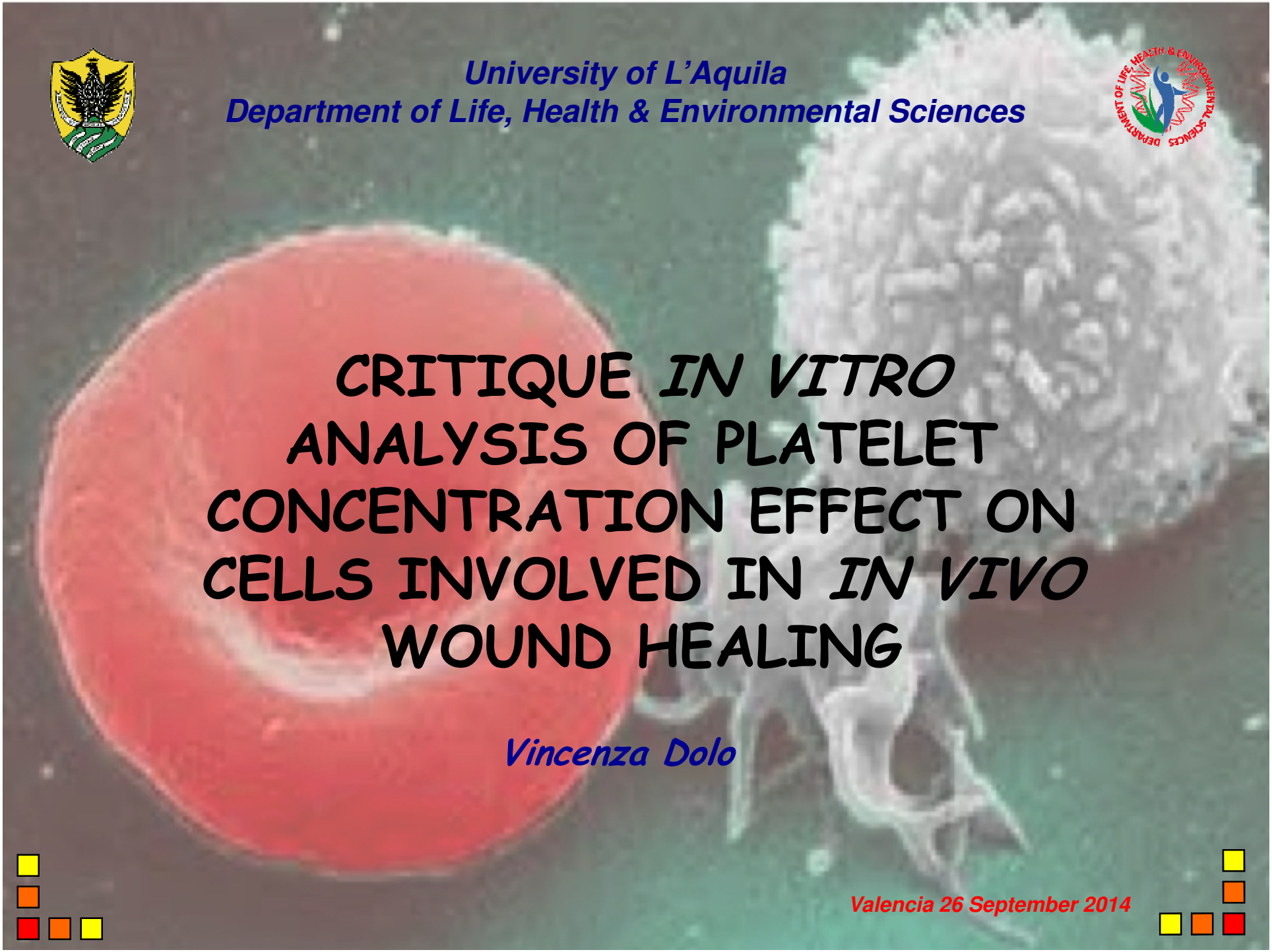


University of L'Aquila
Department of Life, Health & Environmental Sciences

CRITIQUE *IN VITRO* ANALYSIS OF PLATELET CONCENTRATION EFFECT ON CELLS INVOLVED IN *IN VIVO* WOUND HEALING

Vincenza Dolo

Valencia 26 September 2014

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OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

WOUND HEALING

Complex and dynamic process of replacing
devitalized and missing cellular structures and tissue layers after injury

INJURY

It is classically divided into four sequential and overlapping phases

Minutes Hours Days Weeks Months Years

Hemostasis

Platelets — Fibrin clot formation
Vasoactive mediator release
Cytokine and GFs release

Inflammation

Neutrophils — Killing and phagocytosing, wound debridment
Monocytes
Macrophages — Killing and phagocytosing, wound debridment
Cytokine and GFs release

Proliferation

Keratinocytes — Reepithelialization
Endothelial cells — Angiogenesis
Fibroblasts — Fibroplasia

Remodeling

Keratinocytes — Epidermis maturation
Myofibroblasts — Wound contraction
Endothelial cells — Apoptosis and scar maturation

Modified from Li J et al. Pathophysiology of acute wound healing Clinics in Dermatology; 25(1): 9-18. 2007

PLATELETS

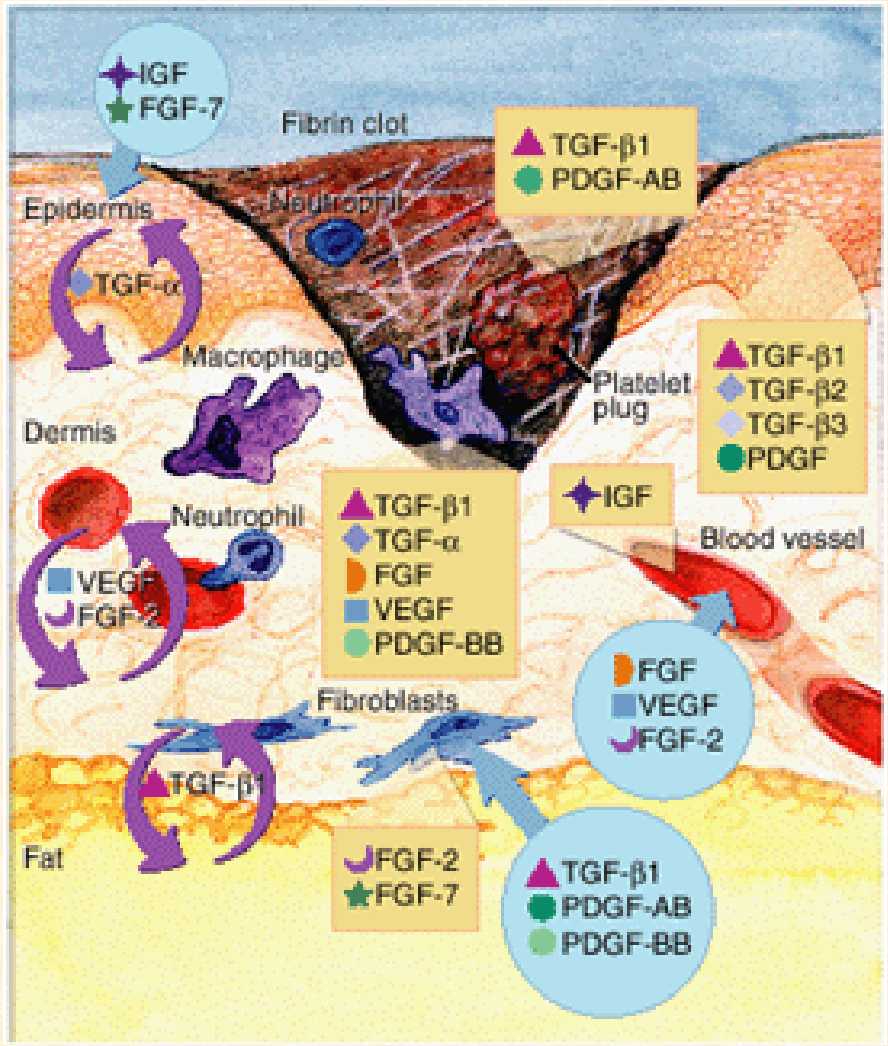


Role in the normal healing response via the secretion of local growth factors

Growth factors released by platelets recruit reparative cells and augmented soft-tissue repair

Platelets are stimulated to release these growth factors by exposure either to collagen or to thrombin and calcium

GROWTH FACTORS IN WOUND HEALING



www.rndsystems.com

Minireview - Cytokines in Wound Healing

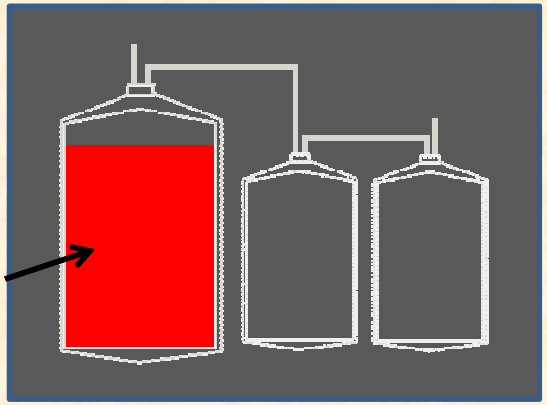
PDGF	Stimulates angiogenesis and chemotaxis / mitogenesis of fibroblasts, endothelial cells, neutrophils, macrophages
EGF	Increases angiogenesis; stimulates re-epithelialization; acts as mitogen for fibroblasts, keratinocytes
TGF-β	Regulates proliferation, differentiation and apoptosis
VEGF	Stimulates endothelial cell proliferation; induces neovascularization
IGF-I	Stimulates osteoblasts growth/differentiation of
KGF	Acts as mitogen for epithelial cells; promotes tissue repair
FGF-β	Acts as mitogen for endothelial cells, fibroblasts, keratinocytes; stimulates angiogenesis

PLATELETS RICH PLASMA (PRP)

