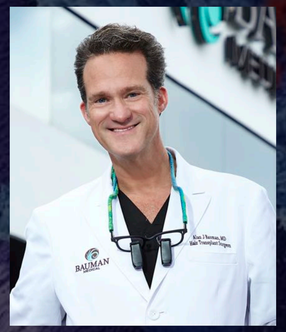
Beyond PRP?

Unveiling PPX:

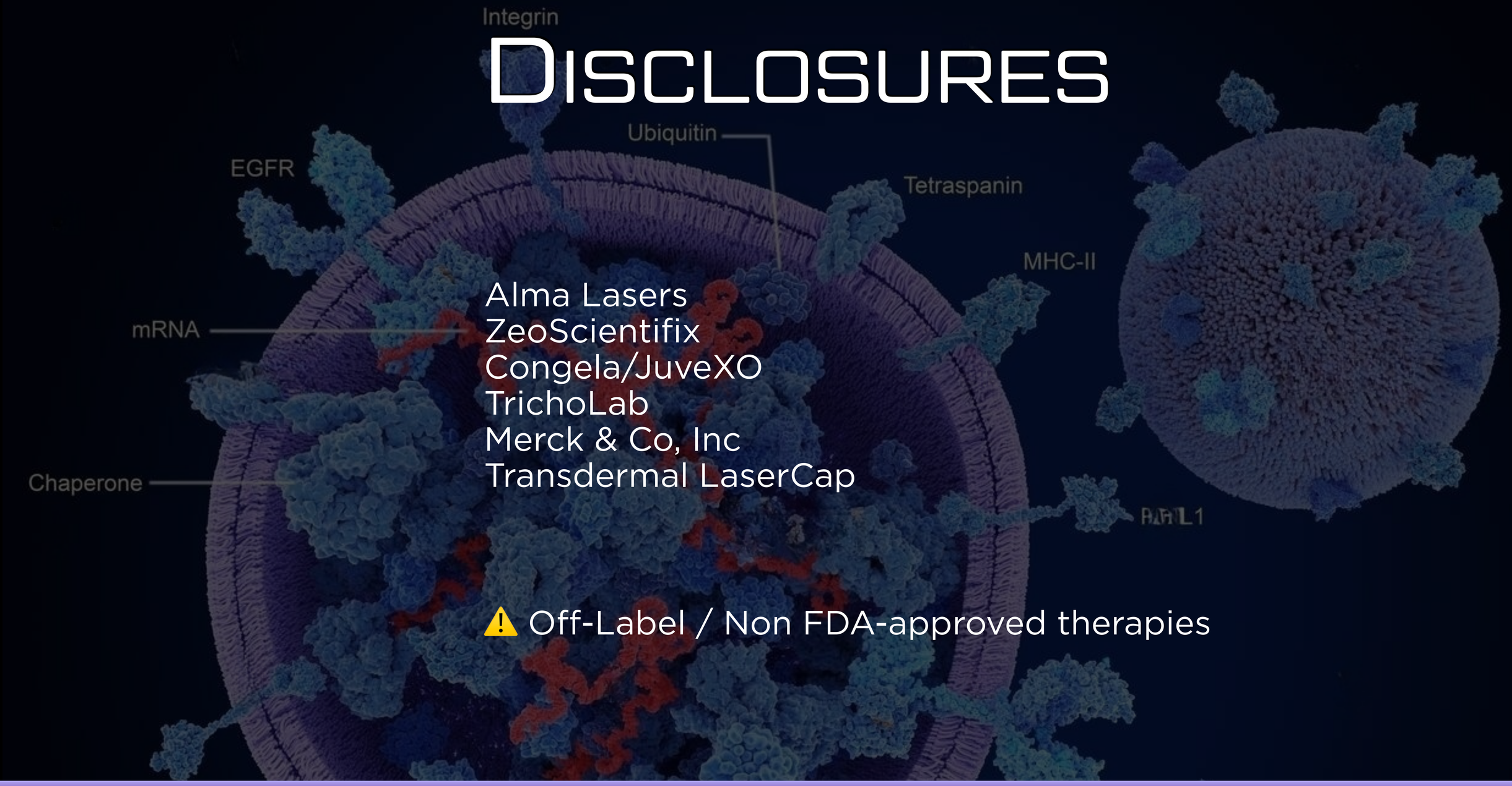
[Plasma Precipitate Fraction]
Standardized Autologous Plasma-Derived Exosomes



Alan J Bauman, MD, ABHRS, FISHRS

32nd World Congress
International Society of Hair Restoration Surgery
Denver | October 17-19, 2024

Disclosures



Alma Lasers
ZeoScientifix
Congela/JuveXO
TrichoLab
Merck & Co, Inc
Transdermal LaserCap

