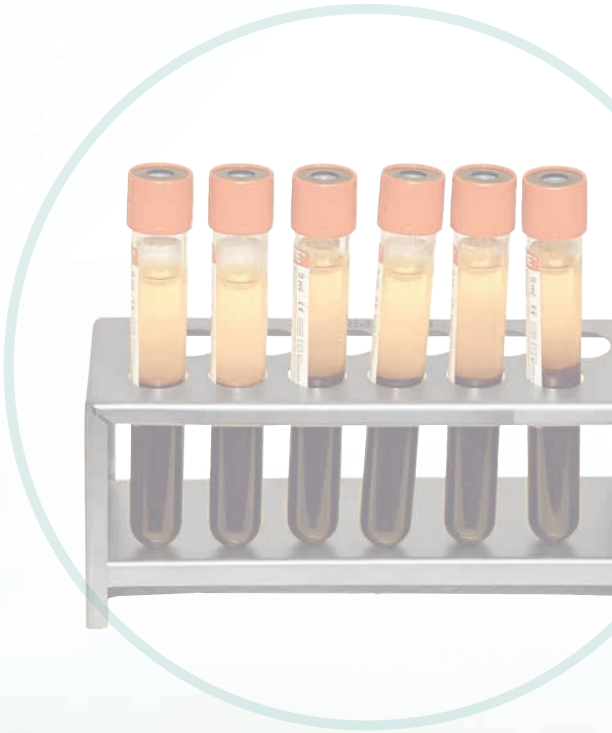
Edited by **Richard J. Miron, DDS, BMSc, MSc, PhD, Dr med dent**

Foreword by ***Robert E. Marx, DDS***

Understanding **Platelet-Rich Fibrin**

Understanding Platelet-Rich Fibrin

Understanding Platelet-Rich Fibrin



Edited by

Richard J. Miron, DDS, BMSc, MSc, PhD, Dr med dent

Group Leader, The Miron Research Lab
Lead Educator, Advanced PRF Education (www.prfedu.com)
Venice, Florida

Department of Periodontology
University of Illinois at Chicago
Chicago, Illinois

QUINTESSENCE PUBLISHING

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

Library of Congress Cataloging-in-Publication Data

Names: Miron, Richard J. (Richard John), 1983- editor.
Title: Understanding platelet-rich fibrin / edited by Richard J. Miron.
Description: Batavia, IL : Quintessence Publishing Co Inc, [2021] | Includes bibliographical references and index. | Summary: "This book outlines the science behind platelet-rich fibrin and then details how to use it in clinical practice to optimize healing outcomes and promote tissue regeneration. Applications include gingival recessions, intrabony and furcation defects, extraction site management, implant dentistry, guided bone regeneration, sinus grafting, oral and maxillofacial surgery, regenerative endodontics, facial esthetics, and medicine"-- Provided by publisher.
Identifiers: LCCN 2020035734 | ISBN 9781647240493 (hardcover)
Subjects: MESH: Platelet-Rich Fibrin | Fibrin--therapeutic use | Tissue Engineering | Bone Regeneration
Classification: LCC QP91 | NLM WH 400 | DDC 612.1/15--dc23
LC record available at https://lccn.loc.gov/2020035734

QUINTESSENCE PUBLISHING
USA

Quintessence Publishing Co, Inc
411 N Raddant Road
Batavia, IL 60510
www.quintpub.com

5 4 3 2 1

Editor: Leah Huffman
Design: Sue Zubek
Production: Angelina Schmelter

Printed in the USA

Contents

Dedication *vii*
Foreword by Robert E. Marx *viii*
Preface *ix*
Contributors *x*
Abbreviations *xiii*

1 Evolution of Platelet Concentrates *1*

SECTION I | BIOLOGY OF PRF

2 Biology of PRF: Fibrin Matrix, Growth Factor Release, and Cellular Activity *11*

3 Horizontal Versus Fixed-Angle Centrifugation of PRF: Optimization of C-PRF *51*

4 Understanding Relative Centrifugal Force (G-Force) *71*

5 Protocols for PRF *83*

6 Importance of Centrifugation Tubes for the Production of PRF *89*

7 Biologic Characterization of e-PRF Membranes *101*

8 Armamentarium in a PRF Kit *117*

9 Phlebotomy *123*

10 Fabricating Various PRF Modalities *135*

11 Overview of Clinical Indications Using PRF *147*

SECTION II | PERIODONTOLOGY

12 Use of PRF for the Treatment of Gingival Recessions *151*

13 Use of PRF for the Treatment of Intrabony and Furcation Defects *167*

14 Use of PRF for Extraction Site Management *189*

SECTION III | IMPLANT DENTISTRY

15 Use of PRF as an Adjunct Therapy to Implant Dentistry *219*

16 Use of PRF in Guided Bone Regeneration *233*

17 Use of PRF for Sinus Grafting *253*

SECTION IV | ADDITIONAL DENTAL AND MEDICAL APPLICATIONS

18 Use of PRF in Oral and Maxillofacial Surgery *275*

19 Use of PRF in Regenerative Endodontics *291*

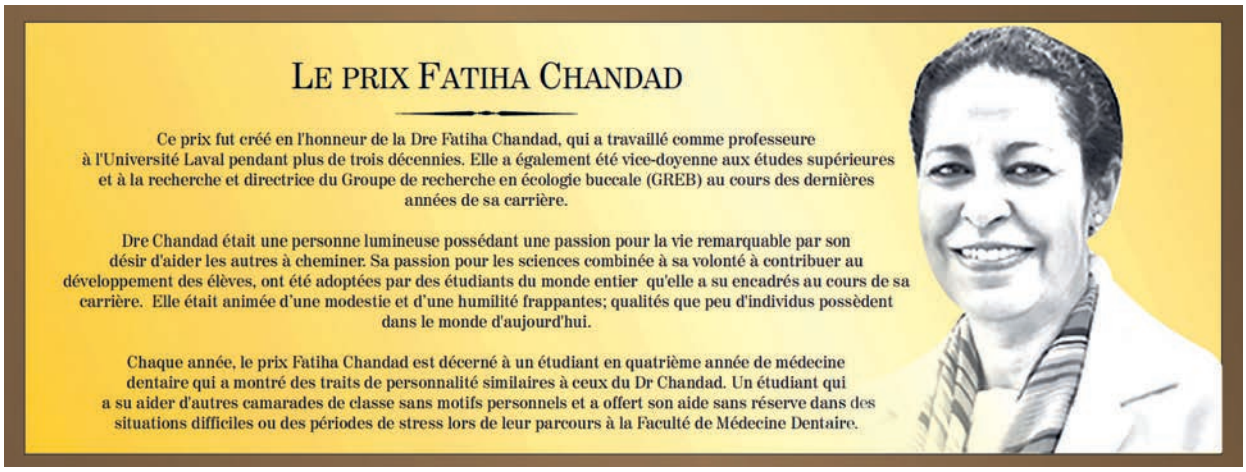
20 Use of PRF in Facial Esthetics *317*

21 Medical Uses of PRF *329*

22 Future Research with PRF *347*

Index *363*

Dedication



I would like to dedicate this textbook specifically to Dr Fatiha Chandad and the Dental Faculty at the University of Laval in Quebec, Canada, who devoted countless hours to training students like me in dentistry.

Dr Chandad, Dean of Dental Research, was someone who motivated me and more importantly made it possible for students to work within her laboratory on research projects during their 4 years of dental studies. It was here that I first started my research activities on PRP/PRF and became fascinated with research as a whole. Dr Chandad is one of the only people I have ever met never wanting to be recognized for her achievements, instead insisting that her students be recognized and at the forefront of their own success. It was during these times that I was awarded the prestigious Hatton Award in Canada and was later named the IADR Young Investigator of the Year in Implant Dentistry.

In appreciation of her contributions to my career, 100% of the royalties from this textbook will be donated to create a scholarship named after her for new dental graduates at the University of Laval. *Milles fois merci pour tout tes efforts!*

Foreword

Many important medical/dental discoveries were stumbled upon by pure chance. An example of this is osseointegration, which launched the modern era of dental implants and orthopedic-embedded prostheses. It occurred when orthopedic researcher Per-Ingvar Brånemark found that his titanium research cages in sheep were so completely ingrown with bone that he couldn't remove them. Similarly, platelet-rich plasma (PRP)/platelet-rich fibrin (PRF) was discovered by the serendipity of observing accelerated and more complete healing in patients who developed hematomas. From that simple observation in the 1980s, the components of the blood clot responsible for the advanced healing have since been determined to be the complete and active growth factors in the alpha granules of viable platelets and several cell adhesion molecules and homing signals in the fibrin clot.

From the early pioneering work in the 1990s to the present, the benchmark of platelet numbers and the functional characteristics of the growth factors, cell adhesion molecules, and homing signals of PRP/PRF have progressed to a mature science and easy-to-use point-of-care devices.

Today, PRP/PRF devices are able to predictably concentrate platelets to known therapeutic levels by a variety of technologic means and to include or exclude leukocytes as per the needs of the wound. PRP/PRF has become a mainstay in bone regeneration in dental implantology and jaw reconstruction, for tendon and joint repair in orthopedics, for soft tissue healing in wound care centers, and in plastic surgery; it has thus benefited hundreds of thousands of patients worldwide.

As one of several individuals who were there at the beginning of the PRP/PRF discovery and who helped to move it along the way somewhat, I am delighted to see that the next generation of clinical researchers as published in *Understanding Platelet-Rich Fibrin* have brought it to the next level.

This text is written for the clinician to understand how and why PRF promotes healing of both bone and soft tissue as well as how to apply it to improve their own results. Written with a balanced blend of science and clinical applications by the most experienced and accomplished PRP/PRF scientists and clinicians of the day, and beautifully illustrated, *Understanding Platelet-Rich Fibrin* is a book for this decade that transcends all specialties of dentistry and many of medicine.