Optimal quantity of platelets and growth factors is debated

Our study wants to investigate in this sense

PLATELET DERIVATIVES

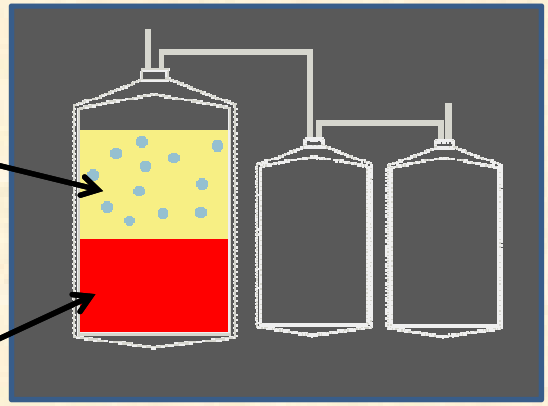


Blood

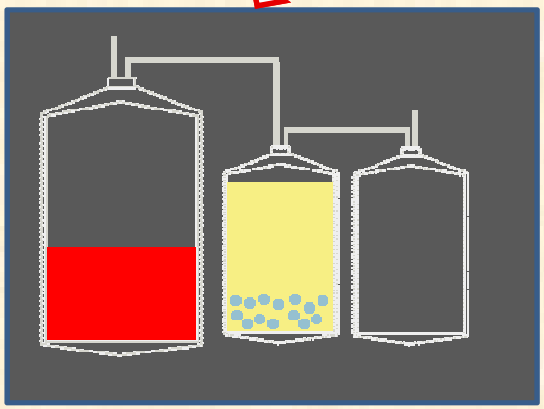
Platelet Rich Plasma (PRP)
(Plasma, PLT, WBC)

Iª centrifugation

Concentrated
RBC

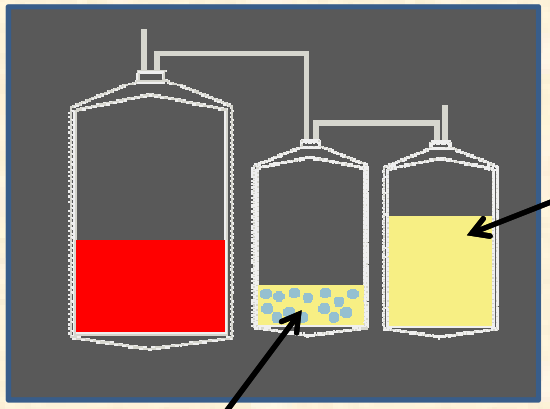


IIª centrifugation



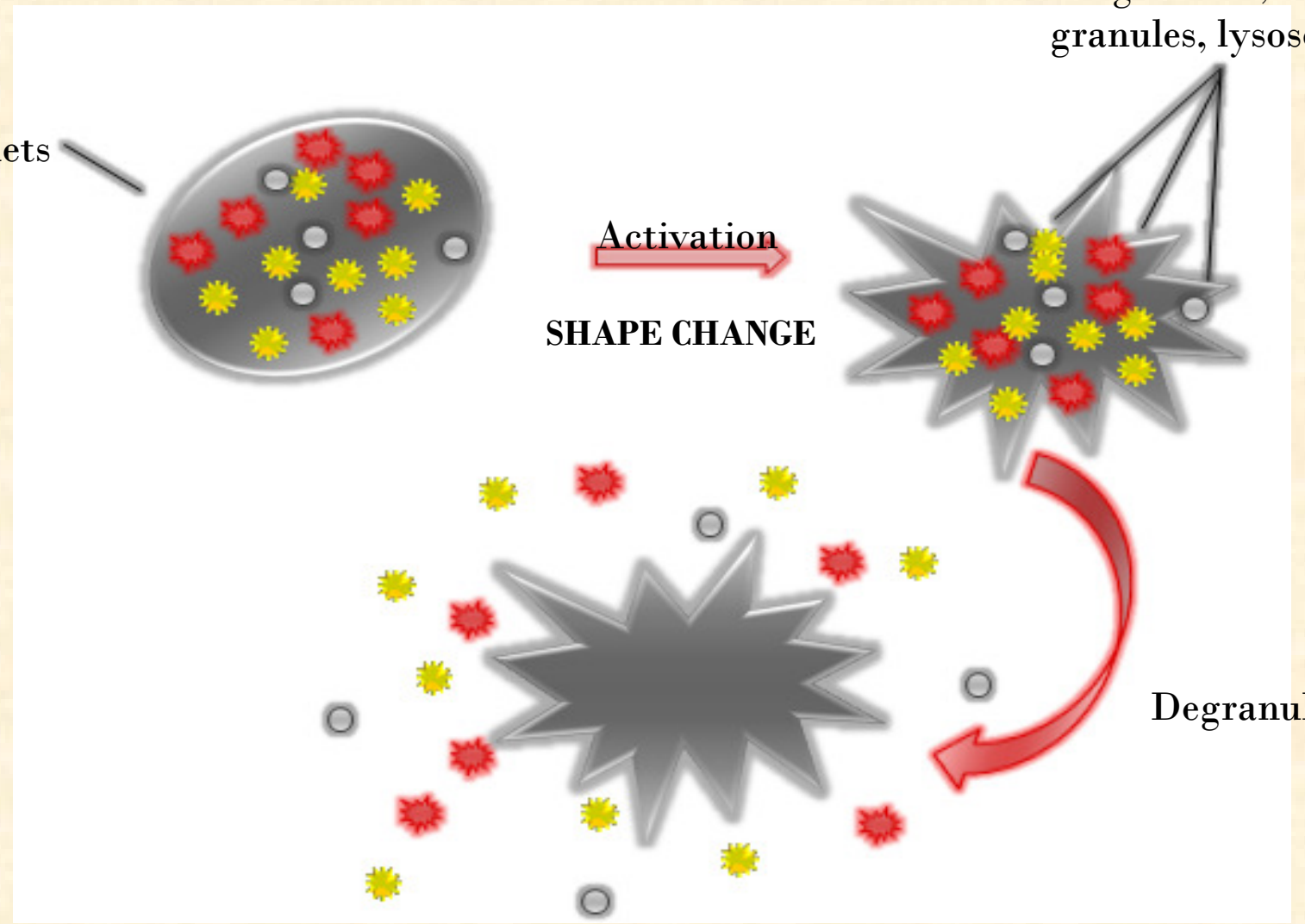
Platelet poor plasma (PPP)

Separation



Platelet concentrate (PC)
(PLT, WBC)