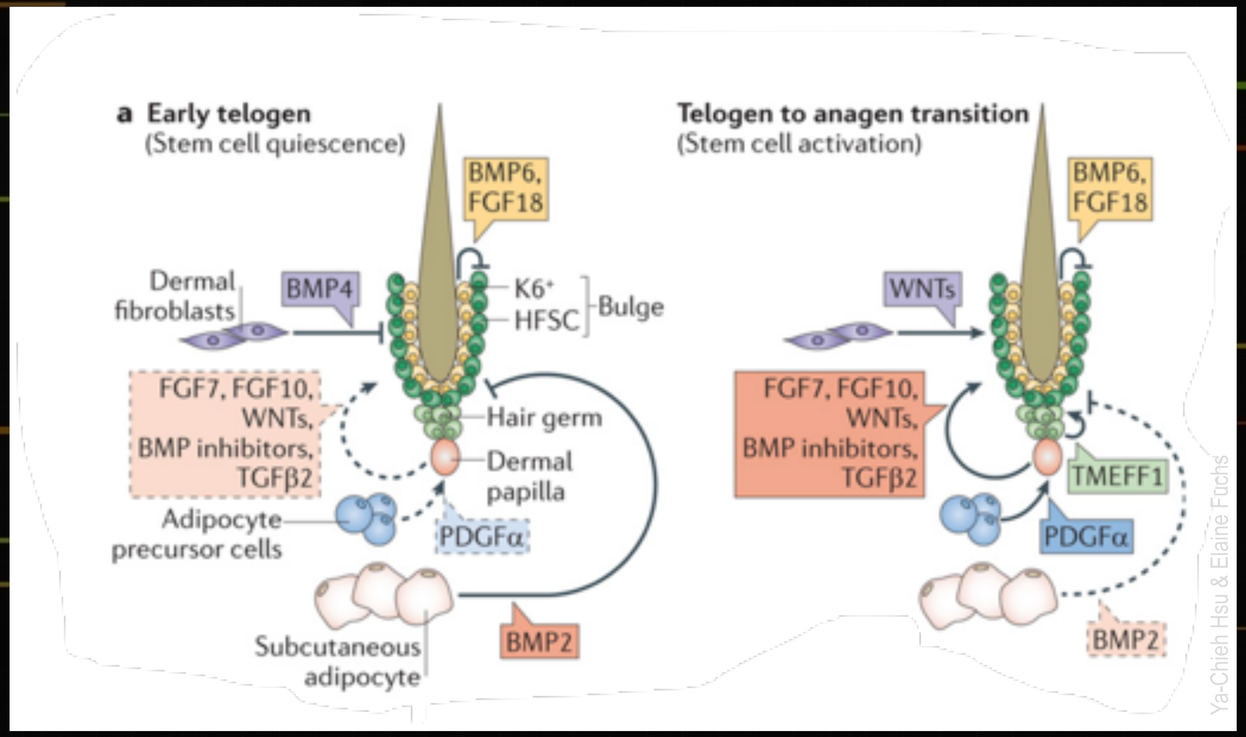
⚠️ Off-Label / Non FDA-approved therapies

Goals & Objectives

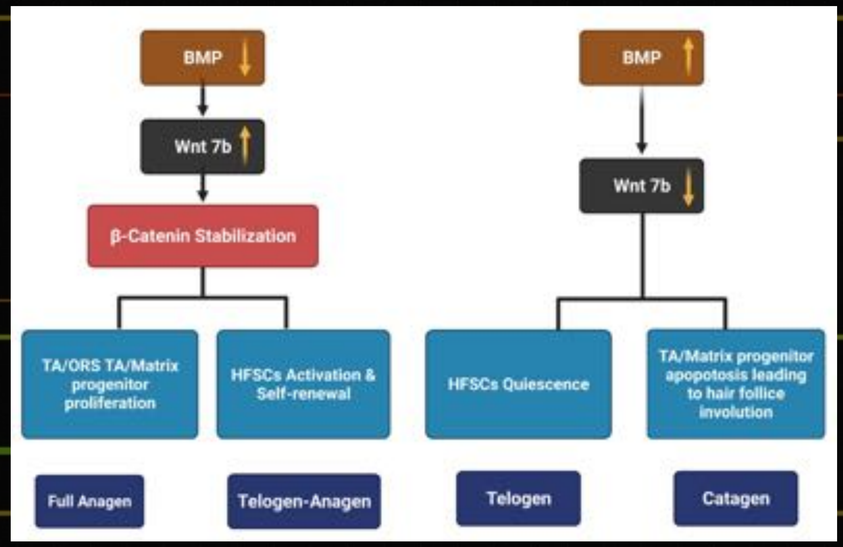
- ~~Regenerative Medicine in Alopecia/Healing~~
- ~~PRP Limitations/Concerns~~
- ~~Exosomes & FDA~~
- Plasma-Derived Exosomes (PPX)
- PPX Production & Composition
- miRNA Payload of PPX
- Conclusion

Goals of Regenerative Medicine in Hair Restoration

Complex interaction between mesenchymal and epidermal derived cells



- Decreased BMP Bone Morphogenic Protein
- Activation of Wnt pathway
- Accumulation of β-Catenin in Nucleus of HFSC's
- HF Stem Cell Activation
- Anagen Onset and Proliferation



Hsu YC, Fuchs E; A family business: stem cell progeny join the niche to regulate homeostasis, Nature Reviews Molecular Cell Biology 13, 103-114 (February 2012) doi:10.1038/nrm3272
Anudeep, TC, et. al. Advancing Regenerative Cellular Therapies in Non-Scarring Alopecia. Pharmaceutics 2022, 14, 612. https://doi.org/10.3390/pharmaceutics14030612

Regenerative Strategies in Hair Restoration

Adult Stem/Signaling Cells, MSCs
- Adipose Derived Stem Cells (ADSC)
  - Nanofat, Stromal Vascular Fraction (SVF)
- Hair Follicle Stem Cells (HFSCs) - autologous or cultured
- Bone Marrow Derived

Perinatal Biologic Tissue
- Umbilical Cord Blood/Tissue (WJ) Derived
- Amniotic Fluid Derived
- Placental MSCs

