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Research Article

Demonstration of Modifications in Capillaries and Fibrous Tracts at the Hypodermis Level after Injection of PRP Alone or Associated with Thrombin on a Human Skin Model

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Abstract

***Introduction**: This study investigates the pathophysiological effects of platelet-rich plasma (PRP) in conjunction with thrombin on lymphatic vessels using in vitro human skin models.*

***Materials and Methods**: Human skin explants were treated with either PRP alone or in combination with thrombin. Histological analysis was employed to assess alterations in vascular and lymphatic structures.*

***Results**: Combined PRP-thrombin treatment demonstrated significant changes in capillaries and fibrous tracts, suggesting an influence on lymphatic remodeling and vascular function.*

***Discussion:** The observed modifications indicate that PRP with thrombin could modulate the tissue environment in a manner conducive to the healing process, with implications for managing post-surgical complications such as seromas.*

***Conclusion:** The PRP-thrombin combination holds therapeutic potential to positively influence tissue regeneration and lymphatic function. Further studies are required to translate these findings into clinical strategies.*

1. Introduction

Modifications at the level of septa or possibly adipocytes, with vasoconstriction of lymphatics and capillaries could help avoid lymphedema observed post-surgical intervention. These lymphatic effusions, also called seromas, correspond to an accumulation of lymph under the skin of the abdomen in the days or weeks following the procedure. This post-abdominoplasty complication is relatively common and generally requires puncturing the effusions.

The objective of this study is to highlight possible histological changes after injections of platelet-enriched plasma (PRP) alone or PRP combined with autologous thrombin serum in the hypodermis of abdominoplasties.

Platelet-rich plasma (PRP) therapy has gained popularity since the first reports of its clinical use in the 1980s and 1990s (1-2). David R. Knighton described the first use of "locally acting growth factors obtained from

human platelets and applied topically", highlighting how it may be of interest to isolate growth factors from whole blood to induce tissue regeneration, particularly in chronic wound healing. At this time, Knighton was using laboratory techniques to prepare PRP.

PRP is easy to obtain, as it only requires a venipuncture. The blood components are then separated by centrifugation to obtain the fraction containing plasma and platelets. The relatively low cost and ease of use have facilitated the rapid expansion of PRP into medical practices. PRP preparation has been greatly simplified in recent years, thanks to the development of commercial PRP preparation devices. These devices also allow the preparation of PRP in compliance with the requirements of health regulations and good practices.

For this, 2 injections at the dermo-hypodermis junction, PRP alone or combined with Thrombin, from the subject's blood collected with the RegenBCT® kit from REGENLAB.

To highlight the modifications secondary to these injections, the fragments of skin collected from in vivo are placed in survival in order to examine at the level of the hypodermis:

• The inflammatory state by dosage of IL8,

• The caliber of the blood capillaries,

• The fibrous tracts after staining the collagen with Sirius red.

2. Material and Methods

2.1 Injected products

Informed consent was obtained from 6 different donor (average age: 39.5) prior to the study initiation.

The patient's platelet-rich plasma (PRP) and Thrombin are collected using REGENLAB's RegenKit® just before their abdominoplasty. In practice, 2x 10 ml of the patient's blood are collected in RegenBCT® (Blood Cell Therapy) tubes containing an anticoagulant solution of sodium citrate and thixotropic gel allowing the plasma to be separated from the blood cells after centrifugation at 1500g. The final result makes it possible to produce 10 ml of a standardized composition of RegenPRP®, plasma enriched to 80% in platelets but depleted in pro-inflammatory granulocytes and erythrocytes (only lymphocytes and monocytes persist). Thrombin

serum (approximately 2 ml) is also collected from the RegenBCT® tubes, at the level of the fibrin clot, taking care not to contaminate the sample with white or red blood cells).

At the time of the surgical procedure, 2 ml of PRP versus a mixture of 2 ml of PRP + Thrombin were injected into 2 areas contiguous to the dermis-hypodermis junction and into the deep hypodermis and using 10 injection points. on an area of 15 cm2.