Robert E. Marx, DDS
Professor of Surgery and Chief of OMFS
University of Miami
Miller School of Medicine
Miami, Florida

Preface

Over 20 years ago, platelet concentrates entered into the medical field as a means to deliver autologous growth factors responsible for favoring wound healing. During this time span, it has gained widespread acceptance in many fields of medicine due to its more natural delivery system.

Most notably, the past 5 years have seen a tremendous increase in publications on PRF, with over 200 scientific peer-reviewed papers being published each and every year. During this span, a marked increase in our understanding of PRF therapy has been made with respect to selection of appropriate centrifugation devices, impact of tube chemistry on clotting, the optimization of protocols to better concentrate PRF, and even the ability to extend the working properties of PRF from 2–3 weeks toward 4–6 months using a simple heating process. Collectively, we continue to gather new knowledge, and as a result, PRF therapy has become one of the fastest-growing therapeutic options in dentistry. Thousands of users have now benefited from this technology, and this number is only expected to continue to increase.

This book is very different from others in its concept design. More than a dozen expert researchers and clinicians alike were gathered as editors across their different fields of expertise. As section editors, these true experts of their respective disciplines were able to produce a much higher overall quality of this textbook. I am grateful for their encouraging team spirit, their effort in bringing this book to an entirely new level, and their level of professionalism and mindset that ultimately led to this comprehensive textbook. I am also grateful to the numerous clinicians who have provided videos to better educate/demonstrate surgical techniques and concepts with PRF, which will greatly enhance the learning experience of the reader.

The book is divided into four primary sections, including *(1)* biology of PRF, *(2)* periodontology, *(3)* implant dentistry, and *(4)* additional dental and medical applications. The book aims to take the reader from a basic biologic understanding of PRF through explanations of the various protocols utilized followed by application of these concepts in numerous clinical scenarios.

I therefore am thrilled to introduce our textbook titled *Understanding Platelet-Rich Fibrin*. I hope you enjoy learning the many aspects centered around the use of PRF in regenerative dentistry.

Acknowledgments

To my parents, family, and friends who have all sacrificed far too often in my pursuit of a career in academic dentistry.

To my classmates, colleagues, and mentors who constantly raised the bar and strived for better.

To Quintessence Publishing for your thorough input in the editing, illustrations, and design of this textbook.

To my family at Lakewood Ranch Dental in Florida who have made clinical practice as enjoyable an experience as can be on a *daily* basis.

To all leaders and researchers alike who have contributed enormously to the field of PRP/PRF and laid the foundation for this textbook to be written.

To the faculty in the Department of Biomedical Sciences and Cell Biology at the University of Western Ontario (London, Canada; BMSc, MSc), the Dental School at the University of Laval (Quebec; DDS), the Department of Music at Berklee College (Boston; MMus), the Department of Periodontology at the University of Bern (PhD, Dr med dent), the Department of Oral Implantology at Wuhan University (China; postdoctoral research fellow), the Plastic Surgery Department at Queen Mary University (London; clinical masters in facial esthetics), and the Department of Periodontology at the University of Illinois at Chicago (clinical masters in periodontology). Your education and mentorship has provided endless opportunities.

And lastly, to the team at Advanced PRF Education (www.prfedu.com) for making excellence in teaching a top priority.

Contributors

Fabrice Baudot, DDS, MSc
Private Practice
Saint-Gély-du-Fesc, France

Luis Bessa, DDS
Director, North Clinic
Porto, Portugal

Mark Bishara, DDS
Private Practice
Bowmanville, Ontario, Canada

Thomas Boas, MSc (Econ)
CEO, Puremed
Roskilde, Denmark

Luigi Canullo, DDS, PhD
Independent Researcher
Rome, Italy

Marco Antonio Castro Pinto, DDS, MSc
Professor, Department of Reconstructive Dentistry
Montemorelos University School of Dentistry
Nuevo Léon, Mexico

Raluca Cosgarea, DDS, MSc, PhD
Professor, Department of Prosthetic Dentistry
Iuliu Haţieganu University
Cluj-Napoca, Romania

Catherine Davies, MBBCh, MBA
Private Practice Specializing in Facial Esthetics
Johannesburg, South Africa

Massimo Del Fabbro, MD, PhD
Professor, Department of Biomedical, Surgical, and Dental Sciences
University of Milan
Milan, Italy

Scott Delboccio, DMD
Private Practice
Naples, Florida

Anika Dham
Research Student, Nova Southeastern University
Fort Lauderdale, Florida

Jonathan Du Toit, DDS, MSc
Department of Periodontics and Oral Medicine
Faculty of Health Sciences
University of Pretoria
Pretoria, South Africa

Meizi Eliezer, DDS, MSc, PhD
Research Associate, Department of Periodontology
School of Dental Medicine
University of Bern
Bern, Switzerland

Masako Fujioka-Kobayashi, DDS, PhD
Professor, Department of Oral and Maxillofacial Surgery
School of Life Dentistry at Tokyo
The Nippon Dental University
Tokyo, Japan

Maria Elisa Galarraga-Vinueza, DDS, MSc, PhD
Professor, School of Dentistry
Universidad de las Américas (UDLA)
Quito, Ecuador

Arun K. Garg, DMD
Private Practice Limited to Implantology
Miami, Florida

Stefan Gerber, MD, DDS, MSc, PhD
Assistant Professor, Department of Cranio-Maxillofacial Surgery
University of Bern
Bern, Switzerland

Ezio Gheno, DDS, PhD
Post-Graduation Program in Dentistry
Fluminense Federal University
Niterói, Rio de Janeiro, Brazil

Alfonso Gil, DDS, MSc
Resident, Fixed and Removable Prosthodontics and Dental Material Science
University of Zurich
Zurich, Switzerland

Howard Gluckman, BDS, MChD (Omp)
Specialist in Periodontics, Implantology, and Oral Medicine
Director of Implant & Aesthetic Academy
Cape Town, South Africa

Reinhard Gruber, PhD
Professor, Department of Oral Biology
Medical University of Vienna
Vienna, Austria

Thomas Lau Hansen, PhD
Puremed
Roskilde, Denmark

Tommy Hardon, DVM
Head Veterinarian, Haslev Dyreklinik
Haslev, Denmark

David Lee Hill, DDS, MSc
Private Practice
Chapel Hill, North Carolina

Søren Jepsen, DDS, MSc, PhD
Director of the Department of Periodontology
University of Bonn
Bonn, Germany

Valerie Kanter, DDS, MSc
Professor, Department of Endodontics
University of California, Los Angeles
Los Angeles, California

Dwayne Karateew, DDS, MSc
Director of Program in Periodontics
University of Illinois at Chicago
Chicago, Illinois

Tomoyuki Kawase, DDS, PhD
Professor, Division of Oral Bioengineering
Institute of Medicine and Dentistry
Niigata University
Niigata, Japan

Johan Lenz, DVM
Veterinarian, Jonas Tornell Veterinär
Ängelholm, Sweden

Marius Leretter, DDS, PhD
University of Medicine and Pharmacy of Timişoara
Vice Dean of Dental School
Timişoara, Romania

Victoria Lima, DDS, MSc
Research Fellow, Division of Periodontics
Institute of Science and Technology
São Paulo State University (UNESP)
São Paulo, Brazil

Richard J. Martin, DDS
Private Practice Limited to Oral and Facial Surgery
Lewisville, Texas

Yuriy May, DMD
Private Practice
Farmington, Connecticut

Brian Mealey, DDS, MS
Professor and Graduate Program Director, Department of Periodontics
University of Texas Health Science Center at San Antonio
San Antonio, Texas

Jacob Coakley Meyer, DVM
Veterinarian, Charlottenlund Dyrehospital
Charlottenlund, Denmark

Richard J. Miron, DDS, BMSc, MSc, PhD, Dr med dent
Group Leader, The Miron Research Lab
Lead Educator, Advanced PRF Education
Venice, Florida

Department of Periodontology
University of Illinois at Chicago
Chicago, Illinois

Omid Moghaddas, DDS, MSc
Assistant Professor, Department of Periodontology
Dental Faculty, Tehran Medical Sciences
Islamic Azad University
Tehran, Iran

Naheed Mohamed, DMD, MSD
Private Practice
Oakville, Ontario, Canada

Vittorio Moraschini, DDS, MSc, PhD
Professor, Department of Oral Surgery
Dental School, Fluminense Federal University
Niterói, Rio de Janeiro, Brazil

Ana Paz, DDS, MS
Private Practice
Lisbon, Portugal

Michael A. Pikos, DDS, MSc
Director, Pikos Institute
Tampa, Florida

Nikola Saulacic, DDS, PhD
Assistant Professor, Department of Cranio-Maxillofacial Surgery
University of Bern
Bern, Switzerland

Benoît Schaller, Dr med, Dr med dent
Professor, Department of Cranio-Maxillofacial Surgery
University of Bern
Bern, Switzerland

Anton Sculean, DMD, Dr med dent, MS, PhD
Professor and Executive Director and Chairman
Department of Periodontology
University of Bern
Bern, Switzerland

Senthil Selvan, DDS
Director, Jeya Dental Clinic
Theni, India

Samer Shaltoni, DMD, MS
Private Practice Limited to Oral Surgery
Buffalo Grove, Illinois

Yoshinori Shirakata, DDS, PhD
Associate Professor, Department of Periodontology
Kagoshima University Graduate School of Medical and Dental Sciences
Kagoshima, Japan

Miguel Stanley, DDS
Private Practice
Lisbon, Portugal

Robert Talac, MD, PhD
Director, Renaxis Spine and Orthopedic Clinic
Houston, Texas

Mustafa Tunali, DDS, PhD
Professor, Department of Periodontology
Haydarpasa Training Hospital
Gulhane Military Medical Academy
Istanbul, Turkey

Delia Tuttle, DDS, MS
Private Practice
Lake Elsinore, California

Hom-Lay Wang, DDS, MSD, PhD
Professor and Director of Graduate Periodontics
Department of Periodontics and Oral Medicine
University of Michigan School of Dentistry
Ann Arbor, Michigan

Hudi Xu, DDS, PhD
Research Associate, Department of Dental Implantology
School of Stomatology
Wuhan University
Wuhan, China

Yufeng Zhang, MD, DDS, PhD
Professor, Department of Dental Implantology
School of Stomatology
Wuhan University
Wuhan, China

Abbreviations

The abbreviations listed here are used throughout the book and are NOT always spelled out in the chapters for ease of reading.