Peptides & Growth Factors

Ultrasonic Sound Waves

RF Radiofrequency

Pulsed Electromagnetic Fields - PEMF

Photobiomodulation - Laser or LEDs

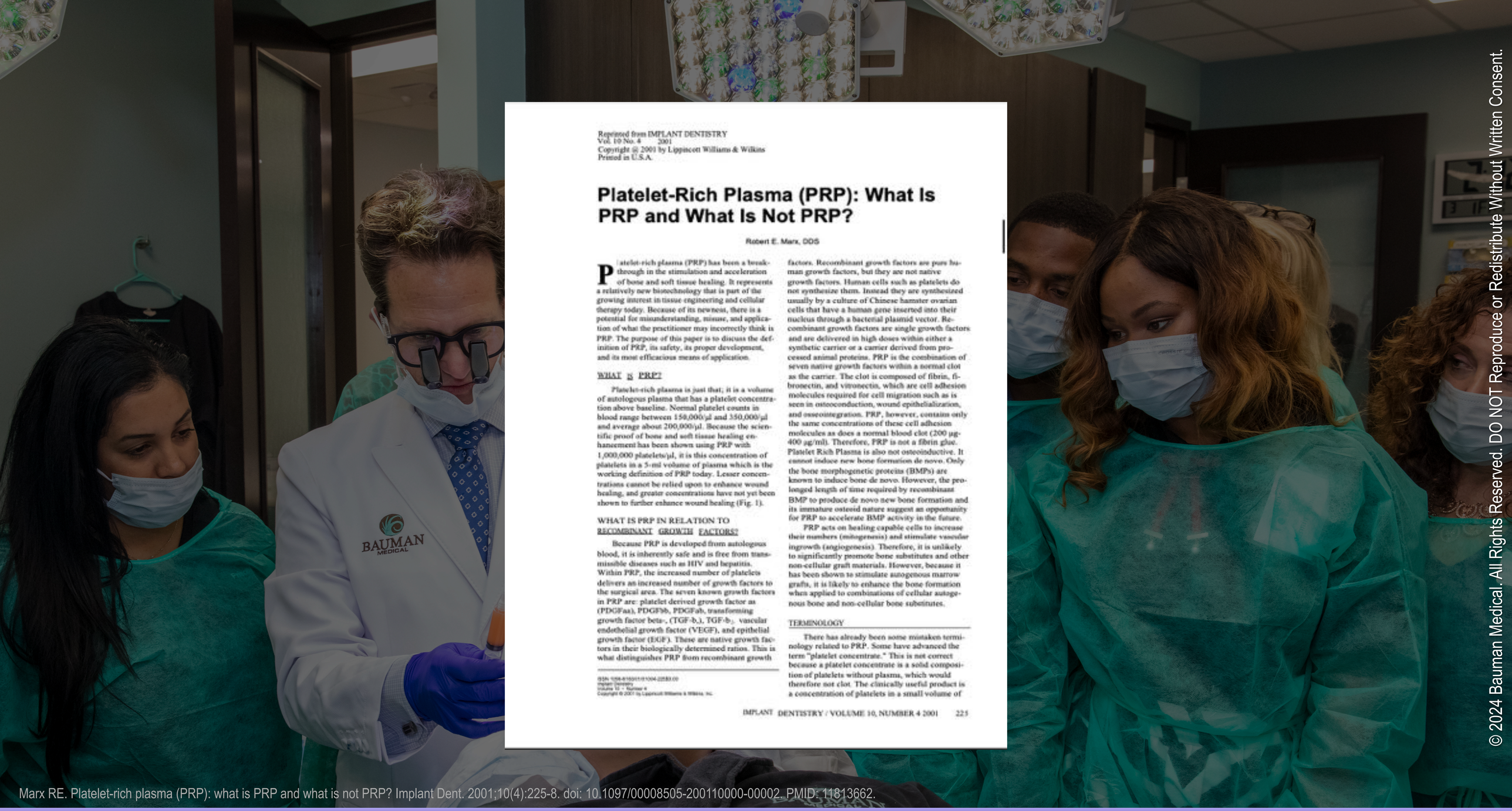
Extracellular Matrix
- xenograft, allograft, synthetic (PDO)

Platelet Rich Plasma (PRP/PRF)

Conditioned Media/Secretome
- ADSC-CM, hUBC-CM, HF-CM, BM-CM

Extracellular Vesicles - EVs
- Exosomes - newborn foreskin SCs, DPC, BM-MSC, Platelet-derived, Autologous Plasma Exosomes PPX, Placenta, WJ…

Hair Follicle Cell Banking, Expansion & Multiplication



Reprinted from IMPLANT DENTISTRY
Vol. 10 No. 4 2001

Platelet-Rich Plasma (PRP): What Is PRP and What Is Not PRP?

Robert E. Marx, DDS

Platelet-rich plasma (PRP) has been a breakthrough in the stimulation and acceleration of bone and soft tissue healing. It represents a relatively new biotechnology that is part of the growing interest in tissue engineering and cellular therapy today. Because of its newness, there is a potential for misunderstanding, misuse, and application of what the practitioner may incorrectly think is PRP. The purpose of this paper is to discuss the definition of PRP, its safety, its proper development, and its most efficacious means of application.

WHAT IS PRP?

Platelet-rich plasma is just that; it is a volume of autologous plasma that has a platelet concentration above baseline. Normal platelet counts in blood range between 150,000/μl and 350,000/μl and average about 200,000/μl. Because the scientific proof of bone and soft tissue healing enhancement has been shown using PRP with 1,000,000 platelets/μl, it is this concentration of platelets in a 5-ml volume of plasma which is the working definition of PRP today. Lesser concentrations cannot be relied upon to enhance wound healing, and greater concentrations have not yet been shown to further enhance wound healing (Fig. 1).

WHAT IS PRP IN RELATION TO RECOMBINANT GROWTH FACTORS?

Because PRP is developed from autologous blood, it is inherently safe and is free from transmissible diseases such as HIV and hepatitis. Within PRP, the increased number of platelets delivers an increased number of growth factors to the surgical area. The seven known growth factors in PRP are: platelet derived growth factor as (PDGFaa), PDGFbb, PDGFab, transforming growth factor beta-, (TGF-b$_1$), TGF-b$_2$, vascular endothelial growth factor (VEGF), and epithelial growth factor (EGF). These are native growth factors in their biologically determined ratios. This is what distinguishes PRP from recombinant growth factors. Recombinant growth factors are pure human growth factors, but they are not native growth factors. Human cells such as platelets do not synthesize them. Instead they are synthesized usually by a culture of Chinese hamster ovarian cells that have a human gene inserted into their nucleus through a bacterial plasmid vector. Recombinant growth factors are single growth factors and are delivered in high doses within either a synthetic carrier or a carrier derived from processed animal proteins. PRP is the combination of seven native growth factors within a normal clot as the carrier. The clot is composed of fibrin, fibronectin, and vitronectin, which are cell adhesion molecules required for cell migration such as is seen in osteoconduction, wound epithelialization, and osseointegration. PRP, however, contains only the same concentrations of these cell adhesion molecules as does a normal blood clot (200 μg-400 μg/ml). Therefore, PRP is not a fibrin glue. Platelet Rich Plasma is also not osteoinductive. It cannot induce new bone formation de novo. Only the bone morphogenetic proteins (BMPs) are known to induce bone de novo. However, the prolonged length of time required by recombinant BMP to produce de novo new bone formation and its immature osteoid nature suggest an opportunity for PRP to accelerate BMP activity in the future.