2.2 Skin fragments maintained in survival (3-7)

Within one hour after excision, the skin explants were rinsed with an antibiotic-containing phosphate buffer solution and divided into fragments allocated to the different experimental conditions. The fragments were placed with the epidermis facing upward on a 3 µm-pore polycarbonate membrane of tissue culture inserts set in wells of 25 mm in diameter in 6-well culture plates (Costar, VWR, Washington, DC, USA). The culture medium (Dulbecco's modified minimum essential medium, GlutaMaxTM, Gibco BRL, Waltham, MA, USA) was supplemented with 0.5% foetal calf serum (DAP), 25 µg/mL of bovine pituitary extract (Gibco BRL), 50 µg/mL of hydrocortisone (H4001, Sigma-Aldrich, St. Louis, MO, USA), 100 µg/mL of penicillin, and 100 µg/mL of streptomycin (Gibco BRL) and added at the bottom of the wells, allowing diffusion between the two compartments separated by the porous membrane (3 µm). The culture plates were maintained at 37 °C in a humidified incubator and a 5% CO_2 atmosphere during 2 days for histological vessels and IL8 analysis and 14 days for fibrous tracts analysis.

3 conditions were compared:

- Skincontrol (non injected),
- Skin after PRP injection,
- Skin after PRP + Thrombine injection.

After 2 days of survival, the samples were fixed in formalin then embedded in paraffin for histological analysis of the capillaries in the hypodermis. The culture media are also collected for the IL8 assay.

After 14 days of culture, the skin fragments were fixed in formalin then embedded in paraffin for morphometric analysis of collagen in the fibrous tracts of the hypodermis.

2.3. Analysis

2.3.1 Measurement of vascular changes in the hypodermis

Fragments of skin were collected, fixed in formol, and embedded in paraffin. Following coloration by hemalun-eosin.

The number of dilated vessels on the whole of the histological slice was counted in the upper part of the hypodermis, at the injection sites (at a magnification of 40, Olympus microscope). This number was compared to the total number of vessels in order to calculate the percentage of dilated vessels in the hypodermis.

This histological analysis was completed by quantitative measurement with the aid of an image analyzer. The slices obtained were observed using a Olympus® BX41 microscope. Photography of the slices was taken with a QImagingRegita 2000R camera directly linked to the microscope. For this, the average area occupied by the light of the vessels (µm²) is measured at different levels (in the superficial dermis and in the upper part of the mid-dermis) on a slice (homogeneity of the results is verified on a second slice if necessary).

This area is determined in relation to an equivalent number of vessels for an examined donor. The average area is evaluated in average on n +/- 10 vessels.

2.3.2. Interleukin -8 quantification

Many cells stimulated by IL1 or TNF□, monocytes, fibroblasts, endothelial cells, keratinocytes, etc.) secondarily produce IL8. IL8 is a chemokine whose main property is to attract circulating leukocytes towards an inflammatory focus.

The culture medium supernatants were kept at −32 °C for interleukin-8 (IL-8) quantification. The levels of this pro-inflammatory mediator were measured using a human IL-8 ELISA kit (Human IL-8/CXCL8 DuoSet ELISA, Bio-Techne, *Minneapolis, MN, USA*) according to the manufacturer's specifications. The results are expressed in pg/mL.

The assay results are reported by fragment weight and finally expressed in pg/mg.

2.3.3. Quantification of fibrous tracts in the hypodermis by computerized image analysis of collagen

The hypodermis, or areolar adipose tissue, is arranged in vertical compartments, distributed perpendicular to the most superficial layers of the skin. Depending on the area analyzed, there may be a variation in thickness. It is formed by fatty lobules interspersed with well-defined fibrous septa (tracts) composed of elastic fibers and collagen and oriented perpendicularly towards the surface, strongly anchored to the dermis and connecting it to the fascia superficialis. These septa serve as a passage for the vessels and nerves of the subcutaneous adipose tissue, with compartments well vascularized by capillary vessels.

The collagen from fibrous tracts in the hypodermis was highlighted on the fragments fixed in formaldehyde and included in paraffin by a coloring in Sirius red. The surface (µm2) of the fibrous tracts was quantified morphometrically by computer-assisted image analysis (Image Pro plus).

2.3.4. Statistical analysis

The results obtained for each parameter are expressed as the mean ± standard deviation (SD) of the individual values determined in each treatment group.