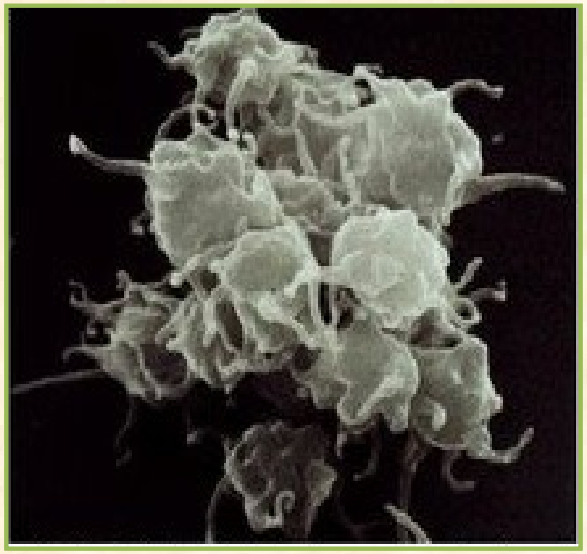
PLATELETS AS SOURCE OF GROWTH FACTORS



PLATELETS AS SOURCE OF GROWTH FACTORS



SHAPE CHANGE

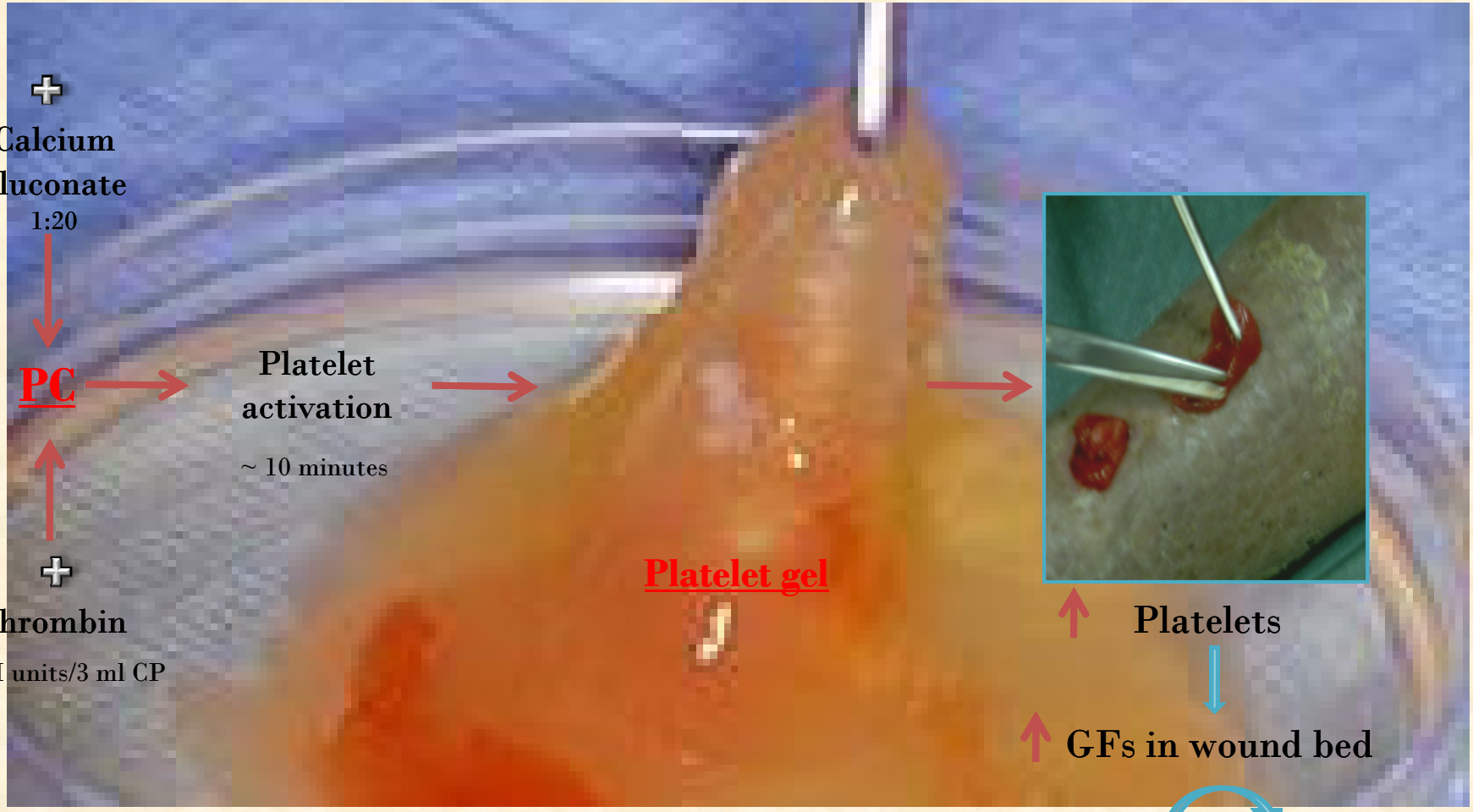


Inactive platelets

Activated platelets

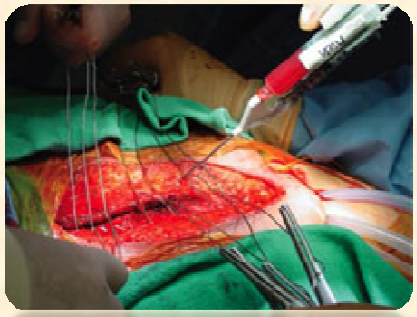
PLATELET GEL

A soft blood component, predominantly autologous, for topical use; it contains a higher concentration of platelets than blood (~ 10-20 fold) and white blood cells, in a small volume of plasma.





Vascular and diabetic ulcers, sores, burns



Cardiac surgery



Oral and maxillofacial surgery

Clinical applications

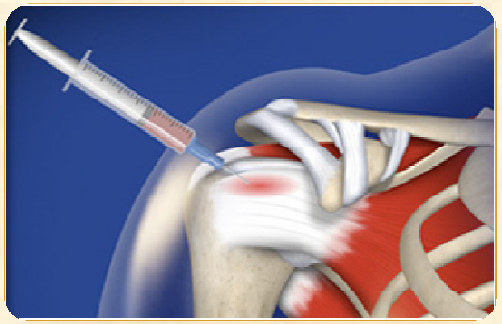
Surgery of the head and neck



Cosmetic surgery



Orthopedics



AIMS

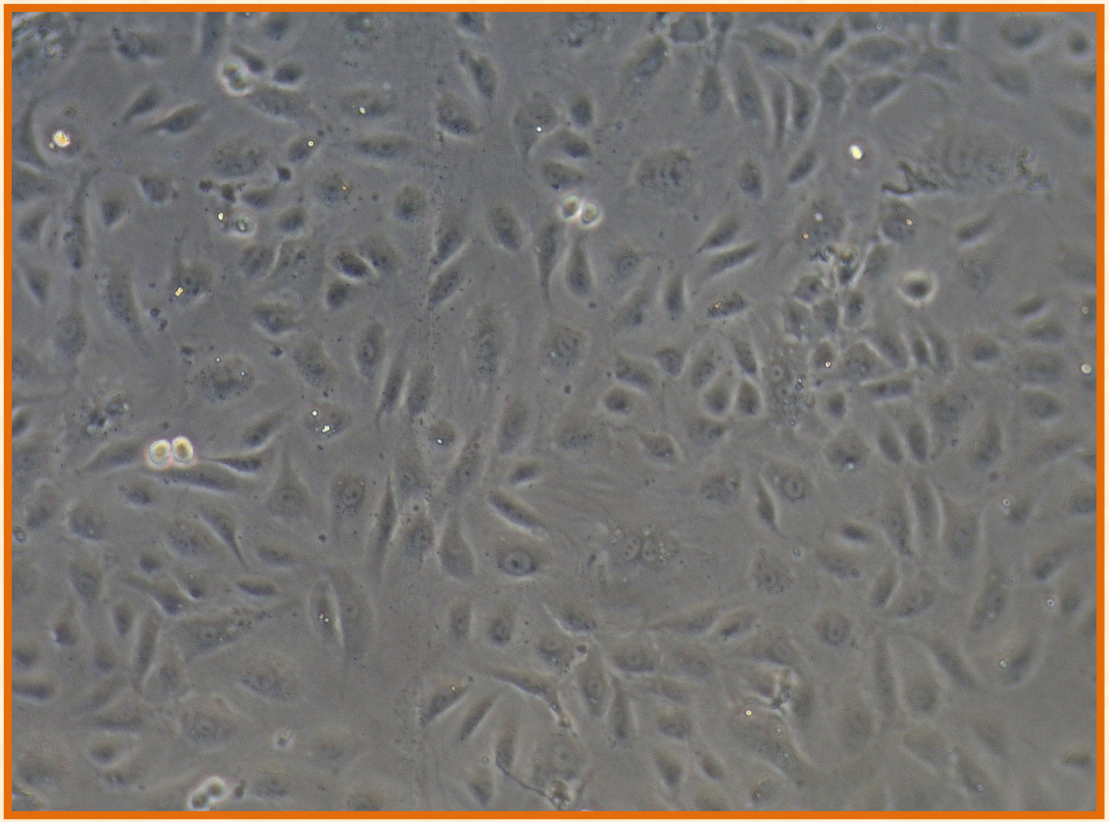
In recent years, many *in vitro* and *in vivo* studies have attempted to explain the biological mechanisms involved in PG-induced tissue regeneration/reparation.

In vitro investigations of cell functions have obtained conflicting results, likely due to several variables, such as the platelet concentration

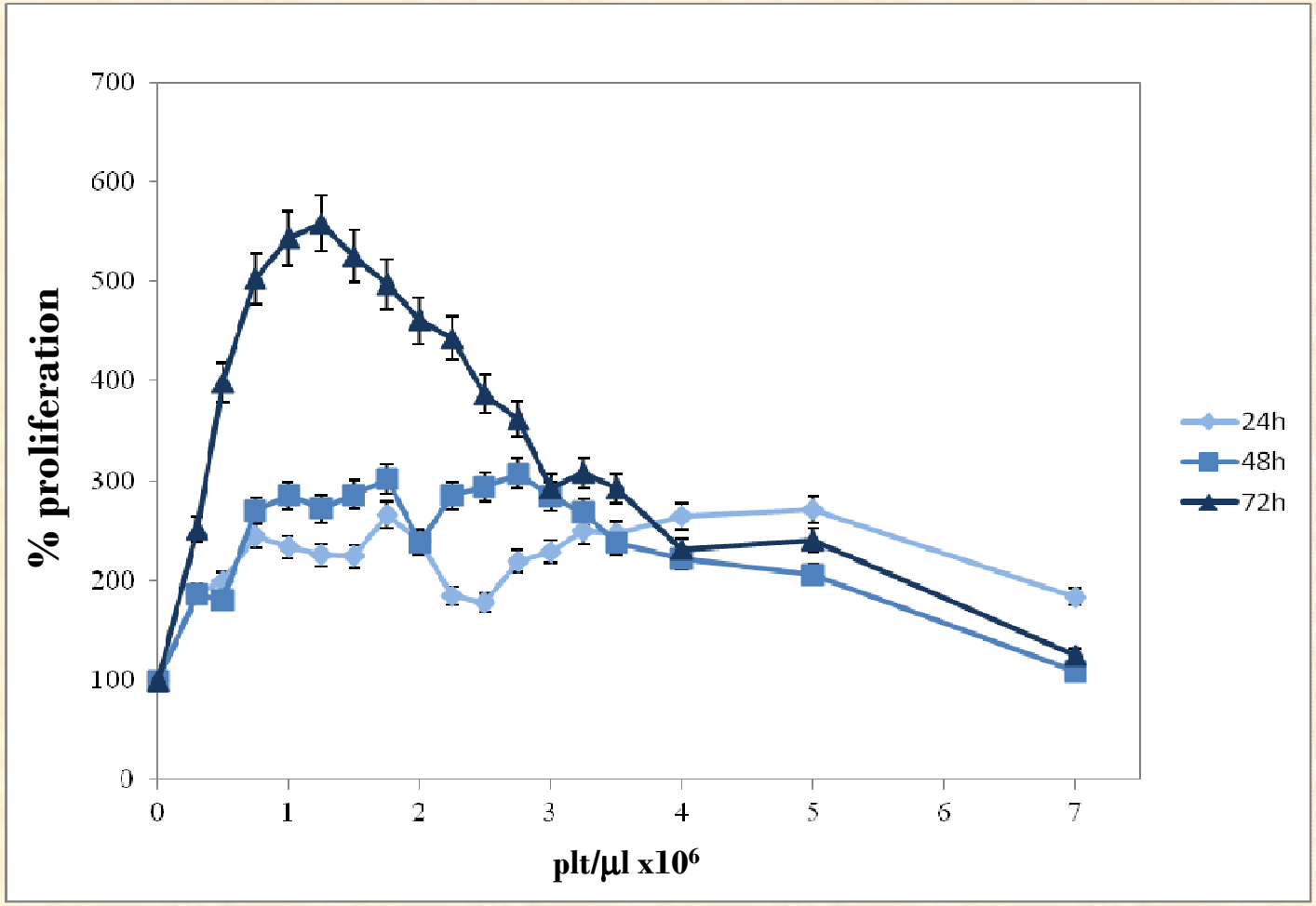
The parameters that should be used in clinical applications to obtain satisfactory results in wound healing remain uncertain.

Understanding the relationship between the platelet concentrations of PG-released supernatants and changes in the cellular parameters of cells involved in wound healing (endothelial cells and fibroblasts) and tendon healing (tenocytes).