PRP acts on healing capable cells to increase their numbers (mitogenesis) and stimulate vascular ingrowth (angiogenesis). Therefore, it is unlikely to significantly promote bone substitutes and other non-cellular graft materials. However, because it has been shown to stimulate autogenous marrow grafts, it is likely to enhance the bone formation when applied to combinations of cellular autogenous bone and non-cellular bone substitutes.

TERMINOLOGY

There has already been some mistaken terminology related to PRP. Some have advanced the term "platelet concentrate." This is not correct because a platelet concentrate is a solid composition of platelets without plasma, which would therefore not clot. The clinically useful product is a concentration of platelets in a small volume of

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Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent. 2001;10(4):225-8. doi: 10.1097/00008505-200110000-00002. PMID: 11813662.

PRP in Male

Androgenetic Alopecia

Dual Spin
1.5M Platelets/μl,
~1.7k Monocytes/μl
[>11B Platelets, >12k Monocytes in 7.5cc PRP]

BEFORE PRP

AFTER 12 MOS

BEFORE PRP

AFTER 12 MOS

BEFORE PRP

AFTER 12 MOS

PRP in Female Androgenetic Alopecia

Dual Spin

1.5M Platelets/μl,

1.7k Monocytes/μl

[>11B Platelets, >12k Monocytes in 7.5cc PRP]

Traction Alopecia in African American Female

BEFORE Bauman-PRP™ | 4 MOS **AFTER** Bauman-PRP™

PRP in Female Traction Alopecia

Dual Spin
1.5M Platelets/μl,
1.7k Monocytes/μl
[>11B Platelets, >12k Monocytes in 7.5cc PRP]

PRP Concerns

Variability in PRP Preparation
- Phlebotomy Technique, Equipment and Methods can alter the platelet concentration
- Leukocyte Rich vs Poor
- PRP analysis at bedside is costly, lab analysis is unreliable/delayed

Variations in Centrifugation
- Speed & Duration affects the concentration, purity, and platelet morphology (i.e., damage)

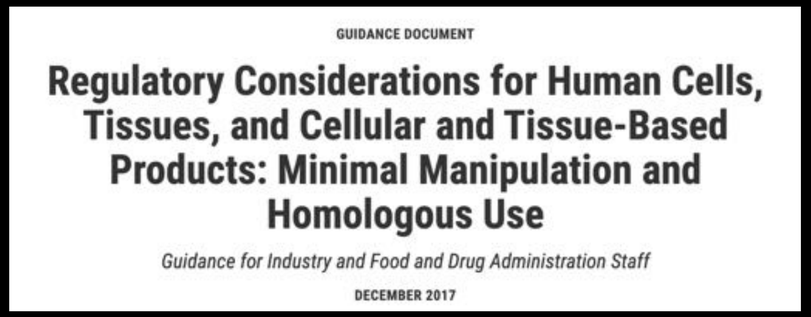
Activation Methods
- Activated (Ca, thrombin), Non-activated, Sonicated

Inconsistent Dosage & Delivery
- Variations in volume, concentration of PRP
- Depth and Method of Injection

Lack of Protocol Standardization
- # Sessions, Frequency, Follow-up, Tracking/Measuring, Re-treatment Timing

Unstandardized Equipment
- Numerous kits, various levels of platelet concentration & quality

FDA HCT/P Guidelines



GUIDANCE DOCUMENT

Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use

Guidance for Industry and Food and Drug Administration Staff

DECEMBER 2017

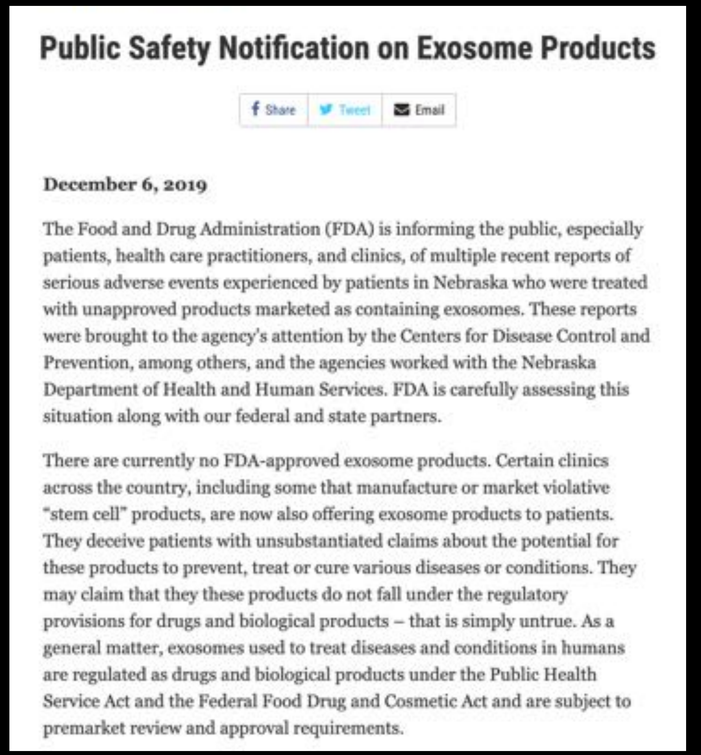
Title 21 CFR 1271.10(a)

HCT/P: Human Cells, Tissues, & Cellular and Tissue-Based Products

- minimal manipulation
- cells, nonstructural tissues
- homologous use
- adipose, SVF, amnion
- manufacturer's objective intent
- licensing, registration & IND

"…PRP is exempt from CFR 1271.10" –K Beitzel

Exosome Warning Letters



Public Safety Notification on Exosome Products

Share Tweet Email

December 6, 2019

The Food and Drug Administration (FDA) is informing the public, especially patients, health care practitioners, and clinics, of multiple recent reports of serious adverse events experienced by patients in Nebraska who were treated with unapproved products marketed as containing exosomes. These reports were brought to the agency's attention by the Centers for Disease Control and Prevention, among others, and the agencies worked with the Nebraska Department of Health and Human Services. FDA is carefully assessing this situation along with our federal and state partners.