ALP	alkaline phosphatase
AM	amniotic membrane
A-PRF	advanced PRF
BoP	bleeding on probing
BMP	bone morphogenetic protein
CAF	coronally advanced flap
CAL	clinical attachment level
CBC	complete blood count
CEJ	cementoenamel junction
C-PRF	concentrated-PRF
CTG	connective tissue graft
DBBM	deproteinized bovine bone mineral
DFDBA	demineralized freeze-dried bone allograft
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
EMD	enamel matrix derivative
e-PRF	extended-PRF
ePTFE	expanded polytetrafluoroethylene
FDA	US Food and Drug Administration
FDBA	freeze-dried bone allograft
GBR	guided bone regeneration
GF	growth factor
H&E	hematoxylin-eosin stain
hPDLC	human periodontal ligament cell
H-PRF	PRF obtained through horizontal centrifugation
IGF	insulinlike growth factor
IL	interleukin
i-PRF	injectable-PRF
ISQ	implant stability quotient
KTW	keratinized tissue width
L-PRF	leukocyte PRF
LPS	lipopolysaccharide
LSCC	low-speed centrifugation concept
mRNA	messenger RNA
MRONJ	medication-related osteonecrosis of the jaw
MSC	mesenchymal stem cell
OFD	open flap debridement
ONJ	osteonecrosis of the jaw
PD	probing depth
PDGF	platelet-derived growth factor
PPE	personal protective equipment
PPP	platelet-poor plasma
PRF	platelet-rich fibrin
PRGF	plasma rich in growth factors
PRP	platelet-rich plasma
PTFE	polytetrafluoroethylene
RBC	red blood cell
RBH	residual bone height
RCF	relative centrifugal force
RCT	randomized controlled trial
rpm	revolutions per minute
RT-PCR	real-time polymerase chain reaction
SD	standard deviation
SE	standard error
SEM	scanning electron microscopy
TGF-β	transforming growth factor β
TMJ	temporomandibular joint
TNF-α	tumor necrosis factor α
T-PRF	titanium-prepared PRF
VEGF	vascular endothelial growth factor
WBC	white blood cell

1

Evolution of Platelet Concentrates

Contributors

Richard J. Miron

Chapter Highlights

- *Evolution of PRF and the reasons for its discovery*
- *Discussion of PRP vs PRGF vs PRF vs L-PRF, A-PRF, etc*
- *Biologic background of key steps involved during wound healing*

Video 1-1

Platelet concentrates were derived more than 20 years ago following the discovery that platelets themselves act as key regulators during the wound healing process. Initial attempts were first made to concentrate these cells using anticoagulants and a centrifugation device; the resulting biomaterial was called *platelet-rich plasma* (PRP). Shortly thereafter, protocols were developed with the aim of avoiding the use of anticoagulants altogether, because clotting is a pivotal step during the wound healing cascade; the resulting biomaterial was called *platelet-rich fibrin* (PRF). Today, platelet concentrates have become incredibly relevant worldwide, with their use spanning across nearly every field of regenerative medicine. Furthermore, one of the main growth factors (GFs) found in platelets—platelet-derived growth factor (PDGF)—has been commercialized as a ready-made laboratory recombinant protein under the trade name GEM 21S (Lynch Biologics). Thus, as medicine has continued to evolve and progress, an obvious and clear trend favoring GF use has been established. Furthermore, by modifying centrifugation devices and spin protocols of PRP/PRF, a greater ability to concentrate not only platelets but also leukocytes became possible, further favoring tissue regeneration. This chapter takes a deep look at the years of research leading to the significant advancement that has been made in this field. The evolution from PRP to PRF, including pioneering concepts such as the low-speed centrifugation concept and horizontal centrifugation, are discussed in terms of their ability to favor higher cell content, GF concentration, and ultimately better wound healing.

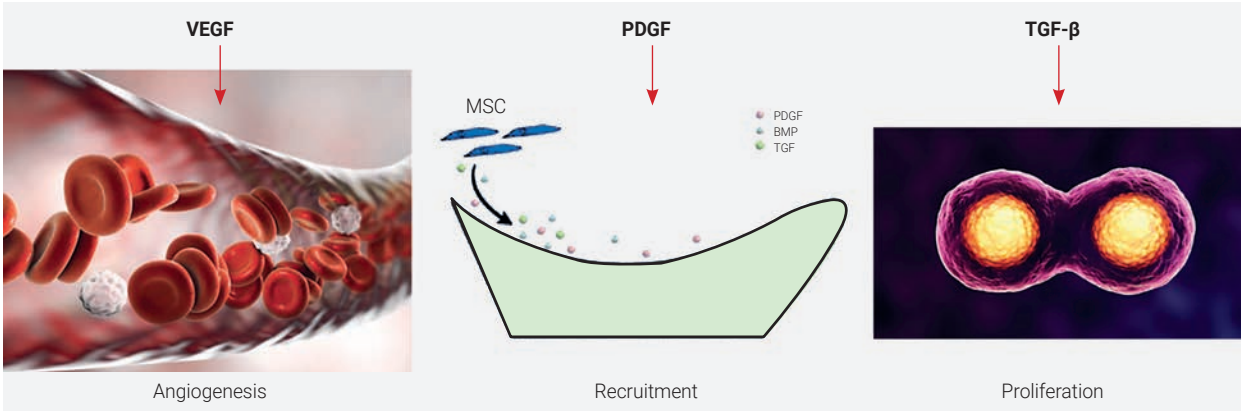


Fig 1-1 The three main GFs that are released from PRF include VEGF, a known inducer of angiogenesis; PDGF, a known inducer of cell recruitment; and TGF-β1, a known stimulator of cell proliferation. MSC, mesenchymal stem cell.

Platelet concentrates have been utilized in medicine for over two decades because of their ability to rapidly secrete autologous GFs and ultimately speed wound healing. They have gained tremendous momentum as a regenerative agent derived from autologous sources capable of stimulating tissue regeneration in a number of medical fields.[1,2] Many years ago, it was proposed that by concentrating platelets using a centrifugation device, GFs derived from blood could be collected from a platelet-rich plasma layer and later utilized in surgical sites to promote local wound healing.[1,2] Today, it has been well established that platelet concentrates act as a potent mitogen capable of the following (Fig 1-1):

- Speeding the revascularization of tissues (angiogenesis)
- Recruiting various cells including stem cells
- Inducing the prompt multiplication of various cell types found in the human body (proliferation)

Wound healing is a complex biologic process whereby many cell types interact with one another as well as their local extracellular matrix (ECM) in order to repair and regenerate damaged tissues.[3–6] While many regenerative agents currently exist on the market to help speed tissue regeneration, it is important to note that the majority are derived from other human sources (allografts) and animal byproducts. These naturally create a foreign body reaction when implanted into host tissues. While the majority of such biomaterials do certainly favor improved healing, it has generally been recognized and accepted that the gold standard for the majority of tissue-regenerative procedures in basically every field of medicine has been the use of autogenous tissues.

Specifically in dentistry, platelet concentrates were introduced over 20 years ago by Robert E. Marx and colleagues with the aim of concentrating blood proteins as a natural source of GFs that would stimulate vascularization (angiogenesis) and tissue ingrowth based on the fact that blood supply is pivotal for tissue regeneration of all tissues.[7] Wound healing has been described as a four-step process that includes *(1)* hemostasis, *(2)* inflammation, *(3)* proliferation, and *(4)* maturation[8–10] (Fig 1-2). Each phase overlaps one another and encompasses various microenvironments, including different cell types that assist in wound healing. Noteworthy are the implications of immune cells during biomaterial integration. In a study titled "OsteoMacs: Key players around bone biomaterials," osteal macrophages were discussed as being key and pivotal cells during the wound healing process.[11] Thus, as tissue biology has continued to evolve, platelet concentrates have also seen significant advancement with respect to their ability to favor healing by incorporating immune cells (leukocytes). Various systematic reviews from multiple fields of medicine have now demonstrated their ability to support tissue regeneration across many tissue types and cell types. This chapter reviews the evolution of platelet concentrates.

PRP (1990s)

The use of platelet concentrates has slowly and gradually gained popularity over time, with a dramatic increase

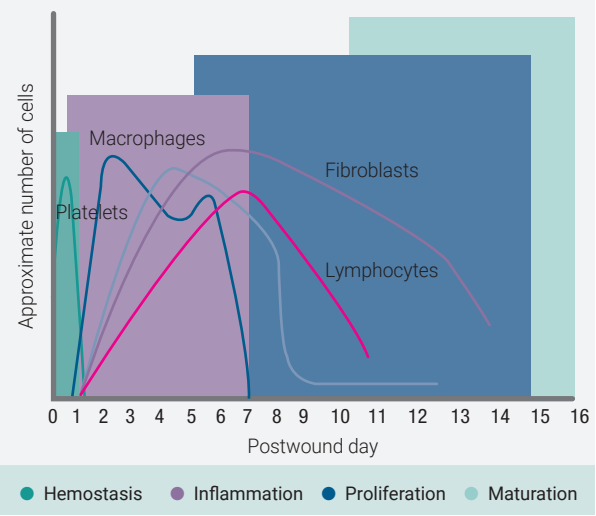


Fig 1-2 Four phases of wound healing: *(1)* hemostasis, *(2)* inflammation, *(3)* proliferation, and *(4)* maturation. Noteworthy are the overlaps between each of the phases and the population of cells found in each category. Whereas lymphocytes typically arise at 7 days, the ability of PRF to introduce a high number at day 0 acts to speed the regenerative phase during this process.

being observed in the past 5 to 10 years. This parallels precisely the massive increase in research articles being published on the topic. Despite this, it is important to review and highlight the pioneering work conducted by Marx and colleagues over 20 years ago, without which none of this textbook would exist.[12–14]

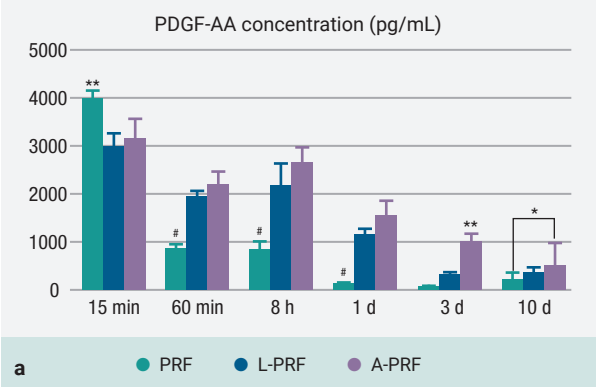
Platelet-rich plasma (PRP), as its name implies, was designed to accumulate platelets in supraphysiologic doses within the plasma layer following centrifugation. The main aim of PRP was to isolate and further concentrate the highest quantity of platelets and their associated GFs for regenerative purposes, thereafter reimplanting this specialized supraconcentrate at sites of local injury. This concept has been the basis of thousands of research articles, with their protocols being utilized to favor wound healing in millions of patients.