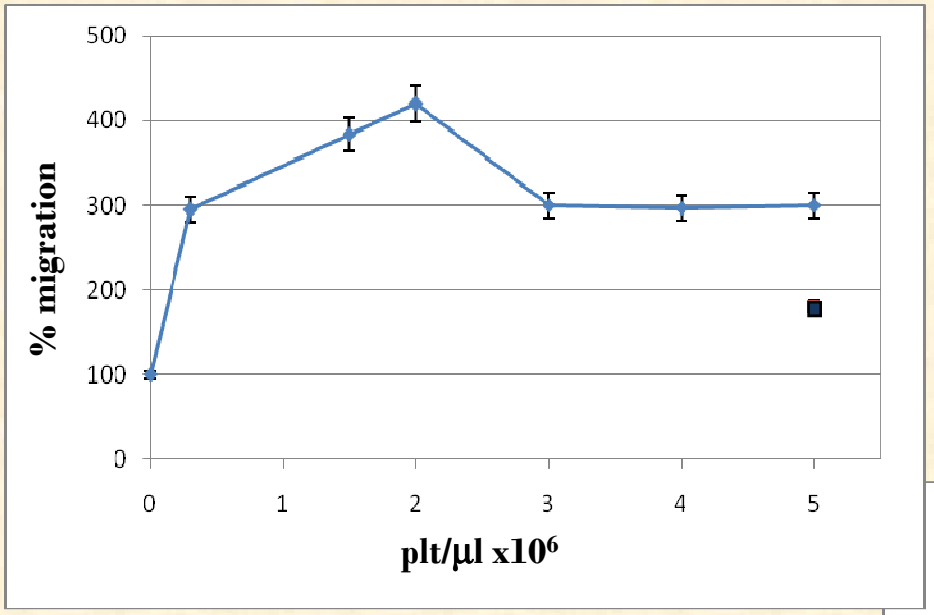
ENDOTHELIAL CELLS



GP STIMULATES ENDOTHELIAL CELLS PROLIFERATION

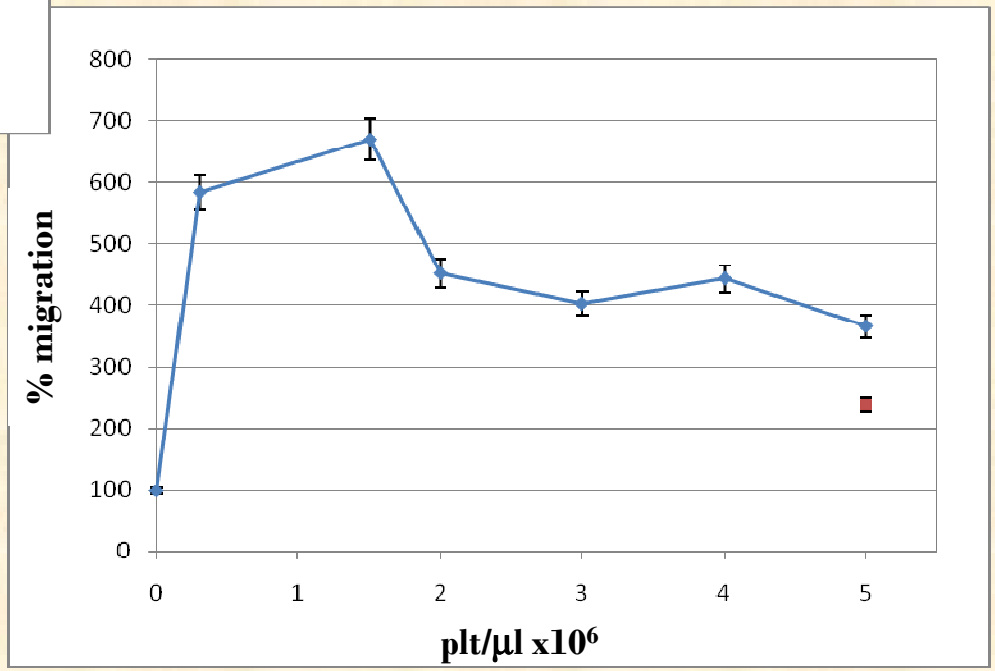


PG AFFECTS HUVEC MOTILITY AND INVASIVENESS

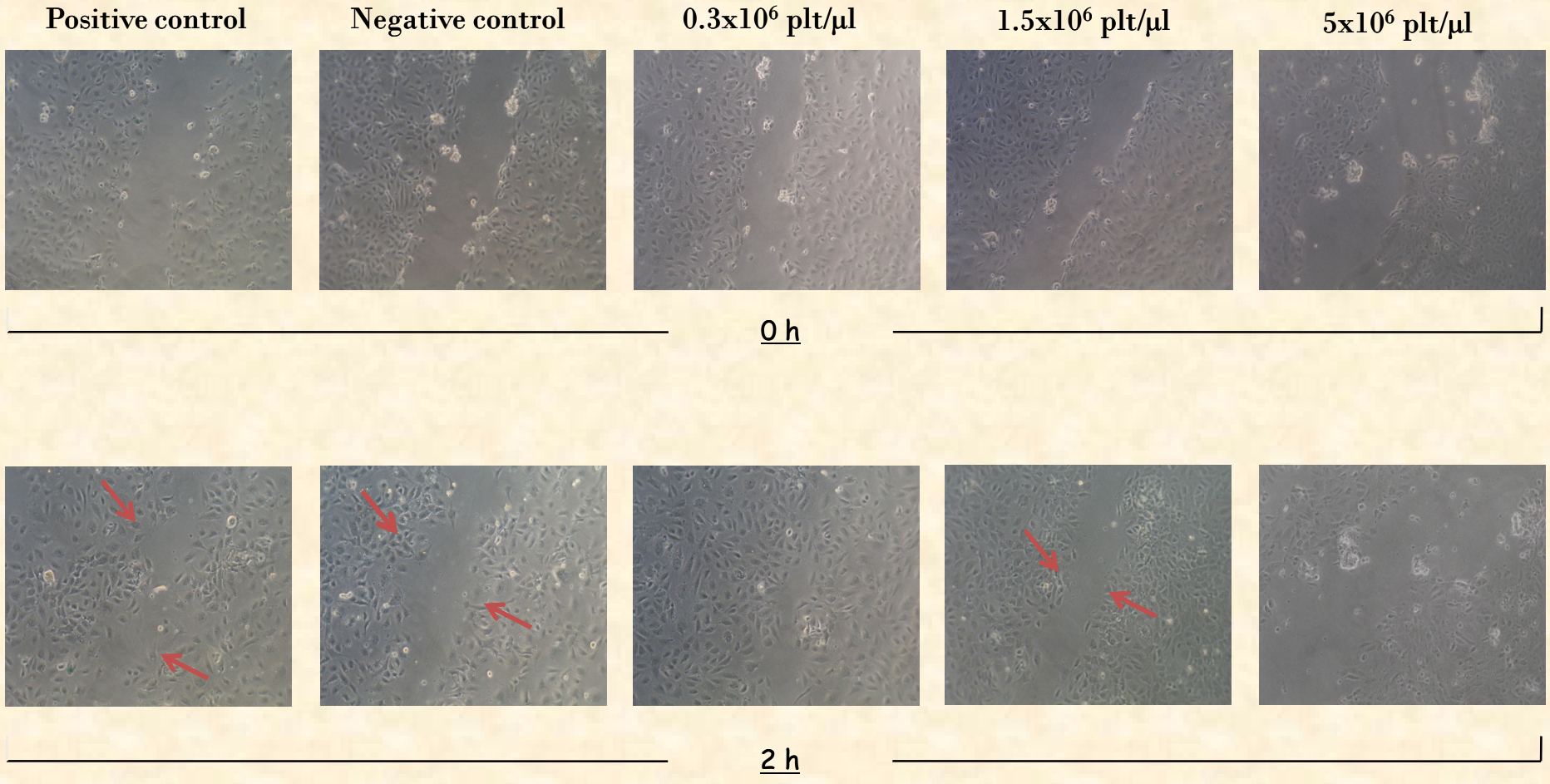


Motility

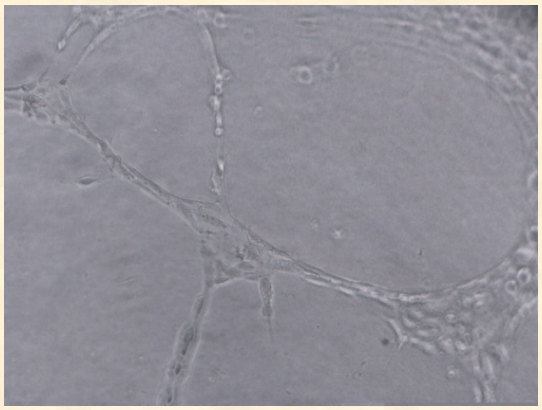
Invasiveness



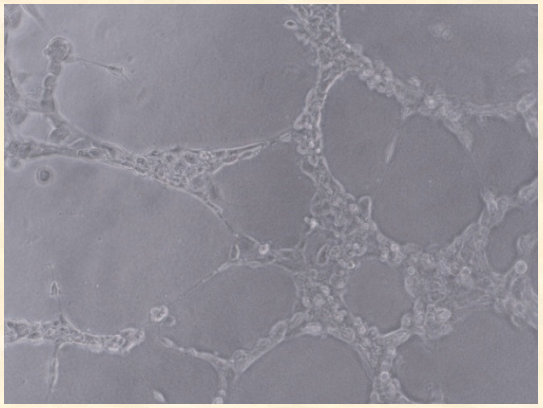
PG AFFECTS HUVEC MOTILITY



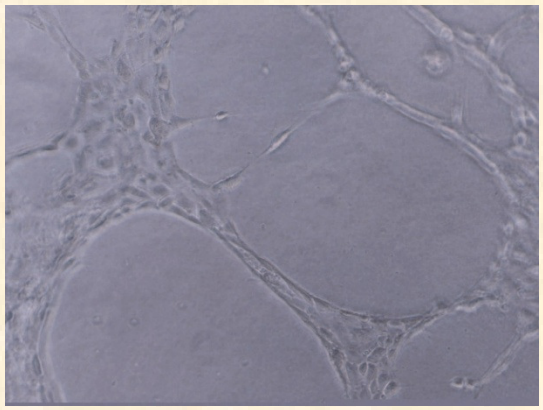
EFFECTS OF PG ON HUVECS CORD FORMATION



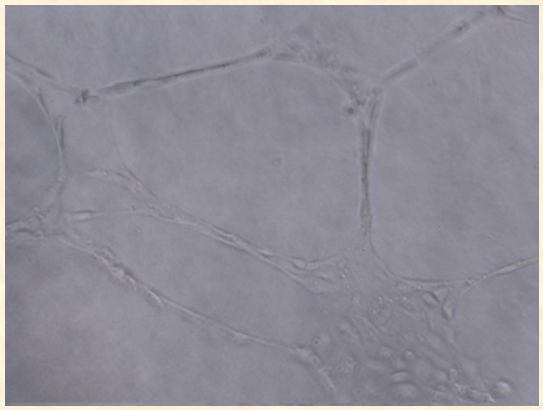
Positive control



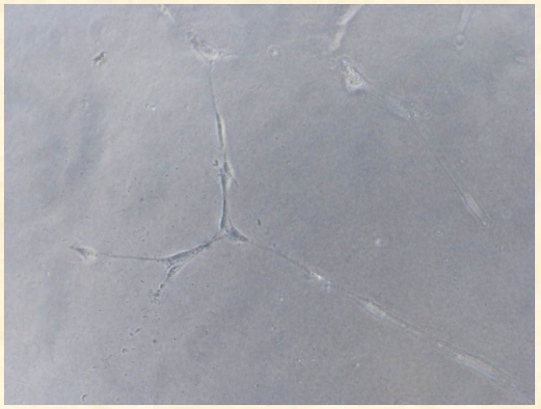
Negative control



$0.3x10^6$ plt/µl



$1.5x10^6$ plt/µl



$3x10^6$ plt/µl

ENDOTHELIAL CELLS

Transfusion. 2009 Apr;49(4):771-8. doi: 10.1111/j.1537-2995.2008.02033.x. Epub 2008 Dec 23.

Identification of an optimal concentration of platelet gel for promoting angiogenesis in human endothelial cells.

Giusti I[1], Rughetti A, D'Ascenzo S, Millimaggi D, Pavan A, Dell'Orso L, Dolo V.

Author information

Abstract

BACKGROUND: Numerous studies have supported the use of topical blood components to improve wound healing and tissue regeneration. Platelet gel (PG), a hemocomponent obtained from mix of