For capillary and IL8 analysis, the statistical analysis was made according the Student's t-test or Mann& Whitney, with an alpha risk of 5% if population was normal.

For the analysis of the surface of the fibrous tracts, considering that their number and size can vary from one skin fragment to another and for the same donor, a Kruskal-Wallis test was carried out (non-parametric Anova).

3. Results

3.1. Analysis of dilated capillaries in the hypodermis

On average, the % of dilated capillaries significantly decreased by 25% after injection of PRP (69.17 ± 4.41) compared to control skin (92.81 ± 4.2).

The % of dilated capillaries significantly decreased by 26% after injection of PRP + Thrombin (68.9 ± 4.86) compared to control skin and similarly compared to PRP alone (no significant difference).

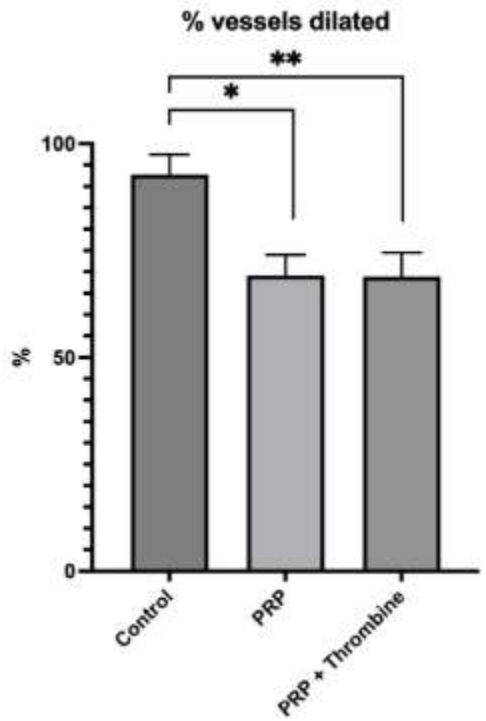

Figure 1

Moreover, the surface area of dilated capillaries significantly decreases by 77% after PRP injection (56.61 ± 15.58) compared to control skin (241.35 ± 53.26). This surface significantly decreases by 74% after PRP + Thrombine injection (63.95 ± 20.28) compared to control skin and similarly compared to PRP alone (no significant difference).