There are currently no FDA-approved exosome products. Certain clinics across the country, including some that manufacture or market violative "stem cell" products, are now also offering exosome products to patients. They deceive patients with unsubstantiated claims about the potential for these products to prevent, treat or cure various diseases or conditions. They may claim that they these products do not fall under the regulatory provisions for drugs and biological products – that is simply untrue. As a general matter, exosomes used to treat diseases and conditions in humans are regulated as drugs and biological products under the Public Health Service Act and the Federal Food Drug and Cosmetic Act and are subject to premarket review and approval requirements.

"…exosomes used to treat diseases and conditions in humans are regulated as drugs…"

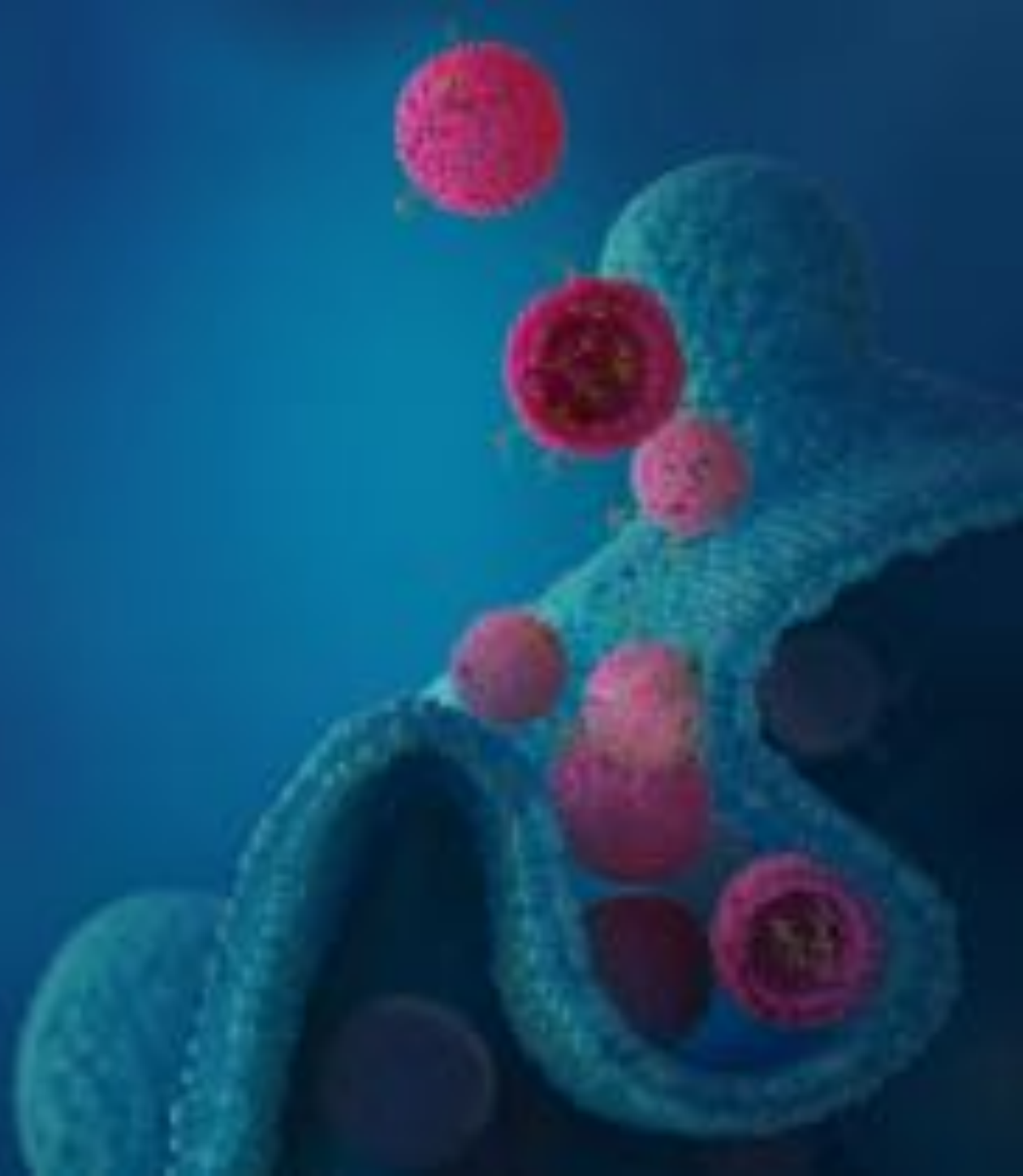
https://www.fda.gov/media/124138/download
https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/public-safety-notification-exosome-products
https://www.fda.gov/news-events/press-announcements/fda-sends-warning-companies-offering-unapproved-umbilical-cord-blood-products-may-put-patients-risk
Beitzel K, Allen D, Apostolakos J, Russell RP, McCarthy MB, Gallo GJ, Cote MP, Mazzocca AD. US definitions, current use, and FDA stance on use of platelet-rich plasma in sports medicine. J Knee Surg. 2015 Feb;28(1):29-34.