activated platelets (PLTs) and cryoprecipitate, is currently being used clinically in an attempt to improve tissue healing. The present study sought to define the most effective PG concentration to promote angiogenesis in vitro.

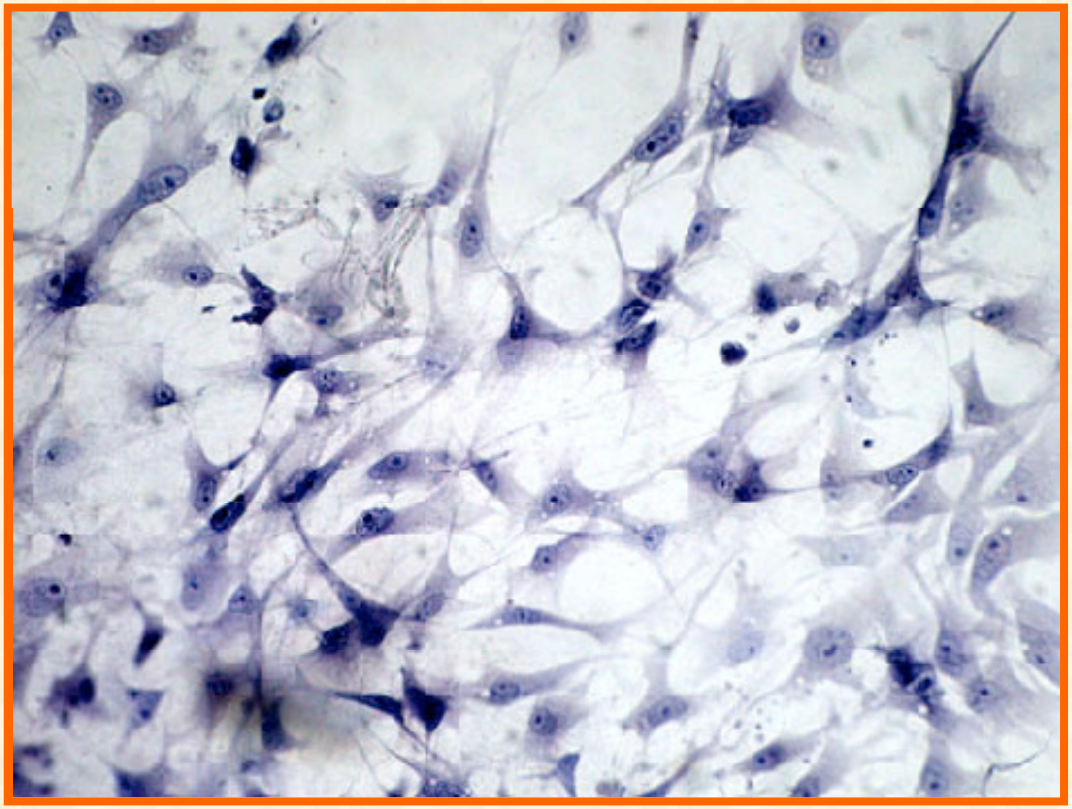
STUDY DESIGN AND METHODS: The effects of PG-released supernatant at different concentrations on human endothelial cells were studied using different in vitro assays (proliferation, migration, invasion, cord formation, and wound healing).

RESULTS: The concentration of PG-released supernatant had a significant influence on the angiogenic potential of endothelial cells. The optimal concentration for the stimulation of angiogenesis was 1.5 x 10(6) PLTs per microL in most of the in vitro experiments used in this study. Lower or higher concentrations of PG displayed a lower angiogenic potential.

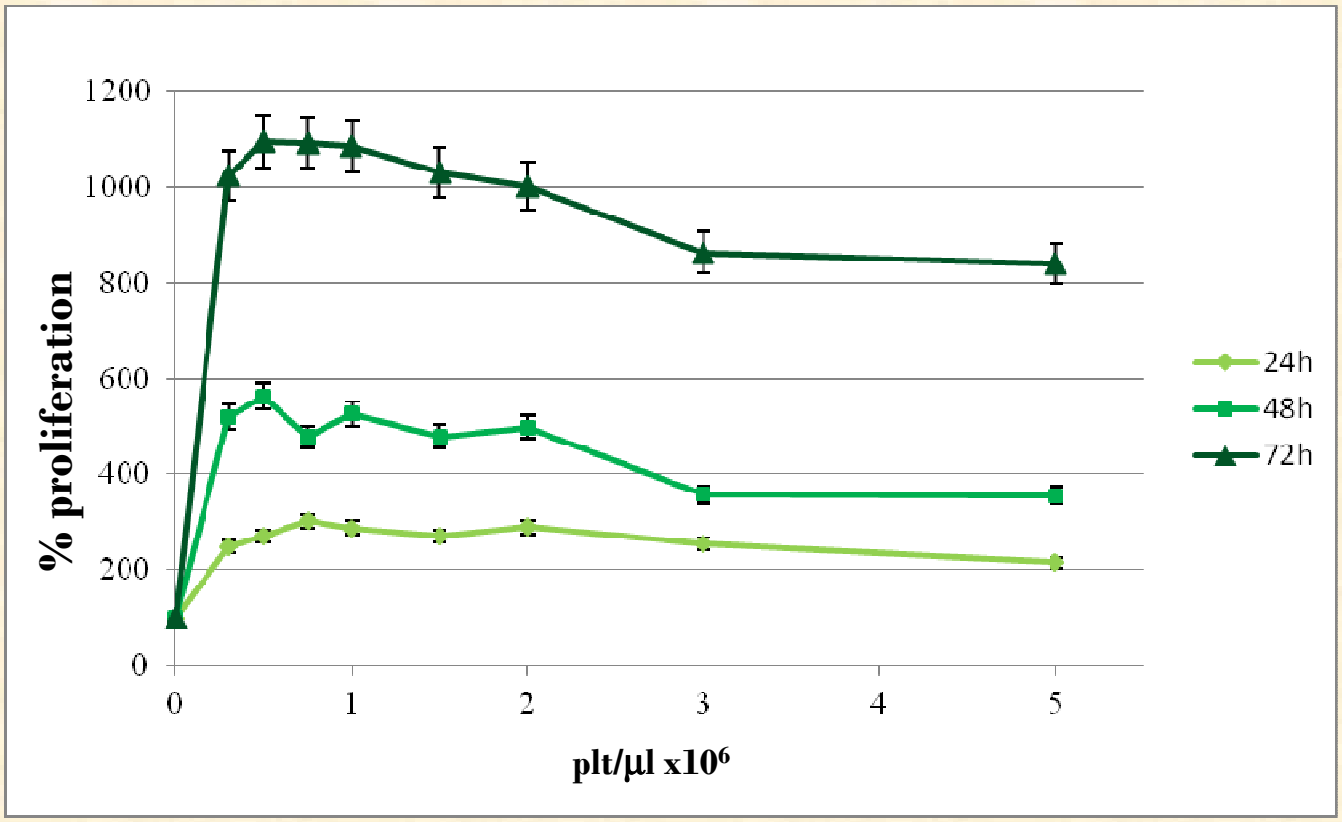
CONCLUSION: An optimal concentration of PG to promote angiogenesis in human endothelial cells was identified. Excessively high PG concentrations may inhibit the angiogenic process, thereby being counterproductive for wound healing in a clinical setting.