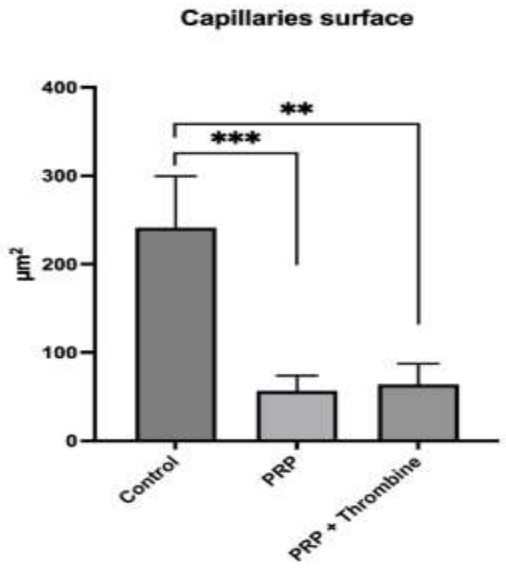

Figure 2

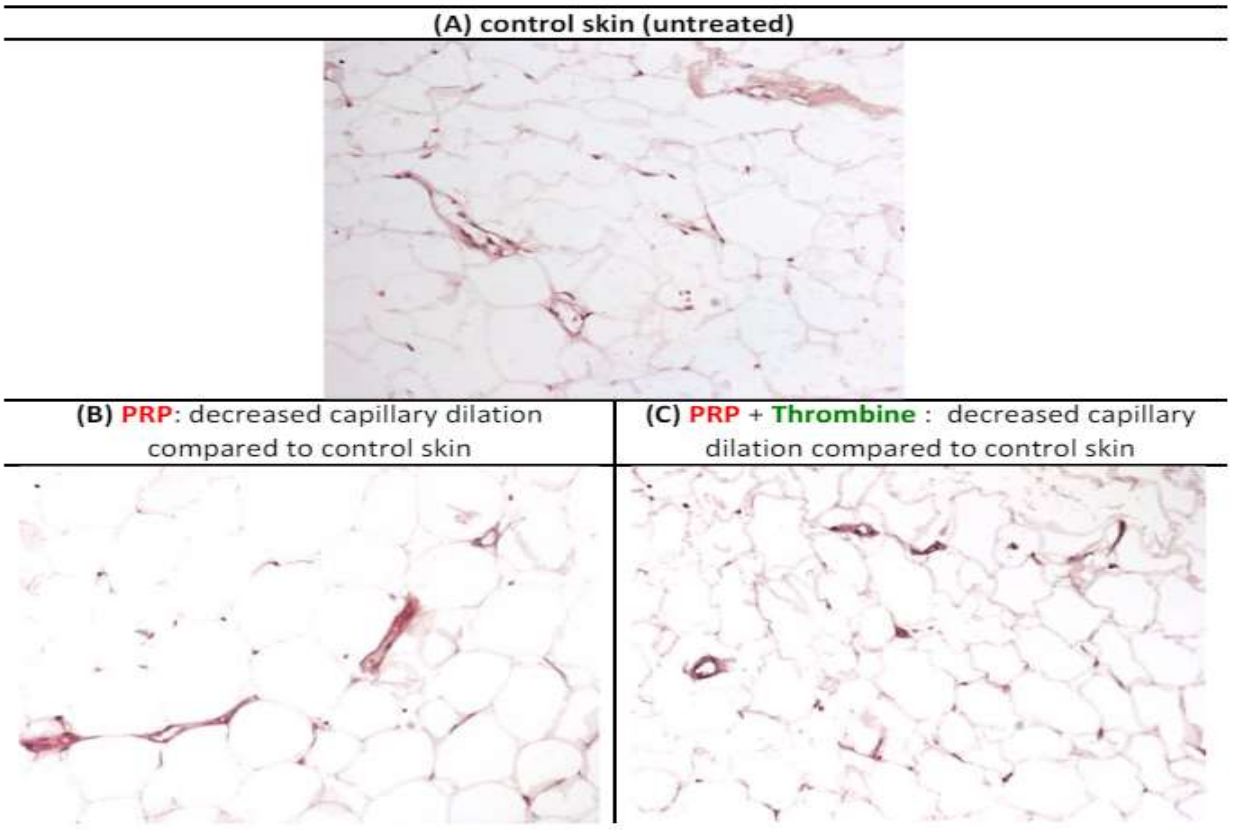

Figure 3: capillary dilation analysis in the hypodermis (Hemalun-eosin x200)

3.2. IL8 Assay

On average, the IL8 level is not modified after PRP (40.0 ± 12.0 pg/mg) or after PRP + Thrombine 42.2 ± 13.25 pg/mg) injection compared to control skin (37.61 ± 10.97 pg/mg), demonstrating an absence of inflammation.

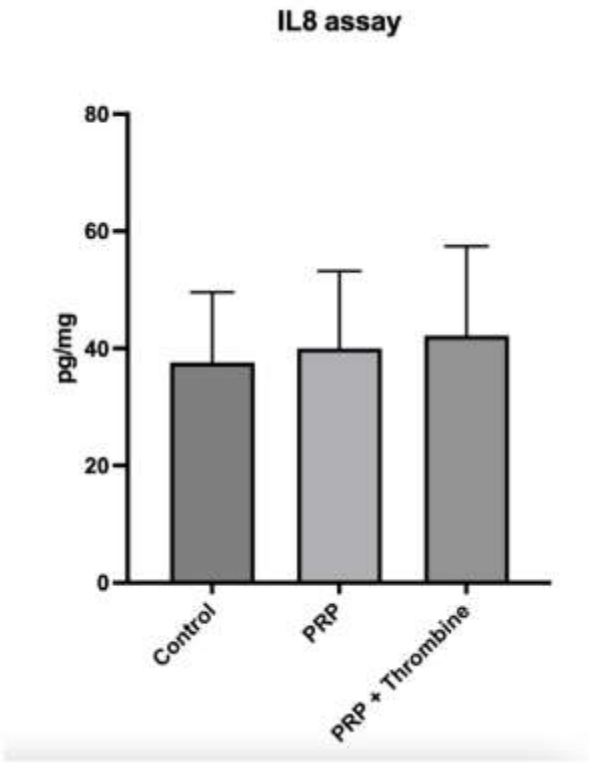

Figure 4

3.3. Quantification of fibrous tracts in the hypodermis by computerized image analysis of collagen

After injection of PRP and PRP + Thrombin, the mean surface area of the fibrous tracts in the hypodermis significantly increases by 41.7% (p=0.0044) and 43.8% (p = 0.035) respectively compared to control skin.