PPX

"**P**ATIENT **P**URE e**X**OSOMES"

AUTOLOGOUS PLASMA PRECIPITATE FRACTION

PLASMA-DERIVED EXOSOMES



INTRODUCING: "PPX"
Autologous Plasma-Derived Exosomes

60cc Phlebotomy

⬇

Transport to Lab

⬇

Screening/Testing for Contaminants/Infectious Agents

⬇

Closed System, cGMP lab process, Minimally-Manipulated
Ultracentrifugation [300 Billion EVs/ml]

⬇

Lyophilization
[2 x 2ml vials of PPX ~600 Billion each]

⬇

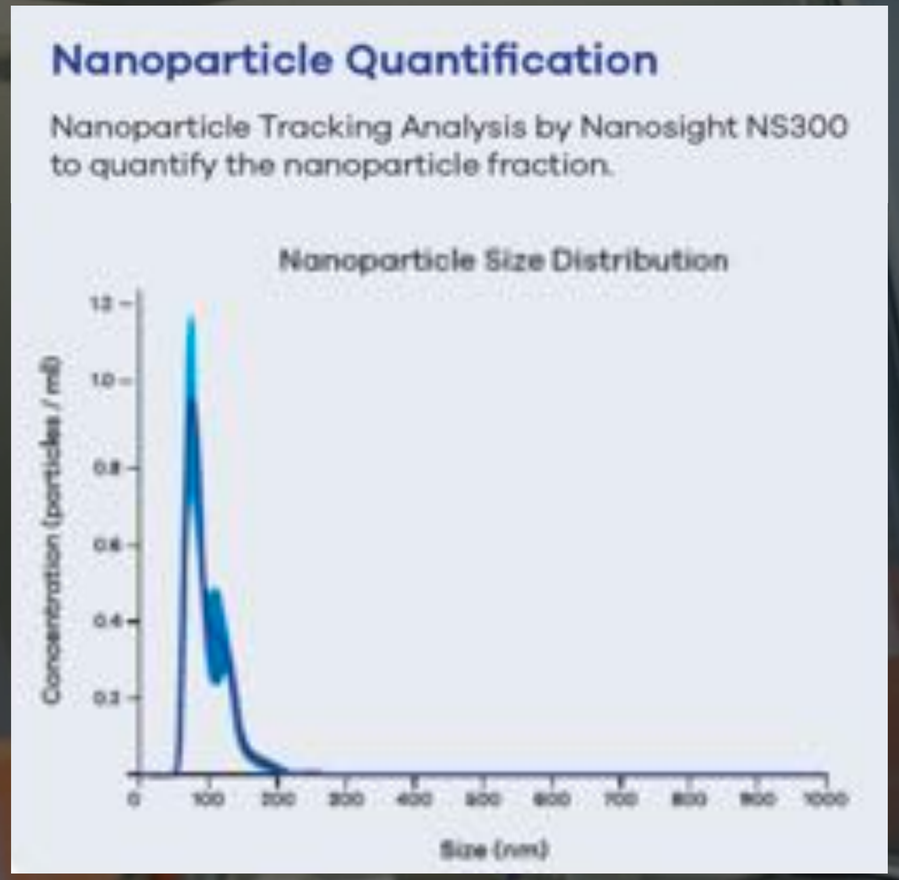
Transport Back to Clinic
[7-10 days]

⬇

Reconstituted & Applied (topical or injection) / Same Patient (Autologous)

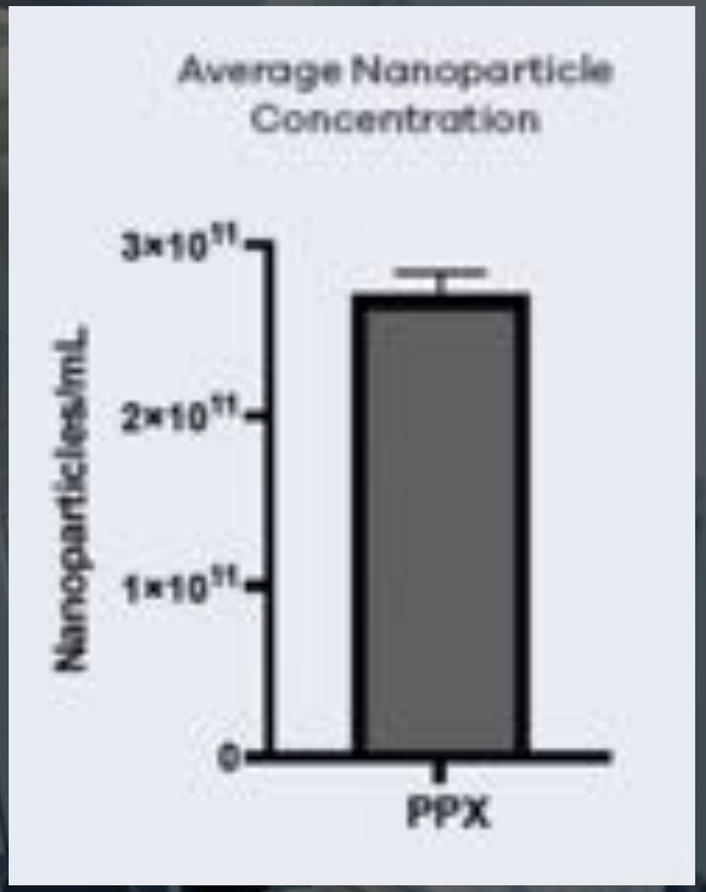
“PPX” Autologous Plasma-Derived Exosomes

[Characterization: Purity, Size & Concentration]



ZEO Scientifix, Inc.

60-100nm



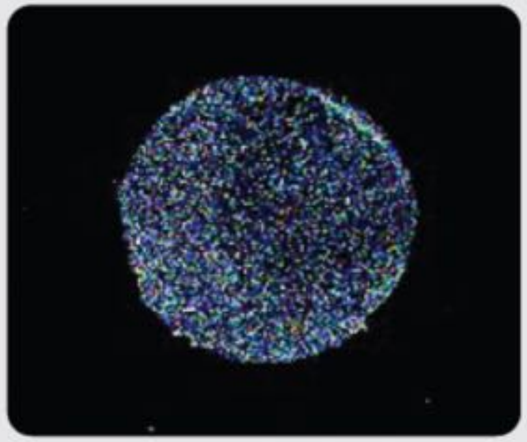
ZEO Scientifix, Inc.