PMID: 19170984 [PubMed - indexed for MEDLINE]

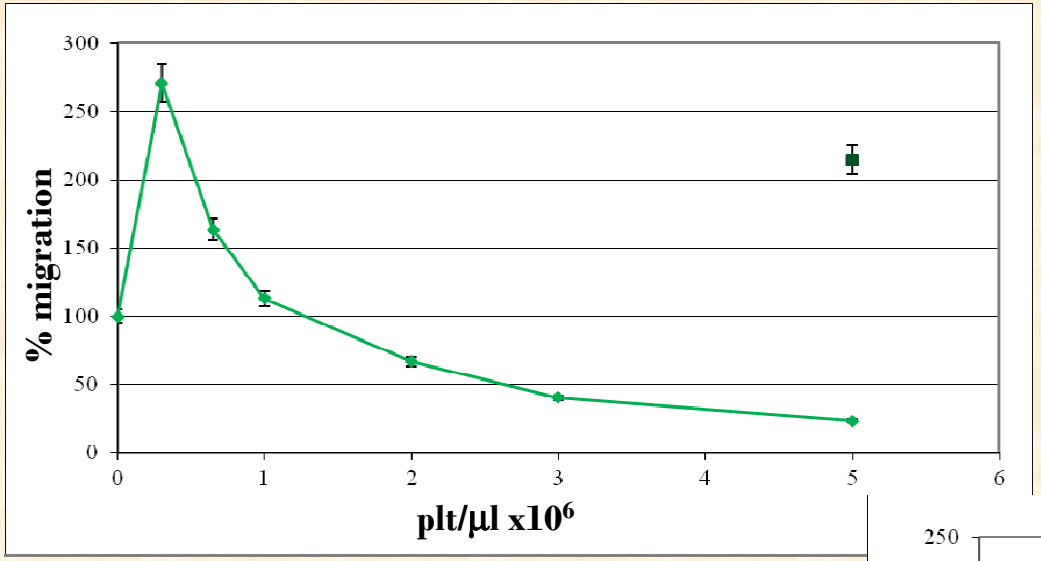
FIBROBLASTS



PG STIMULATES FIBROBLAST CELL PROLIFERATION

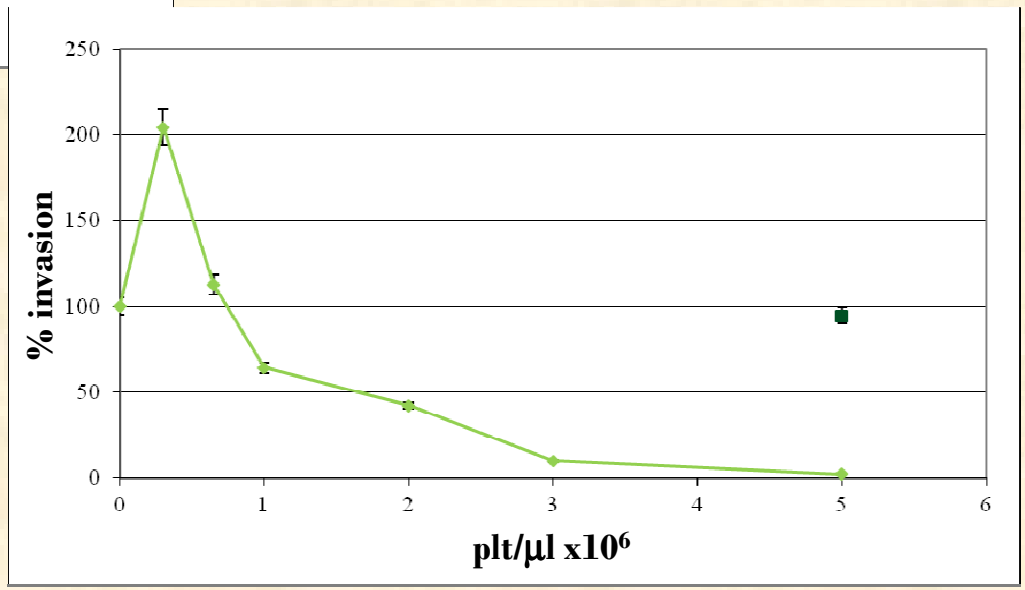


PG AFFECTS FIBROBLAST MOTILITY AND INVASIVENESS

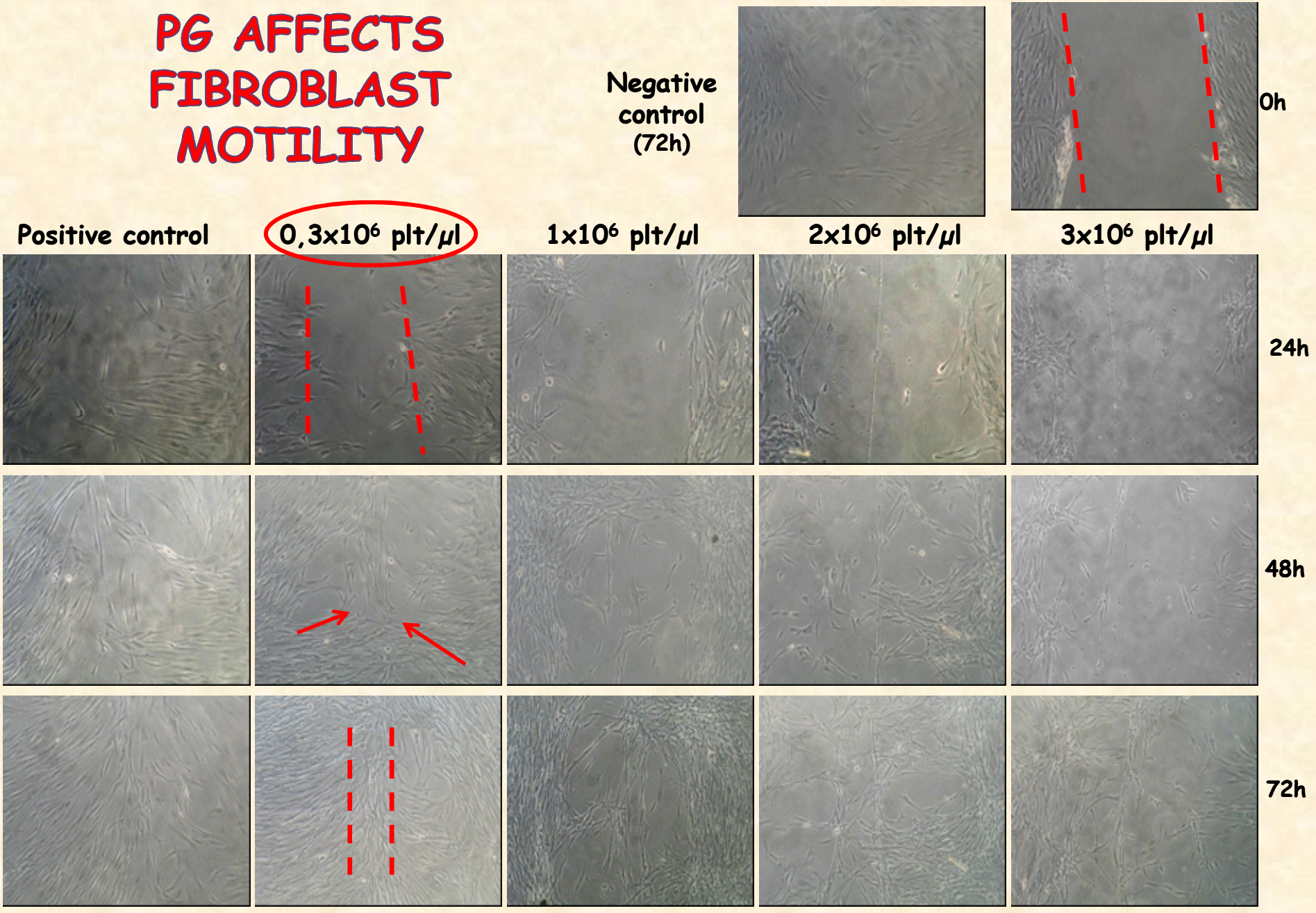


Motility

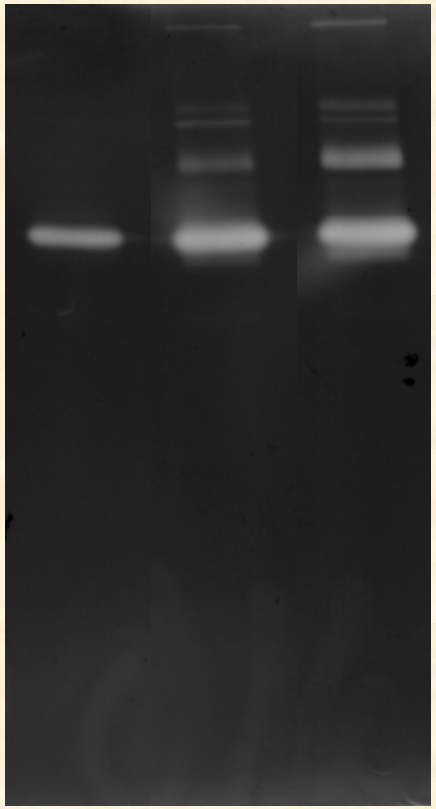
Invasiveness



PG AFFECTS FIBROBLAST MOTILITY

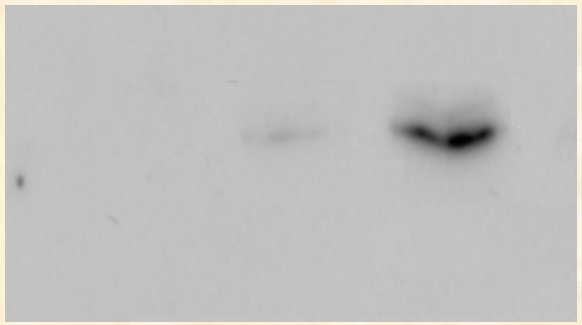


PG AFFECTS ECM REMODELING



ProMMP-9

ProMMP-2
MMP-2



Collagen I

1: untreated cells
2: 0,3x10^6 plt/µl
3: 1,5x10^6 plt/µl

FIBROBLASTS

Wound Repair Regen. 2013 Mar-Apr;21(2):300-8. doi: 10.1111/wrr.12025. Epub 2013 Feb 25.

The effects of platelet gel-released supernatant on human fibroblasts.

Giusti I[1], Rughetti A, D'Ascenzo S, Di Stefano G, Nanni MR, Millimaggi D, Dell'orso L, Dolo V.