Initial protocols typically ranged in duration from 30 minutes to 1 hour based on the centrifugation/collection systems and protocols utilized. The original concept was pioneered by Harvest Technology, where it was shown that over 95% platelet concentration could be accumulated, having the potential to help favor the regenerative phase of many cell types including soft tissues, epithelial cells, periodontal ligament cells, and bone cells.[15,16] Because these initial protocols were lengthy, anticoagulants were added to the blood collection tubes. These typically were various forms of concentrated bovine thrombin or sodium citrate.

Despite its growing success and continued use after its discovery, several reported limitations existed with these initial formulations of PRP. The 30-minute or longer technique was generally considered lengthy for routine dental or medical practice, and more importantly, the use of anticoagulants was shown to limit wound healing from reaching its maximum potential. Simply put, when injury is created following an open wound, a blood clot is one of the first steps that occurs in order for healing to take place. Shortly thereafter, cells and GFs get trapped within this newly formed ECM, and the wound healing process/cascade begins. By limiting the body's ability to form a stable clot, wound healing is limited. Several studies have now demonstrated the superior outcomes of platelet-rich fibrin (PRF) when compared to PRP simply by removing anticoagulants from their formulations.[17–21] Even the pioneering research team behind the plasma rich in growth factors (PRGF) concept (Anitua et al) have since demonstrated more physiologic healing ability with anticoagulant removal.[17]

Another drawback of PRP was the fact that it remained liquid by nature (due to the use of anticoagulants), so when it was combined with biomaterials, a much faster delivery of GFs was observed (Fig 1-3). While an initial burst of GFs is typical of PRP therapy, a slower release of GFs over an extended period of time has been shown to better stimulate cell growth and tissue regeneration.[22,23]

Much advancement related to PRP therapy has been made over the past 20 years, and two excellent textbooks have been written by its pioneers—*Dental and Craniofacial Applications of Platelet-Rich Plasma* by Robert E. Marx and Arun K. Garg (Quintessence, 2005), and *Autologous Blood Concentrates* by Arun K. Garg (2018). Its breakthrough features include the novel ability to concentrate platelets



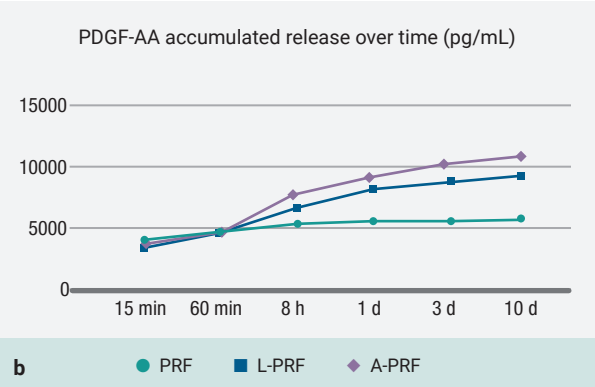


Fig 1-3 *(a and b)* GF release from PRP and PRF at each time point of PDGF-AA over a 10-day period. Notice that while PRP has significantly higher GF release at early time points, over a 10-day period, significantly higher levels are most commonly found with A-PRF due to the slow and gradual release of GFs utilizing slower centrifugation speeds. (Adapted from Kobayashi et al.[19])

to supraphysiologic doses and further stimulate tissue regeneration across virtually all tissue types. For these reasons, PRP has not surprisingly been utilized in practically every field of medicine.

Snapshot of PRP

- Marx was the first to show that a concentration of platelets could favor tissue regeneration in the oral cavity.
- A subsequent device was brought to market thanks to these breakthrough research projects conducted at the University of Miami (Harvest system).
- PRP is credited for having exponentially grown the entire field of platelet concentrates, including its subcategories such as PRF.

L-PRF (2000–2010)

Because the anticoagulants utilized in PRP prevented clotting, pioneering work performed by Dr Joseph Choukroun and Dr David Dohan Ehrenfest led to the development of PRF.[24] The aim was to develop a second-generation platelet concentrate focused on anticoagulant removal. Because anticoagulants were removed, a much quicker working time was needed, and centrifugation had to begin shortly after blood draw (otherwise, the blood would naturally clot). Furthermore, high g-force centrifugation protocols were initially utilized in an attempt to separate blood layers prior to clotting. The final spin cycle (initial studies ranged from 2500–3000 rpm for 10–12 minutes = ~700g) resulted in a plasma layer composed of a fibrin clot with entrapment of platelets and leukocytes. The main advantage of this fibrin matrix was its ability to release GFs over an extended period of time while the fibrin clot was being degraded.[25] Over the years, PRF has been termed *L-PRF* (for *leukocyte platelet-rich fibrin*) due to the discoveries that several leukocytes remained incorporated in PRF and that white blood cells play a central and key role in the tissue healing process. The most commonly utilized protocol today is a spin cycle at 3000 rpm for 10 minutes or 2700 rpm for 12 minutes (RCF-max = ~700g, RCF-clot = ~400g).

Several other advantages also existed during clinical use because it avoided the need for dual-spin protocols requiring pipetting or various specialized tube compartments, which made the overall procedure much more user-friendly, cheaper, and faster when compared to PRP. Original protocols were purposefully designed to spin at high centrifugation speeds with the main aim of phase separation to occur as quickly as possible in order to separate the red corpuscle base layer from the upper plasma layer prior to clotting. Following centrifugation, a platelet-rich fibrin mesh was formed, giving it the working name *PRF*[26–28] (Fig 1-4). PRF has since been highly researched, with over 1,000 publications dedicated to this topic alone.

Additionally, research teams from around the world have demonstrated the impact of leukocytes on tissue healing.[29–34] While it was once thought that the additional benefit of leukocyte incorporation into PRF was its main properties in improved host defense to foreign pathogens,[29–34] it has since been shown in well-conducted basic research studies

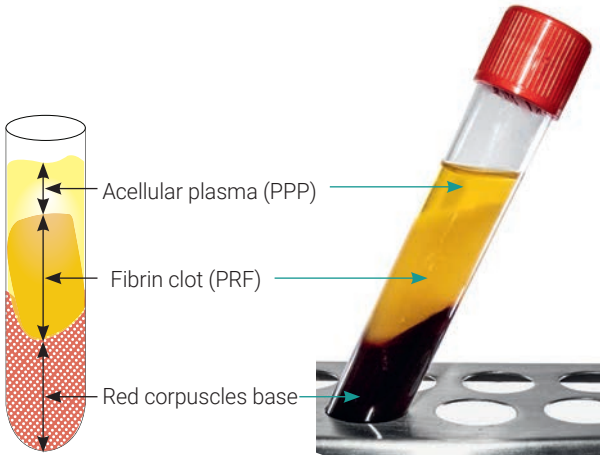


Fig 1-4 Layers produced after centrifugation of whole blood. A PRF clot forms in the upper portion of tubes after centrifugation.

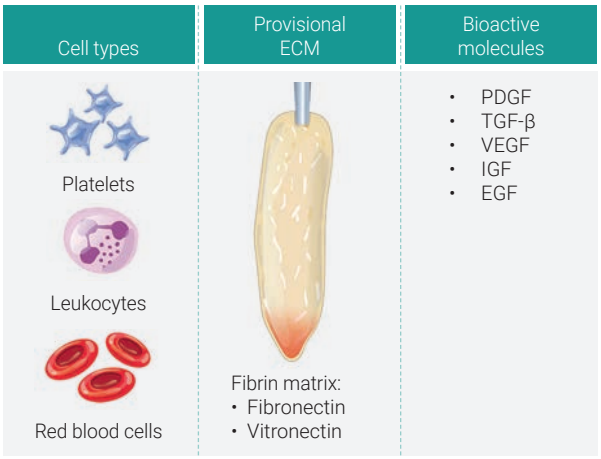


Fig 1-5 Three main components of PRF all derived naturally from the human body. These include *(1)* cell types (platelets, leukocytes, and red blood cells); *(2)* a provisional ECM 3D scaffold fabricated from autologous fibrin (including fibronectin and vitronectin); and *(3)* a wide array of over 100 bioactive molecules, including most notably PDGF, TGF-β, VEGF, IGF, and EGF.

that leukocytes are pivotal to tissue regeneration and favor faster wound healing also.[11,35–37] In dentistry, where the oral cavity is filled with bacteria and microbes, the inclusion of leukocytes was initially thought to play a pivotal role in wound healing by participating in the phagocytosis of debris, microbes, and necrotic tissues, as well as directing the future regeneration of these tissues through the release of several cytokines and GFs and orchestrating cell-to-cell communication between many cell types.