300 billion/ml

“PPX” Autologous Plasma-Derived Exosomes
[Characterization: Identity/Markers, Concentration vs PRP]

Extracellular Vesicle Identification

Exo Dot immunofluorescent analysis reveals the presence of Extracellular Vesicles (CD9+) in PPX™

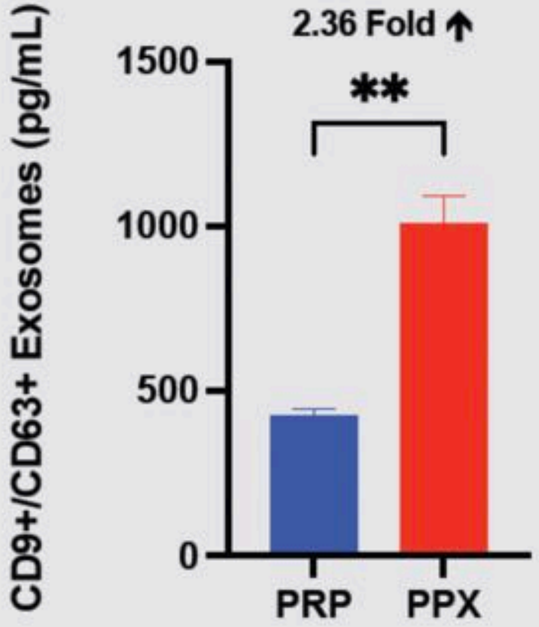


ZEO Scientifix, Inc.

CD9+

Exosome Concentration

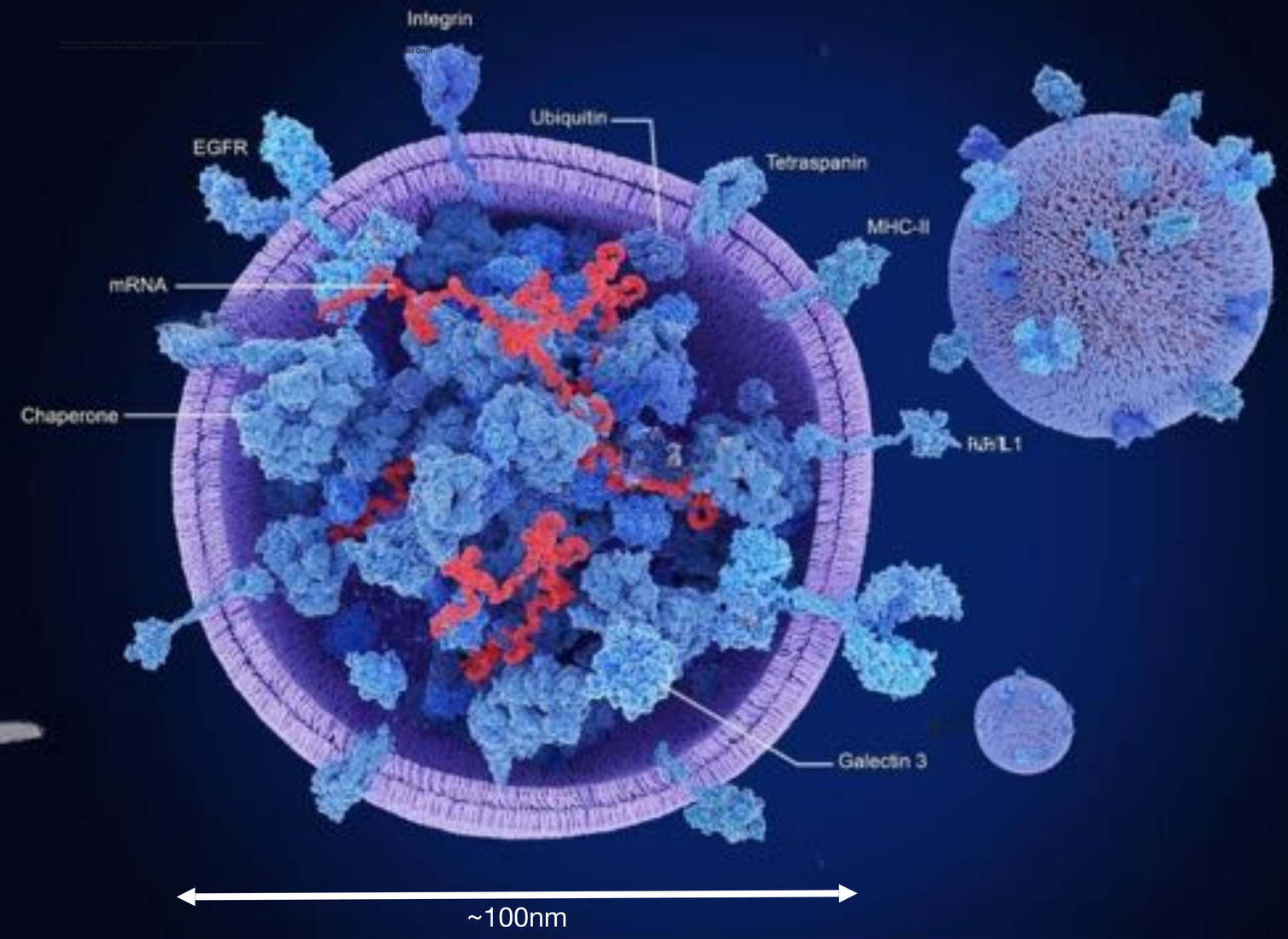
CD9-CD63 ELISA Analysis quantifies the concentration of exosomes in PRP vs PPX™



ZEO Scientifix, Inc.