Author information

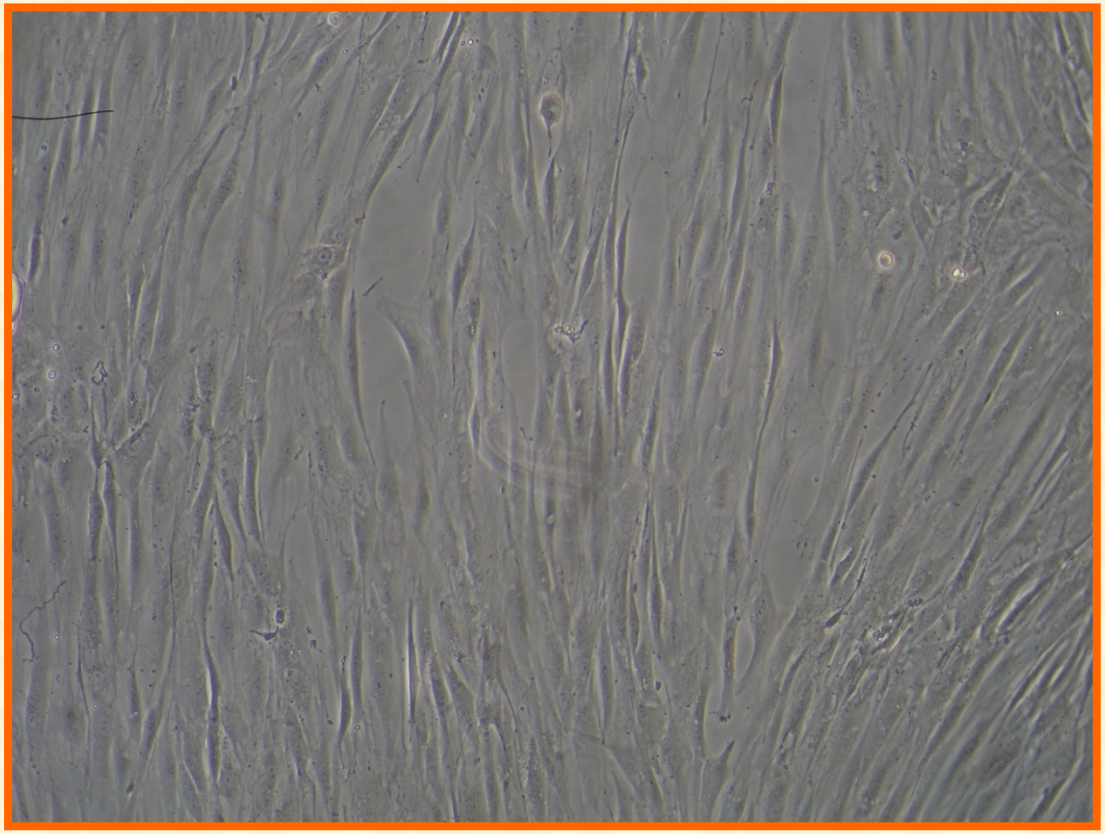
Abstract

In recent years, interest in the topical use of platelet gel (PG) to stimulate wound healing has rapidly extended into various clinical applications and specialized fields. Many recent in vitro and in vivo studies have attempted to explain the biological mechanisms involved in PG-induced tissue regeneration/reparation. However, it remains unclear which parameters should be used in clinical applications to obtain satisfactory results in the healing of wounds. Toward this end, the present study focused on understanding the relationship between platelet concentrations and the cellular parameters of the cell types, i.e., fibroblasts, involved in wound healing. Normal human dermal fibroblasts were treated with PG-released supernatant at various concentrations in different assays (proliferation, migration, invasion, and in vitro scratch wound closure) to identify the most effective concentration to promote the fibroblasts' activities. Different concentrations of platelets per microliter in PG have different levels of efficacy in inducing fibroblast activity. The most effective concentration was obtained from PG at a concentration of approximately 0.5-1.5 × 10(6) plt/μL; higher concentrations were less effective. This study shows that excessively high concentrations of platelets per microliter have an inhibitory effect on the wound healing processes and are, therefore, counterproductive.

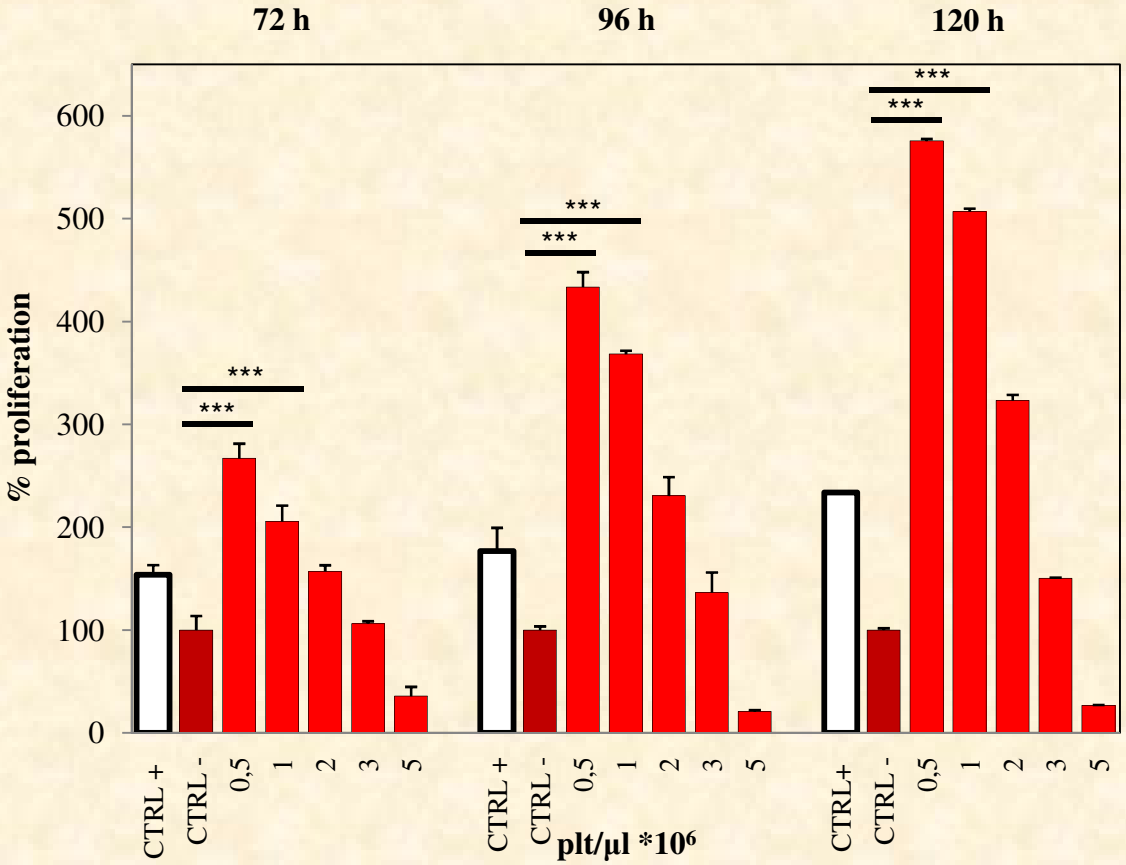
© 2013 by the Wound Healing Society.

PMID: 23438188 [PubMed - indexed for MEDLINE]