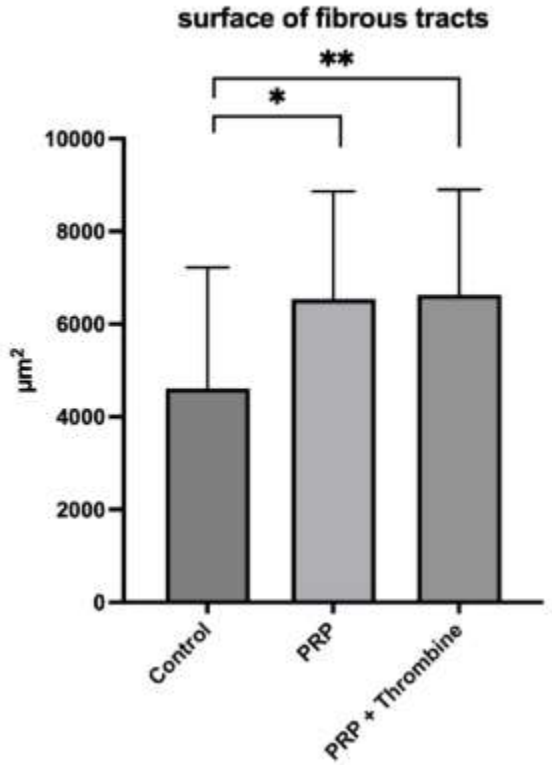
surface of fibrous tracts
**
*
10000
8000
6000
4000
2000
0
µm²
Control
PRP
PRP + Thrombine