Tissue engineering with PRF

Tissue engineering has been an emerging discipline over the past decade, with major breakthroughs routinely being made every year. At its simplest foundation, tissue engineering requires three parameters: *(1)* a scaffold responsible to support tissue ingrowth, *(2)* cells that may act to promote tissue regeneration, and *(3)* GFs that stimulate the overall wound healing events. Unlike the majority of biomaterials currently available on the market, PRF actually contains each of these three properties (Fig 1-5). For comparative purposes, routine bone allografts contain a scaffold (mineralized cortical/cancellous bone) and GFs embedded in its bone matrix (such as bone morphogenetic protein 2 [BMP-2]) but have no cells. Recombinant human GFs typically have a GF (for instance, rhBMP-2) and a carrier (collagen sponge) but also lack cells. Certain stem technologies typically contain cells and also a delivery system (for instance a nanocarrier delivery system) but lack GFs. The ability to actually contain each of the three tissue engineering properties within a single biomaterial is quite rare and, more importantly, usually extremely expensive (think recombinant GFs and/or stem cell technology).

PRF, on the other hand, is a particularly simple and inexpensive way to utilize the three principles of tissue engineering by utilizing a 3D scaffold (fibrin) that incorporates both regenerative host cells (platelets and leukocytes) and various GFs. These include PDGF, TGF-β, and VEGF, each of which is crucial during the regeneration process. Furthermore, the concentrated leukocytes (as opposed to simply platelets) in PRF have been well implicated as key regulators of tissue healing and formation.[26–28,31,38]

Snapshot of PRF

- PRF is considered a second-generation platelet concentrate with a longer GF release profile.
- Centrifugation protocols are shorter and do not need any chemical additives such as anticoagulants.
- PRF falls more in line with tissue engineering principles in that it is not only an accumulation of cells and GFs but also a scaffold (fibrin matrix).
- PRF incorporates leukocytes, which are key cells in pathogen defense and biomaterial integration.

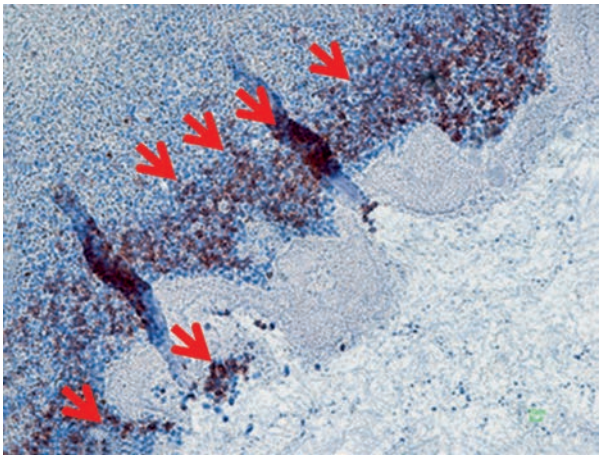


Fig 1-6 Histologic observation of leukocytes following centrifugation. Resulting white blood cells have been shown to be contained basically in the layers between the plasma PRF layer and the red blood cell clot. This finding demonstrated quite clearly that the g-force was excessive, necessitating the development of newer protocols aimed to improve the retention of leukocytes within the PRF matrix. (Reprinted with permission from Ghanaati et al.[39])

Fig 1-7 Newer centrifugation protocols allow production of a liquid formulation of PRF found in the top 1- to 2-mL layer of centrifugation tubes following a 3- to 5-minute protocol. This liquid can be collected in a syringe and reinjected into defect sites or mixed with biomaterials to improve their bioactive properties. (Reprinted with permission from Davies and Miron.[40])

A-PRF and i-PRF (2014–2018)

While much of the research performed in the late 2000s and early 2010s was dedicated to the clinical uses and indications of L-PRF discussed later in this textbook, major discoveries were made several years later from basic research laboratories. Following extensive clinical use and research with the original L-PRF protocol, it was discovered in 2014 by Dr Shahram Ghanaati that centrifugation carried out at relatively high centrifugation speeds (~700g) led to the great majority of leukocytes being located either at the buffy coat zone (between the red blood cell layer and the upper plasma layer) or more commonly at the bottom of centrifugation tubes (Fig 1-6).[39] It was expressed that the longer the centrifugation time is carried out, the more likely it is that cells get pushed further down the centrifugation tube. Similarly, the faster the spin centrifugation speed (higher g-force), the greater the proportion of cells found in the lower levels of centrifugation tubes.

Pioneering research within his laboratory led to the development of an advanced PRF (A-PRF) whereby lower centrifugation speeds (~200g) led to a higher accumulation of platelets and leukocytes more evenly distributed throughout the upper PRF layers. These newer protocols more favorably led to a higher release and concentration of GFs over a 10-day period when compared to PRP or L-PRF.[19] In 2015 to 2017, our research team further demonstrated that optimization of PRF could be achieved by reducing not only centrifugation speed but also the time involved. The A-PRF protocol was therefore modified from 14 minutes at 200g as originally described in 2014 down to an 8-minute protocol.[19]

Following an array of basic research studies on this topic, it was observed that by further reducing the g-force and also the time, it was possible to obtain a plasma layer that had not yet converted into fibrin (ie, scientifically liquid fibrinogen but often referred to as *liquid-PRF* for simplicity). In a study titled "Injectable platelet rich fibrin (i-PRF): Opportunities in regenerative dentistry?",[20] it was demonstrated that at lower centrifugation speeds and times (~60g for 3 minutes), a liquid-PRF (termed *injectable-PRF* or *i-PRF*) could be obtained. While these protocols typically produced minimal volumes (~1.0–1.5 mL), it was shown that both platelets and leukocytes were even more highly concentrated when compared to L-PRF or A-PRF (Fig 1-7).[40] This liquid-PRF layer could be utilized clinically for approximately 15 to 20 minutes, during which time fibrinogen and thrombin had not yet converted to a fibrin matrix (ie, remained liquid). This has since been utilized for injection into various joints/spaces similar to PRP, however with the reported advantages of a longer GF release time. Furthermore, the concept of "sticky" bone was also developed. Importantly, a different type of tube (plastic) was needed to minimize clotting, as will be discussed in detail in chapter 5.

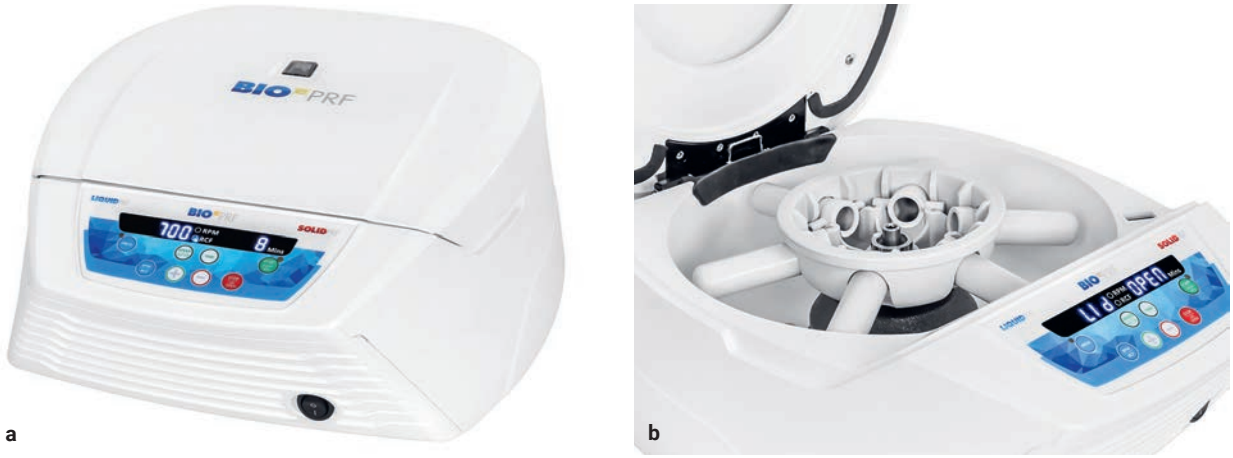


Fig 1-8 *(a)* Clinical photograph of a Bio-PRF centrifuge. *(b)* Photograph demonstrating the horizontal centrifugation concept. The tubes are inserted vertically (up and down), but once the device begins to rotate, the tubes swing out completely horizontally. This favors better blood cell layer separation with higher platelet and GF concentrations.

Snapshot of A-PRF and i-PRF

- Original L-PRF protocols were shown to be too fast, leading to all the cells being accumulated only at the buffy coat zone, with the majority of leukocytes found within the red blood cell layer.
- The low-speed centrifugation concept was shown in 2014 to favor a higher concentration of cells within PRF membranes.
- By further lowering speed and time, a liquid-PRF formulation became available, commonly known as *injectable-PRF* (or i-PRF).

Video 1-2

H-PRF and C-PRF (2019–Present)

Very recently, our research group discovered through a series of basic laboratory experiments that horizontal centrifugation led to significantly greater concentrations of platelets and leukocytes when compared to currently available fixed-angle centrifugation devices most commonly utilized to produce L-PRF and A-PRF. Simply, horizontal centrifuges are routinely utilized in high-end research laboratories as well as in medical hospitals because of their greater ability to separate layers based on density (Fig 1-8; see also chapters 2 and 3). Unlike fixed-angle centrifugation systems whereby the tubes are actually inserted at a 45-degree angle, in horizontal centrifugation systems (often referred to as *swing-out bucket centrifugation*), the tubes have the ability to swing out to 90 degrees once they are in rotation (Video 1-2). Amazingly, the original PRP systems developed by Harvest and Marx utilized and still use this technology.