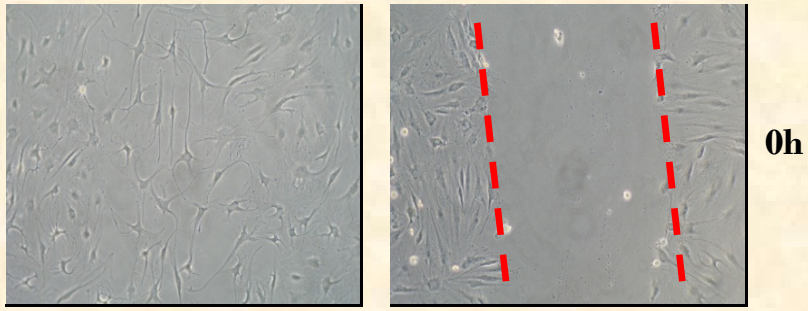
TENOCYTES

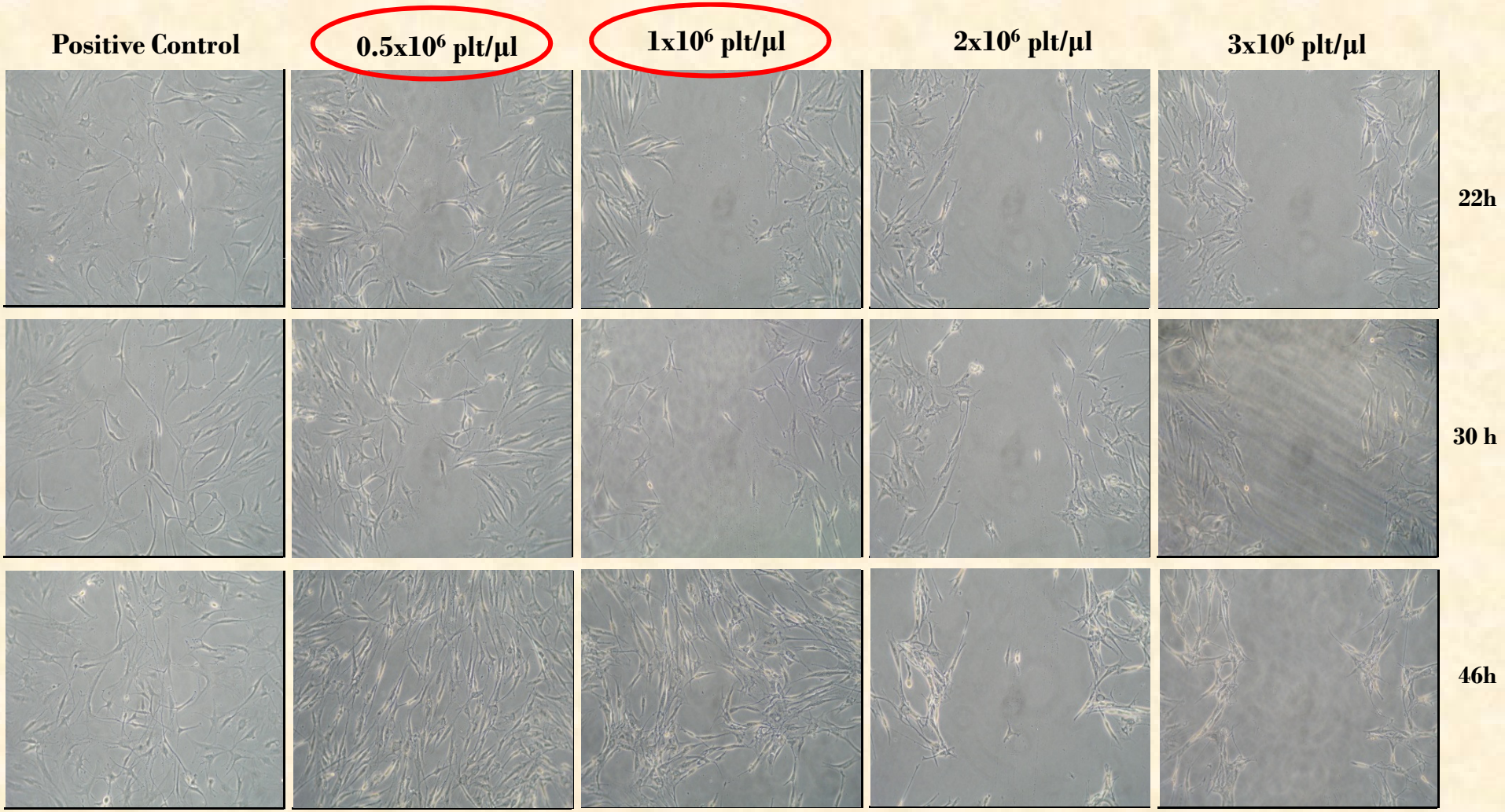


PG STIMULATES TENOCYTE PROLIFERATION

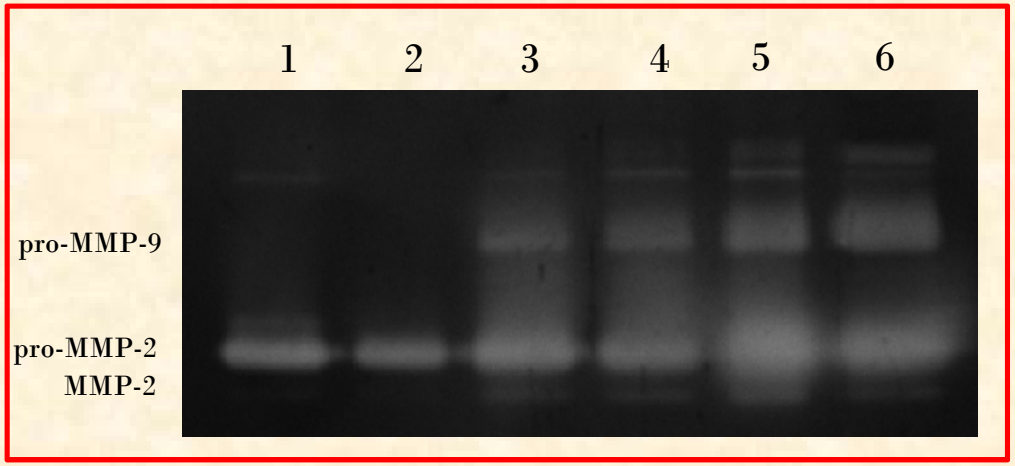


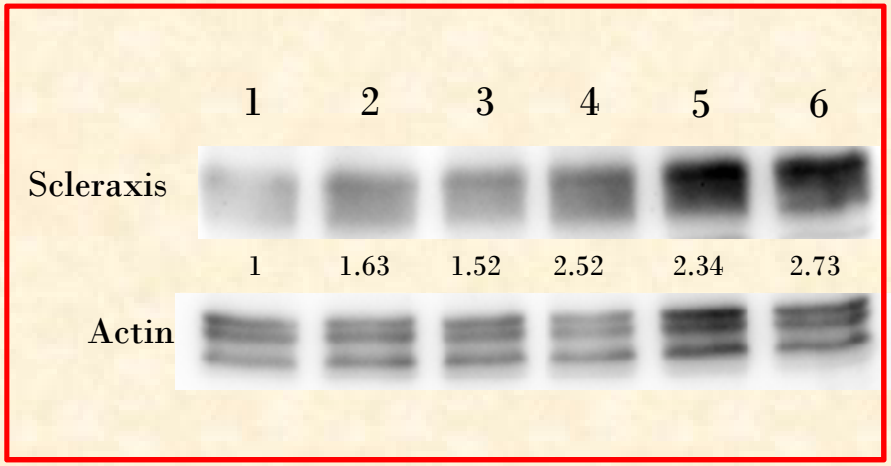
THE EFFECT OF PG ON TENOCYTE MIGRATION



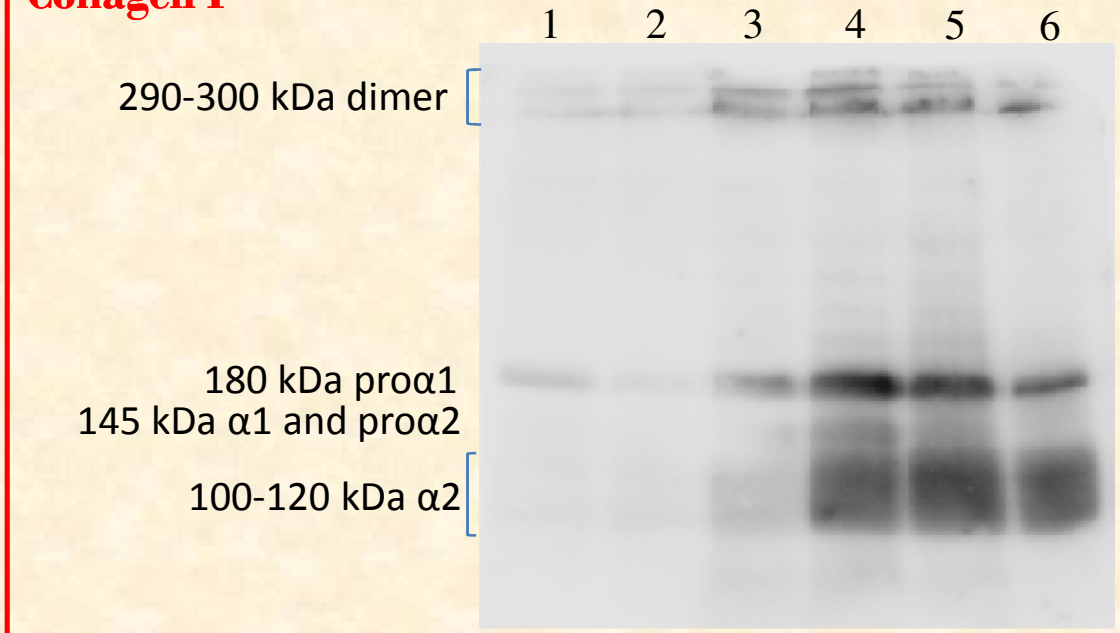


PG AFFECTS THE EXPRESSION OF MOLECULES INVOLVED IN ECM REMODELING





Collagen I



1: positive control
2: negative control
3: 0.5 × 10^6 plt/μL
4: 1 × 10^6 plt/μL
5: 2 × 10^6 plt/μL
6: 3 × 10^6 plt/μL

TENOCYTES

Biomed Res Int. 2014;2014:630870. doi: 10.1155/2014/630870. Epub 2014 Jul 23.

Platelet concentration in platelet-rich plasma affects tenocyte behavior in vitro.

Giusti I[1], D'Ascenzo S[1], Mancò A[2], Di Stefano G[1], Di Francesco M[1], Rughetti A[3], Dal Mas A[4], Properzi G[1], Calvisi V[5], Dolo V[1].

⊕ Author information

Abstract

Since tendon injuries and tendinopathy are a growing problem, sometimes requiring surgery, new strategies that improve conservative therapies are needed. Platelet-rich plasma (PRP) seems to be a good candidate by virtue of its high content of growth factors, most of which are involved in tendon healing. This study aimed to evaluate if different concentrations of platelets in PRP have different effects on the biological features of normal human tenocytes that are usually required during tendon healing. The different platelet concentrations tested (up to 5 × 10(6) plt/μL) stimulated differently tenocytes behavior; intermediate concentrations (0.5 × 10(6), 1 × 10(6) plt/μL) strongly induced all tested processes (proliferation, migration, collagen, and MMPs production) if compared to untreated cells; on the contrary, the highest concentration had inhibitory effects on proliferation and strongly reduced migration abilities and overall collagen production but, at the same time, induced increasing MMP production, which could be counterproductive because excessive proteolysis could impair tendon mechanical stability. Thus, these in vitro data strongly suggest the need for a compromise between extremely high and low platelet concentrations to obtain an optimal global effect when inducing in vivo tendon healing.

PMID: 25147809 [PubMed - in process] PMCID: PMC4132404 Free PMC Article

CLINICAL APPLICATION FOR HARD-TO-HEAL WOUNDS

Male patient, 57 years old, diabetic foot ulcer

Before PG application





After 4 applications

After 10 applications





After 11 applications

Female patient, dehiscence of the surgical wound after appendectomy

Before PG application





After 1 application

After 2 applications





After 3 applications

Male patient, diabetes, amputation of the first toe of the left foot following a necrosis, failure of healing after surgery

Before PG application





After 4 applications

After 6 applications





After 9 applications

CONCLUSIONS

Data provide scientific indications of the real ability of PG to induce, *in vitro*, all the necessary mechanisms that are required for endothelial cells, fibroblasts and tenocytes to restore normal tissue during wound healing *in vivo*.

CONCLUSIONS

Different concentrations of plt/μL (most likely, different concentrations of the growth factors that are released from the platelets) exhibit different levels of efficacy in inducing these processes. Excessively high concentrations seem to be counterproductive

Further studies, *in vitro* and *in vivo,* are needed to define reliable guidelines in the clinical use of platelet gel.

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Sandra D'Ascenzo
Marianna Di Francesco
Katia Ragone

Division of Transfusion Medicine
San Salvatore Hospital, L'Aquila;

Anna Rughetti
Luigi Dell'Orso



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