Figure 5

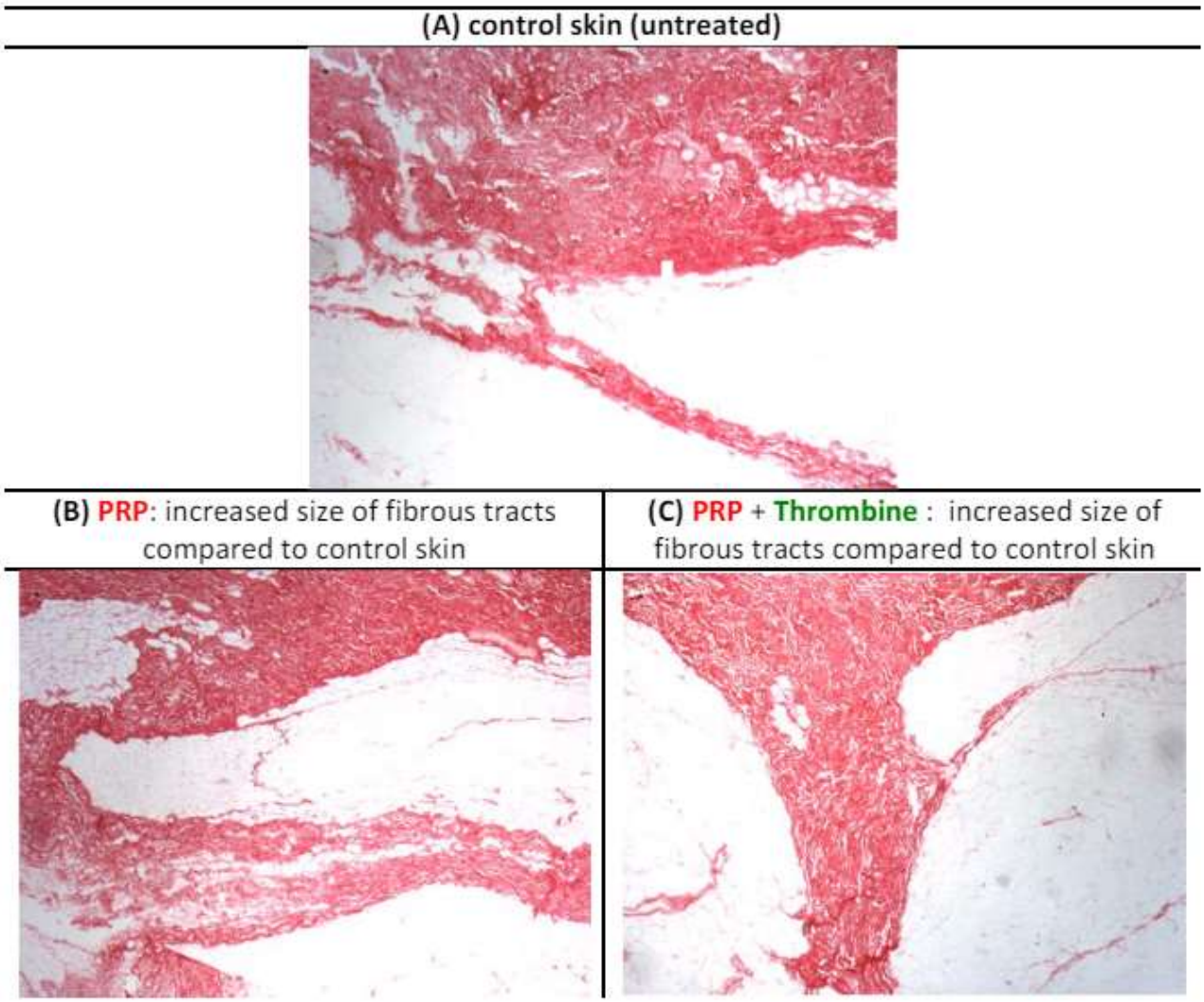

Figure 6: Visualisation of fibrous tracts (blue arrow) in the hypodermis after staining the collagen with sirius red (x200)

4. Discussion

In the quest for optimizing the post-operative healing process in plastic surgery, the autologous application of platelet-rich plasma (PRP) glue has emerged as a promising path. Our study aims to investigate the pathophysiological mechanisms by which autologously activated PRP may modulate the tissue microenvironment and thus contribute to the prevention of seromas (9-16).

The literature on the subject, while abundant, is not unanimous and reflects a certain disparity in findings, particularly due to the variability of protocols and the methodology of studies. This heterogeneity underlines the crucial importance of standardizing the procedures for PRP use, as well as the need for more in-depth studies for a comprehensive understanding of its effects.

According to our hypothesis, injections of PRP (alone or combined with Thrombin) at the dermis-hypodermis junction would make it possible to obtain vasoconstriction of the lymphatics and capillaries and thus avoid lymphedema observed post-surgical intervention.

In order to verify this postulate, 2 injections of PRP alone or associated with Thrombin were injected at the level of the dermo-hypodermis junction, using the subject's blood recovered with the RegenBCT® kit from REGENLAB.

After surviving skin fragments from abdominoplasties, it was revealed:

• A reduction in the caliber of blood capillaries by 70% in the hypodermis,

• An absence of inflammation (stable IL8 level compared to control skin),

Moreover, the increase in the surface area of the fibrous tracts of approximately 40% reflecting collagen neosynthesis in the hypodermis, may also explain the vasoconstriction observed after injection (bearing in mind that the control and injected skin fragments were different and may include fibrous tracts of different sizes).

Beyond plastic and aesthetic surgery, PRP enriched with autologous thrombin is emerging as a cross-disciplinary tool in medicine, with potential applications in craniofacial surgery, orthopedics, otolaryngology, and maxillofacial surgery (17- 33). These fields could benefit from its hemostatic and adhesive properties to improve tissue repair and regeneration, suggesting its role as a universal facilitator of healing. However, despite the enthusiasm generated by these 12 varied applications, standardization remains a major challenge to validate these promises and achieve widespread and effective use.

5. Conclusion

Our research confirms the potential of PRP of Associated with thrombin while calling for a rigorous scientific approach for larger-scale randomized clinical trials. Future work will need to establish solid and consistent

evidence of its effectiveness, with the goal of integrating PRP into the suite of advanced solutions for improved post-operative recovery and optimized management of surgical complications."

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