In 2019, an article on the topic demonstrated clearly that horizontal centrifugation could lead to up to a four-times greater cell content when compared to fixed-angle centrifugation.[41] This represented a marked ability to greatly concentrate cells found within PRF, which were primarily being accumulated on the back distal surfaces of PRF tubes (Fig 1-9). The major disadvantage of fixed-angle centrifugation is that during the spin cycle, cells are typically driven along the back wall of the centrifugation tubes at high g-forces (Fig 1-10). This also exposes cells to higher compressive forces against the back wall, and

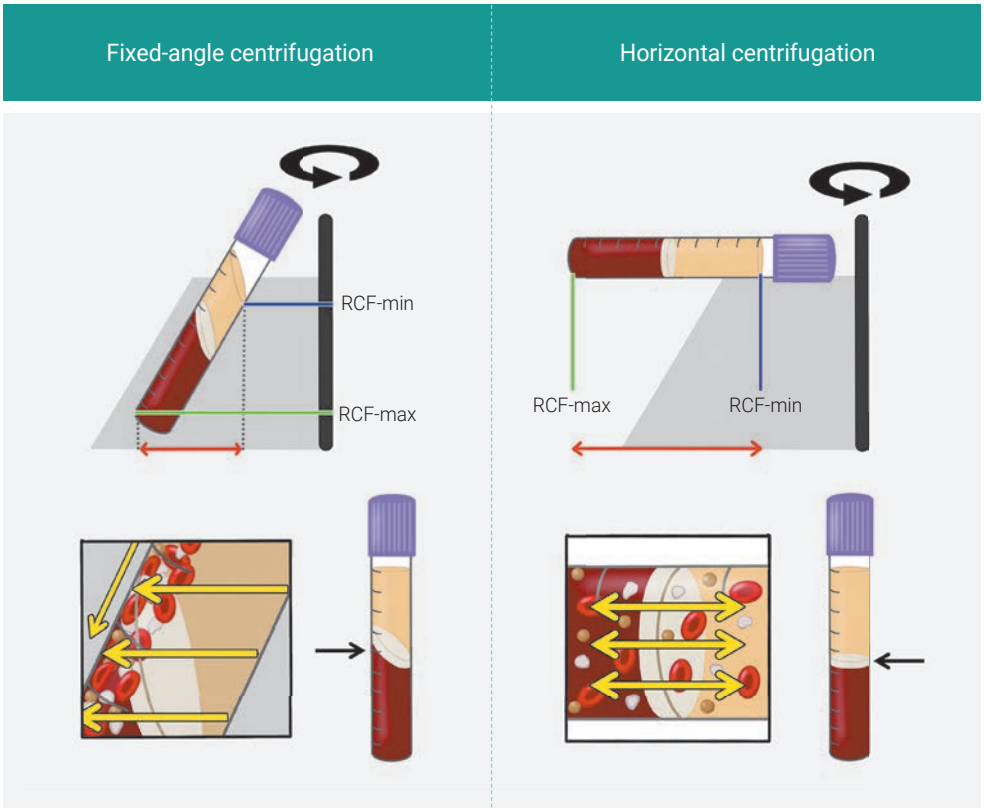


Fig 1-9 Illustrations comparing fixed-angle and horizontal centrifuges. With horizontal centrifugation, a greater separation of blood layers based on density is achieved owing to the greater difference in RCF-min and RCF-max. Following centrifugation on fixed-angle centrifuges, blood layers do not separate evenly, and as a result, an angled blood separation is observed. In contrast, horizontal centrifugation produces even separation. Owing to the large RCF values (~200g–700g), the cells are pushed toward the outside and downward. On a fixed-angle centrifuge, cells are pushed toward the back of centrifugation tubes and then downward/upward based on cell density. These g-forces produce additional shear stress on cells as they separate based on density along the back walls of centrifugation tubes. In contrast, horizontal centrifugation allows for the free movement of cells to separate into their appropriate layers based on density, allowing for better cell separation as well as less trauma/shear stress on cells. (Modified from Miron et al.[41])

cells must then separate by traveling either up or down the inclined centrifugation slope based on their respective cell density differences. Because red blood cells are larger and heavier than platelets and leukocytes, they travel downward, whereas lighter platelets travel toward the top of the tube where PRF is collected. This makes it relatively difficult for the small cell types such as platelets and leukocytes to reach the upper layer, especially granted that red blood cells outnumber in particular white blood cells typically by 1,000-fold (see chapter 2). Therefore, it is not possible to reach optimal accumulation of platelets or leukocytes using a fixed-angle centrifuge.

Furthermore, by utilizing a novel method to quantify cell types found in PRF, it was possible to substantially improve standard i-PRF protocols that favored only a 1.5- to 3-fold increase in platelets and leukocytes. Noteworthy is that several research groups began to show that the final concentration of platelets was only marginally improved in i-PRF when compared to standard baseline values of whole blood.[41,42] In addition, significant modifications to PRF centrifugation protocols have further been developed, demonstrating the ability to improve standard i-PRF protocols toward liquid formulations that are significantly more concentrated (C-PRF) with over 10- to 15-times greater concentrations of platelets and leukocytes when compared to i-PRF (see chapters 2 and 3). Today, C-PRF has been established as the most highly concentrated PRF protocol described in the literature.

Fig 1-10 Visual representation of layer separation following either L-PRF or H-PRF protocols. L-PRF clots are prepared with a sloped shape, and multiple red dots are often observed on the distal surface of PRF tubes, while H-PRF results in horizontal layer separation between the upper plasma and lower red corpuscle layer.

Snapshot of H-PRF and C-PRF

- Horizontal centrifugation leads to up to a four-times greater accumulation of platelets and leukocytes when compared to fixed-angle centrifugation systems commonly utilized to produce L-PRF and A-PRF.
- Cells accumulate evenly when PRF is produced via horizontal centrifugation as opposed to along the back distal surface of PRF tubes on fixed-angle centrifuges.
- Standard i-PRF can be further improved with horizontal centrifugation.

Conclusion

Platelet concentrates have seen a wide and steady increase in popularity since they were launched more than two decades ago. While initial concepts launched in the 1990s led to the working name *platelet-rich plasma*, subsequent years and discoveries have focused more specifically on their anticoagulant removal (ie, PRF). Several recent improvements in centrifugation protocols, including the low-speed centrifugation concept and horizontal centrifugation, have led to increased concentrations of GFs and better healing potential. Both solid-PRF as well as liquid-based formulations now exist, with an array of clinical possibilities created based on the ability to accumulate supraphysiologic doses of platelets and blood-derived GFs. Future strategies to further improve PRF formulations and protocols are continuously being investigated to additionally improve clinical practice utilizing this technology.

References

1. Anfossi G, Trovati M, Mularoni E, Massucco P, Calcamuggi G, Emanuelli G. Influence of propranolol on platelet aggregation and thromboxane B_2 production from platelet-rich plasma and whole blood. Prostaglandins Leukot Essent Fatty Acids 1989;36:1–7.
2. Fijnheer R, Pietersz RN, de Korte D, et al. Platelet activation during preparation of platelet concentrates: A comparison of the platelet-rich plasma and the buffy coat methods. Transfusion 1990;30: 634–638.
3. Coury AJ. Expediting the transition from replacement medicine to tissue engineering. Regen Biomater 2016;3:111–113.
4. Dai R, Wang Z, Samanipour R, Koo KI, Kim K. Adipose-derived stem cells for tissue engineering and regenerative medicine applications. Stem Cells Int 2016;2016:6737345.
5. Rouwkema J, Khademhosseini A. Vascularization and angiogenesis in tissue engineering: Beyond creating static networks. Trends Biotechnol 2016;34:733–745.
6. Zhu W, Ma X, Gou M, Mei D, Zhang K, Chen S. 3D printing of functional biomaterials for tissue engineering. Curr Opin Biotechnol 2016;40:103–112.
7. Upputuri PK, Sivasubramanian K, Mark CS, Pramanik M. Recent developments in vascular imaging techniques in tissue engineering and regenerative medicine. Biomed Res Int 2015;2015:783983.
8. Gosain A, DiPietro LA. Aging and wound healing. World J Surg 2004;28:321–326.
9. Eming SA, Brachvogel B, Odorisio T, Koch M. Regulation of angiogenesis: Wound healing as a model. Prog Histochem Cytochem 2007;42:115–170.
10. Eming SA, Kaufmann J, Lohrer R, Krieg T. Chronic wounds: Novel approaches in research and therapy [in German]. Hautarzt 2007;58:939–944.
11. Miron RJ, Bosshardt DD. OsteoMacs: Key players around bone biomaterials. Biomaterials 2016;82:1–19.
12. de Vries RA, de Bruin M, Marx JJ, Hart HC, Van de Wiel A. Viability of platelets collected by apheresis versus the platelet-rich plasma technique: A direct comparison. Transfus Sci 1993;14:391–398.
13. Whitman DH, Berry RL, Green DM. Platelet gel: An autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surgery 1997;55:1294–1299.
14. 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 Endodontol 1998;85:638–646.
15. Jameson C. Autologous platelet concentrate for the production of platelet gel. Lab Med 2007;38:39–42.
16. Marx RE. Platelet-rich plasma: Evidence to support its use. J Oral Maxillofac Surg 2004;62:489–496.
17. Anitua E, Prado R, Troya M, et al. Implementation of a more physiological plasma rich in growth factor (PRGF) protocol: Anticoagulant removal and reduction in activator concentration. Platelets 2016;27:459–466.
18. Abd El Raouf M, Wang X, Miusi S, et al. Injectable-platelet rich fibrin using the low speed centrifugation concept improves cartilage regeneration when compared to platelet-rich plasma. Platelets 2019;30:213–221.
19. Kobayashi E, Fluckiger L, Fujioka-Kobayashi M, et al. Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clin Oral Investig 2016;20:2353–2360.
20. Miron RJ, Fujioka-Kobayashi M, Hernandez M, et al. Injectable platelet rich fibrin (i-PRF): Opportunities in regenerative dentistry? Clin Oral Investig 2017;21:2619–2627.
21. Wang X, Zhang Y, Choukroun J, Ghanaati S, Miron RJ. Effects of an injectable platelet-rich fibrin on osteoblast behavior and bone tissue formation in comparison to platelet-rich plasma. Platelets 2018;29:48–55.
22. Lucarelli E, Beretta R, Dozza B, et al. A recently developed bifacial platelet-rich fibrin matrix. Eur Cell Mater 2010;20:13–23.
23. Saluja H, Dehane V, Mahindra U. Platelet-rich fibrin: A second generation platelet concentrate and a new friend of oral and maxillofacial surgeons. Ann Maxillofac Surg 2011;1:53–57.
24. Choukroun J, Adda F, Schoeffler C, Vervelle A. Une opportunité en paro-implantologie: Le PRF. Implantodontie 2001;42:e62.
25. 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.
26. 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:e56–e60.
27. 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:e37–e44.
28. 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:e45–e50.
29. Martin P, Leibovich SJ. Inflammatory cells during wound repair: The good, the bad and the ugly. Trends Cell Biol 2005;15:599–607.
30. Tsirogianni AK, Moutsopoulos NM, Moutsopoulos HM. Wound healing: Immunological aspects. Injury 2006;37(suppl 1):S5–S12.
31. Adamson R. Role of macrophages in normal wound healing: An overview. J Wound Care 2009;18:349–351.
32. Davis VL, Abukabda AB, Radio NM, et al. Platelet-rich preparations to improve healing. Part I: Workable options for every size practice. J Oral Implantol 2014;40:500–510.
33. Davis VL, Abukabda AB, Radio NM, et al. Platelet-rich preparations to improve healing. Part II: Platelet activation and enrichment, leukocyte inclusion, and other selection criteria. J Oral Implantol 2014;40:511–521.
34. Ghasemzadeh M, Hosseini E. Intravascular leukocyte migration through platelet thrombi: Directing leukocytes to sites of vascular injury. Thromb Haemost 2015;113:1224–1235.
35. Batoon L, Millard SM, Raggatt LJ, Pettit AR. Osteomacs and bone regeneration. Curr Osteoporos Rep 2017;15:385–395.
36. Chang MK, Raggatt LJ, Alexander KA, et al. Osteal tissue macrophages are intercalated throughout human and mouse bone lining tissues and regulate osteoblast function in vitro and in vivo. J Immunol 2008;181:1232–1244.
37. 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.
38. 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:e51–e55.
39. 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.
40. Davies C, Miron RJ. PRF in Facial Esthetics. Chicago: Quintessence, 2020.
41. Miron RJ, Chai J, Zheng S, Feng M, Sculean A, Zhang Y. A novel method for evaluating and quantifying cell types in platelet rich fibrin and an introduction to horizontal centrifugation. J Biomed Mater Res A 2019;107:2257–2271.
42. Varela HA, Souza JCM, Nascimento RM, et al. Injectable platelet rich fibrin: Cell content, morphological, and protein characterization. Clin Oral Investig 2019;23:1309–1318.

