INTRODUCTION

Cascade Medical Enterprises (CME) has a commitment to study the basic science and mechanisms of how our products work. As part of this commitment, CME sought the expertise of Dr. Chandan Sen and his team at Ohio State University (OSU). Dr Sen is a highly respected wound healing expert who has hundreds of papers to his credit. Dr. Sen is Professor and Vice Chairman of Surgery (Research), Associate Dean (Medicine) and Executive Director of the OSU Comprehensive Wound Center. He serves as a chief advisor to several multinational wound care companies and sits on the editorial board of a number of wound care (Wound Healing Society) and molecular biochemical/biologic publications.

In this study, Dr Sen and his group wanted to challenge the CASCADE® platelet-rich fibrin matrix (PRFM) by examining the following:

1. Characterization of platelets and fibrin matrix
2. Functionality of the platelets in vitro
3. Functionality of the platelets and fibrin matrix in an animal model

The study, for the first time, showed the kinetics of the viability of platelets embedded in the PRFM. It also showed:

- A slow and steady release of growth factors from the platelets in the PRFM due to the use of non-thrombin activation
- VEGF was primarily responsible for endothelial mitogenic response
- Induction of endothelial cell proliferation in wounds and improved wound angiogenesis and healing in ischemic wounds indicating potential mechanisms of action of PRFM in healing of chronic ulcers.
STUDY SUMMARY

This study investigated the CASCADE® / FIBRINET® Autologous Platelet System in the platelet-rich matrix (PRFM) membrane format. The PRFM membrane was prepared by transferring the platelet-rich plasma (PRP) from two yellow top tubes into a vial containing calcium chloride, which was centrifuged at 4500 x g for 25 minutes. This results in a PRFM disc or membrane, which takes the shape of the base of the vial.

The PRFM membranes were used in three different parts of the study for either in vitro culture or porcine wound studies.

PART 1 – In vitro characterization of PRP and PRFM

In Part 1 of the study, the researchers characterized the recovery of platelets in the PRP, as well as the viability of platelets and growth factor release from platelets in the PRFM membrane. Blood collected from 20 human donors was used for this part of the study.

- The mean PRP platelet recovery in this study was 63%. (Refer to additional information in VALUE PROPOSITION section below.)
- Platelet viability in the PRFM membrane was determined using a lactate dehydrogenase (LDH) system. Under these conditions, viability of platelets in PRFM was observed over 7 days.
- The growth factor levels in media incubated with PRFM were determined using ELISA methods. An increase in the levels of PDGF-BB, TGFβ1 and VEGF-A was noted in culture media indicating a gradual release of growth factors by platelets embedded in PRFM. A slow and steady release of growth factors from PRFM was observed over 7 days.

Part 2 – In vitro endothelial cell proliferation

In order to determine whether the growth factors released by the PRFM membrane are biologically active, the media incubated with PRFM were tested in a human micro-vascular endothelial cells (HMEC) proliferation assay.

The authors found the following:

- Treatment of HMEC with media from the PRFM resulted in increase in HMEC proliferation; however, the cell proliferation was not correlated to PDGF or TGFβ levels in PRFM media.
• VEGF released from PRFM was primarily responsible for endothelial mitogenic response. Activation of endothelial cells in response to PRFM-released growth factors occurred via the ERK activation pathway.

Part 3 – In vivo porcine wound study

To determine the effects of PRFM on wound healing, the authors used a porcine ischemic wound model, as these ischemic wounds have previously been shown to have impairment in angiogenesis (formation of blood vessels) and subsequent closure. PRFM membranes were prepared from autologous blood collected from pigs in the study. The PRFM membranes were applied to ischemic excisional wounds and the quality of the regenerated tissue was determined by histological evaluation on day 14.

• PRFM-treated ischemic wounds at day 14 showed presence of mature collagen fibers in the treated wounds compared to untreated wounds (controls)
• On day 14 post-wounding, PRFM-treated ischemic wounds displayed increased number of cellular structures, which are indicative of vascular formation.
• The authors also used other methods including laser capture micro dissection (LCM) and laser Doppler blood flow imaging. The LCM, laser Doppler data and immunohistochemical data indicate presence of functional vessels and angiogenesis in PRFM treated wounds.

MAIN CONCLUSIONS FROM PAPER:

1. The study characterized recovery and viability of platelets as well as growth factor release from platelets in the PRFM membrane.
2. A slow and steady release of growth factors from PRFM was observed because of the use of non thrombin activation approach
3. The growth factors released by platelets in PRFM induced endothelial cell proliferation.
4. The VEGF released from PRFM was primarily responsible for endothelial mitogenic response. The PRFM induced endothelial cell proliferation occurred via ERK activation pathway.
5. PRFM effectively induced endothelial cell proliferation in wounds and improved wound angiogenesis in ischemic wounds indicating potential mechanisms of action of PRFM in healing of chronic ulcers.
6. Angiogenesis, or the formation of new blood vessels from existing ones, is a critical step in the regeneration of hard and soft tissues.2

VALUE PROPOSITION SUMMARY for the CASCADE® SYSTEM

1. The mean PRP platelet recovery in this study was 63%.
   • Other studies have shown that the CASCADE® system has the greatest platelet capture efficiency with the least variability.3 The CASCADE® system employs an overall technique independent technology
   • Other studies have shown platelet recoveries greater than 95% for the CASCADE® system, especially with adequate mixing of the PRP
2. As seen in the work of Lucarelli et al. with mesenchymal stem cells\textsuperscript{4}, Visser and Arnoczky with tendon chondrofibroblasts\textsuperscript{5}, and in this study of endothelial cells, CASCADE® PRFM has the ability to stimulate the migration and proliferation of a wide variety of different cell types involved in both soft tissue and bone repair.

3. The PRFM response in this study, both in vitro and in vivo, appears dependent upon the continual release of functional growth factors from the intact platelets within the fibrin matrix.
   - Platelet poor plasma remaining from PRFM membrane production was not able to stimulate the endothelial cells.
   - Platelet viability and growth factor levels were demonstrated up to 7 days with the CASCADE® system, which uses no exogenous thrombin
   - PRFM and prolonged release of growth factors enabled migration into the wound and new blood vessel growth

REFERENCES