2

Biology of PRF: Fibrin Matrix, Growth Factor Release, and Cellular Activity

Contributors

Masako Fujioka-Kobayashi
Yufeng Zhang
Reinhard Gruber
Richard J. Miron

Chapter Highlights

- *What is PRF?*
- *How does PRF differ from PRP at the biologic and cellular level?*
- *What is the role of each cell type found in PRF?*
- *What is the role of each GF found in PRF?*
- *How does centrifugation speed and time affect PRF?*
- *What advantages exist for horizontal centrifugation versus fixed-angle centrifugation?*

Video 2-1

Much can be discussed with respect to the biology of PRF and its ability to impact tissue regeneration. During the natural wound healing process, vascularization of tissues plays a pivotal role, facilitating the invasion of incoming cells, growth factors (GFs), cytokines, and other regenerative factors. The main aim of platelet concentrates, discovered over two decades ago, is to favor new blood flow (angiogenesis) to damaged tissues, thereby improving their healing potential by delivering a supraphysiologic concentration of blood-derived cells (namely platelets) and regenerative GFs. This chapter takes a deep look into the actual separation of blood layers during the centrifugation process to provide the clinician a general overview of the cell types and GFs found in PRF, including their roles, and also discusses the effects of centrifugation speed and time on cell layer separation. Furthermore, the advantages of producing an autologous fibrin scaffold are presented as it being a key regulator of wound healing because of its autologous source and its ability to promote the slow and gradual release of GFs over time. The advantages of horizontal centrifugation versus fixed-angle centrifugation are also discussed based on recent data from various laboratories from around the world.

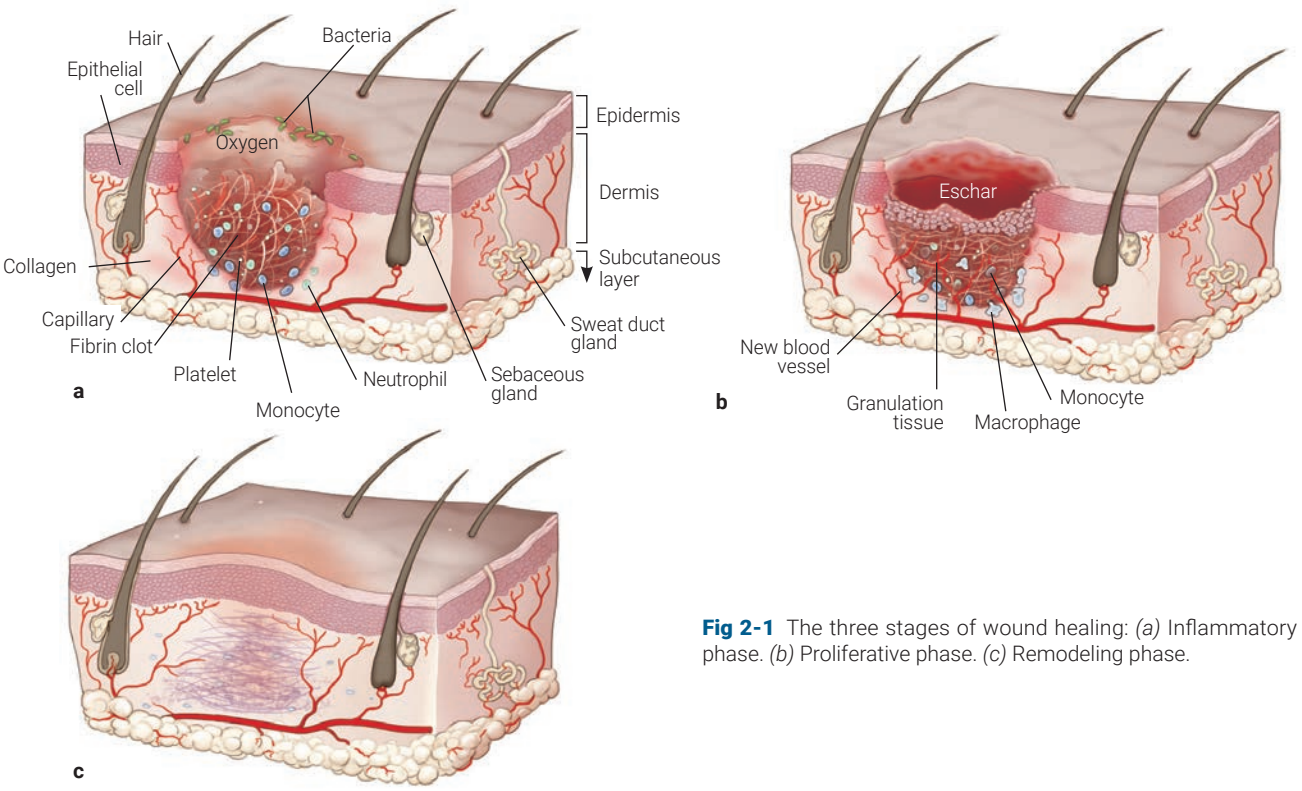


Fig 2-1 The three stages of wound healing: *(a)* Inflammatory phase. *(b)* Proliferative phase. *(c)* Remodeling phase.

The wound healing process is divided into three stages: the inflammatory phase, the proliferative phase, and the remodeling phase (Fig 2-1). The inflammatory phase starts at the time of injury and generally involves a wide array of cytokines and growth factors (GFs) that are released within the first 24 to 48 hours. Accordingly, a dynamic interaction occurs between endothelial cells, angiogenic cytokines, and the extracellular matrix (ECM) in an attempt to accelerate wound healing via an orchestrated delivery of multiple GFs in a well-controlled fashion.[1]

In general, blood provides essential components to the healing process that comprise both cellular and protein products that essentially are the base components of wound healing. During the healing process, blood will undergo clotting within a few minutes to prevent further blood loss. This is an important step that will be later discussed in the PRF tube section, because in order for clotting to occur and even be improved in both speed and quality (in particular in patients taking anticoagulants), a proper understanding of the clotting cascade is required. In its simplest of forms, oxygen helps improve blood clotting, and for this reason, the simple removal of centrifugation tube lids following the spin process will lead to faster clotting of PRF and a superior fibrin mesh. One of the major roles of platelets is to assist during hemostasis through a fibrin clot formation.[1,2] Not surprisingly, the additional use of PRF for wound healing (for example, following tooth removal and extraction socket healing) in patients taking anticoagulants can drastically improve the healing outcomes simply by improving clotting. Because PRF contains many platelets and a fibrin nucleus is already formed, bleeding has been shown to be significantly reduced postoperative when PRF is utilized in patients on anticoagulant therapy.[3]

Tips

- Oxygen helps improve blood clotting, so the simple removal of centrifugation tube lids following the spin process will lead to faster clotting of PRF.
- In patients undergoing anticoagulant therapy, the simple addition of PRF during surgery can help favor faster clotting, thereby reducing bleeding times postoperative.

Table 2-1 Properties of cells found in whole blood

	Platelets	WBCs	RBCs
Density (kg/m³)	1040–1065	1055–1085	1095–1100
Frequency (1/µL)	200,000	5,000	5,000,000
Surface (µm²)	28	330	140
Radius (µm)	11.5	5–7.5	4
Volume (µm³)	14	200	92
Shape	Irregular disc	Spherical	Biconcave

Platelets also release various GFs and cytokines that further lead to tissue regeneration but also attract macrophages and neutrophils to the defect site. These cells are responsible for clearing debris, replacing necrotic tissue, and removing bacteria from the wound site.

The proliferative phase begins by day 3, where the blood clot within the wound is further supplied with a provisional matrix typically composed in part with fibrin, which facilitates cell migration, while the clot within the vessel lumen contributes to hemostasis.[2] Fibroblast cells are recruited to the wound site and begin producing new collagen in a random and somewhat disorganized order. Simultaneously, new blood vessel formation leads to new angiogenesis, and the wound gradually begins to gain initial stability.

During the third and final stage (the remodeling phase), disorganized collagen is replaced by newly organized collagen fibrils that provide enhanced stability and strength to the injured site, where tissue regeneration takes place[4] (see Fig 2-1).

Whole blood is comprised of four main components: blood plasma, red blood cells (RBCs), white blood cells (WBC), and platelets. Initially, platelets were reported as the major responsible component for the activation and release of crucial GFs for wound healing, including PDGF, coagulation factors, adhesion molecules, cytokines, and angiogenic factors. Their role has been extremely well described in the literature, so typically the entire field has been referred to as *platelet concentrates* or *platelet-rich plasma/fibrin*. Interestingly, however, over the years more attention has been placed on leukocytes, which are not only responsible for host defense but also highly implicated in the wound healing and regenerative phases.

Table 2-1 highlights the various cell types found in blood, including their density, frequency, and surface area. Note that while platelets are the lightest of the group, WBCs and RBCs are very similar in density. For these reasons they are also harder to separate in a centrifuge based on density. Noteworthy is the fact that per µL, there are 5,000,000 RBCs when compared to only 5,000 WBCs. Therefore, RBCs outnumber WBCs in a 1,000:1 ratio, which make them difficult to separate, especially on a fixed-angle centrifugation device as discussed later in this chapter (Video 2-2).

Leukocytes and RBCs are similar in density. This makes these two cell types extremely difficult to separate, especially because RBCs outnumber them 1,000 to 1.

Video 2-2

Cells in PRF

As shown in Table 2-1, the three main cell types found in PRP and PRF are platelets, leukocytes (WBCs), and RBCs. The entire initial goal of platelet concentrates was of course to concentrate platelets. Because they are the lightest of all cells found in blood, it was possible to utilize a centrifugation device to separate these layers based on their density. Lighter cells (platelets) could therefore be accumulated to the top, followed by leukocytes. Because RBCs are the densest of the group, they tend to migrate downward during the centrifugation process. In an ideal situation, the final PRF matrix should be composed of a high concentration of

platelets, leukocytes, and fibrin. It has been shown that the initially developed PRF (also termed L-PRF for *leukocyte PRF*) concentrates contained greater than 90% platelets and more than 50% leukocytes within a high-density fibrin network when compared to whole blood.[5] By utilizing more advanced quantification devices and recently developed methods, our research team has been better able to harvest leukocytes specifically. The lower yield of leukocytes is typically a result of their more similar density to RBCs, making them harder to separate from and accumulate in the upper layers where PRF is collected. This is particularly difficult on fixed-angle centrifuges. Several other methods have been proposed to favor accumulation of cells, including shorter centrifugation times as well as lower centrifugation forces, as discussed later in this chapter (see section on the low-speed centrifugation concept).[6]

Leukocytes have been shown to be an integral component of PRF therapy and play a prominent role in wound healing. Studies from basic sciences and animal research have revealed how impactful a role leukocytes play during tissue regeneration by comparing PRP/PRF therapy with and without WBCs.[7–9] In these split design studies, the contralateral side receiving leukocytes performed significantly better, promoting researchers and clinicians to develop protocols to better incorporate or harvest leukocytes. Naturally, PRF contains a higher number of leukocytes when compared to the first-generation platelet concentrates PRP and PRGF.

While the role of leukocytes has been well described as host defense against incoming pathogens, they also play a central role in immune modulation of biomaterials and participate in the wound healing process due to their ability to secrete key immune cytokines such as IL-1β, IL-6, IL-4, and TNF-α.[2,10,11] They have been highly investigated in PRF therapy, with the impact of centrifugation speed and time affecting both their concentration and location, mainly owing to the fact that they are very similar in density and size to RBCs. Previously, it was demonstrated how faster protocols initially utilized to produce L-PRF were far too high in both g-force and time (2700 rpm for 12 minutes; ~700g).[6] This led to the histologic observation that the majority of cells were concentrated either at the buffy coat region or at the bottom of centrifugation tubes within the RBC layer component.[6] Based on these observations, it became clear that centrifugation speeds (g-forces) were evidently too high, pushing leukocytes especially down to the bottom of centrifugation tubes and away from the PRF clot. In order to redistribute leukocyte cell numbers across the entire PRF matrix, both a change in centrifugation speed and/or time (lower) as well as a change in the centrifugation device (horizontal centrifugation as opposed to fixed-angle) were deemed necessary to further improve platelet formulations, as reviewed later in this chapter.

Advantages of a 3D Fibrin Network

Fibrin is the activated form of a plasmatic molecule called *fibrinogen* that converts into fibrin with thrombin. Fibrin formation is one of the first key components to tissue wound healing. When an individual cuts himself, the first event taking place prior to any regeneration is fibrin clot formation. This is why patients on anticoagulant therapy typically do not heal quite as effectively because delayed clotting leads to delayed healing. The obvious advantage of PRF therapy is its ability to accumulate various cell types including platelets without anticoagulants, thereby improving clotting properties. Once a fibrin clot is formed during the centrifugation cycle, cells and GFs are able to be trapped within the 3D fibrin matrix, favoring the slower and gradual release of GFs from PRF over time.[12] Fibrin is a soluble fibrillary molecule that is present in high quantity both in plasma itself as well as in the α-granules of platelets. Fibrin therefore plays a determining role in platelet aggregation during hemostasis and is critical to healing. Not surprisingly, the use of fibrin alone (without GFs or living cells as a fibrin glue) has been shown to lead to matrix stabilization favoring tissue stability, cellular invasion, and ultimately tissue regeneration.[13–15] However, PRF has numerous advantages in that during the fibrin clot formation, a supraphysiologic concentration of platelets, leukocytes, and GFs are also present, forming a sort of "superclot" consisting of an intimate assembly of cytokines, glycanic chains, and structural glycoproteins enmeshed within a slowly polymerized fibrin network[16,17] (Fig 2-2).

PRF forms a "superclot" consisting of an intimate assembly of cytokines, glycanic chains, and structural glycoproteins enmeshed within a slowly polymerized fibrin network.

The fibrin scaffold produced following centrifugation has further been identified as a biologic 3D network with the

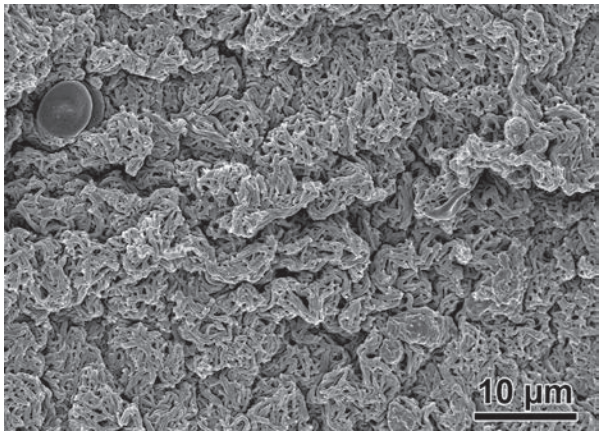


Fig 2-2 SEM examination of the fibrin clot revealing a dense and mature fibrin matrix with various cell types entrapped within its matrix.

ability for the fibrillar micropores to support cell migration, proliferation, differentiation, and delivery of GFs. Platelets have theoretically been described as being massively trapped within the fibrin network, and the release of GFs is largely dictated by the actual timespan in which the 3D PRF scaffold is broken down (typically within 2–3 weeks).[18] One of the major advantages of PRF when compared to PRP is the fact that by simply removing anticoagulants, a fibrin matrix is formed with a natural autologous delivery system capable of slowly and gradually releasing GFs over time.[7–9] This leads to GF delivery over a period of 2 to 3 weeks as opposed to only a few hours observed in PRP.[12]

Finally, stem cells exist naturally in whole blood, albeit at extremely low levels.[19,20] Stem cells have the ability and potential to differentiate into many cell types, including adipocytes, osteoblasts, and chondrocytes. Many commercial enterprises report that mesenchymal stem cells (MSCs) exist in extremely high numbers in PRF or that only certain protocols or machinery favor their accumulation, but these reports have not been validated in any high-quality peer-reviewed journal. While future research investigating the impact of MSCs in blood is necessary, it may represent a potential future strategy to isolate MSCs relatively easily at low cost.

The commercial claims that MSCs exist in extremely high numbers in PRF or that only certain protocols or machinery favors their accumulation have not been validated.

Growth Factors in Blood

Naturally, GFs are critical to wound healing, and a variety of GFs have been commercialized as recombinant human sources once their roles were established. GFs are largely responsible for the migration of cells and also play a critical role in their adhesion, proliferation, and differentiation. While GFs exist in all tissues, it is important to note that blood serves as a main reservoir of numerous GFs and cytokines promoting angiogenesis and tissue regeneration for wound healing. It is also important to note that certain GFs may exist as inactive or partially active precursors that require proteolytic activation, or may further require binding to matrix molecules for activity or stabilization. For this reason, interfering with the natural clotting cascade such as when utilizing PRP may affect the bioactivity of certain GFs.[12] Typically, GFs also have extremely short biologic half-lives in the order of a few minutes.[21] The body has been trained to secrete various GFs in programmed orders to activate very complex cellular processes.[22] Unlike recombinant human GFs that typically only comprise a single GF, platelet concentrates create the opportunity to deliver many autologous GFs simultaneously. Furthermore, leukocytes are known immune cells capable of "sensing" their microenvironment during the regenerative phase. Together with platelets, leukocytes serve as a major cell type during the natural wound healing process. The GFs most accumulated and delivered in PRF include VEGF, PDGF, TGF-β1, EGF, and IGF.[23,24] Their individual roles are discussed below.

Unlike recombinant human GFs that typically only comprise a single GF, platelet concentrates create the opportunity to deliver many autologous GFs simultaneously.

VEGF

VEGF is secreted by activated thrombocytes and macrophages to damaged sites to promote angiogenesis. The VEGF family is related to PDGF and includes VEGF-A, -B, -C, -D, and -E. VEGF has previously been isolated and utilized as a recombinant GF described as the most potent GF