2.4x concentration of CD9+/ CD63+ Exosomes vs PRP

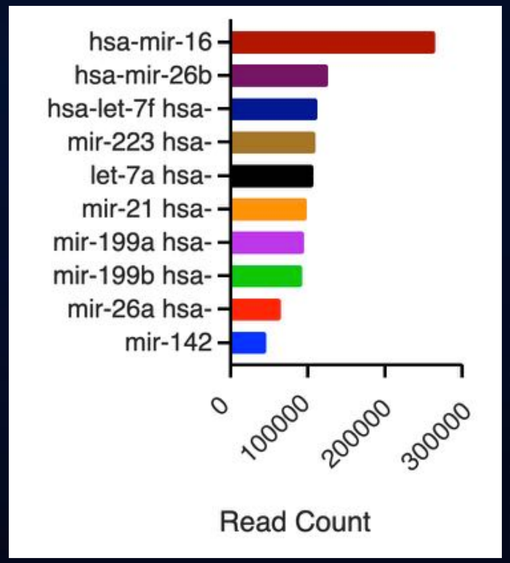
PPX Exosome Cargo



>300 Bioactive Ingredients:

proteins, nucleic acids, lipids, and metabolites

Top 10 miRNA in PPX



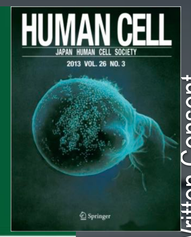
ZEO Scientifix, Inc.

Functions of miRNAs in Alopecia

Current insight into the functions of microRNAs in common human hair loss disorders: a mini review

Review Article | Published: 27 April 2021

Volume 34, pages 1040–1050, (2021) Cite this article



Sujay Paul ✉, Iván Licona-Vázquez, Francisco I. Serrano-Cano, Natalia Frías-Reid, Carolina Pacheco-Dorantes, Surajit Pathak, Samik Chakraborty & Aashish Srivastava

Current insight into the functions of microRNAs in common human hair loss disorders: a mini review

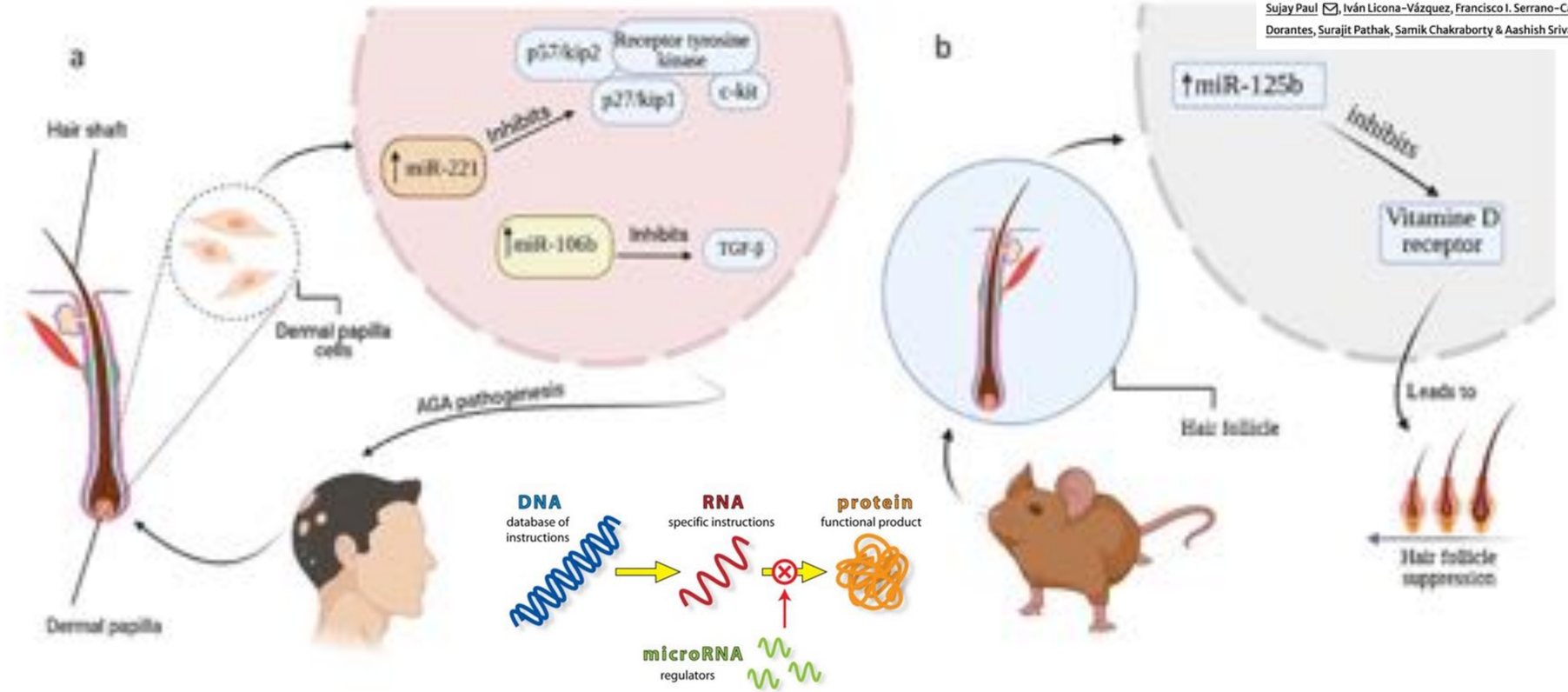


Fig. 2 miRNAs dysregulation and their implications in AGA. MiRNAs are significantly dysregulated in humans and mice affected by AGA. (2a) shows that miR-221 and miR-106b are upregulated in human dermal papilla cells and inhibit multiple target proteins, such as p57/kip2, p27/kip1, receptor tyrosine kinase, and c-kit proteins, and TGF-b leads to the AGA pathogenesis. While in (2b), it has been shown that upregulation of miR-125b inhibits vitamin D receptor (VDR) in mice hair follicles, promoting hair follicle suppression. Therefore, it is suggested that these interactions might explain AGA pathogenesis (↑: upregulated; ↓: downregulated)

Sujay Paul

Paul S, et al. Current insight into the functions of microRNAs in common human hair loss disorders: a mini review. Hum Cell. 2021 Jul;34(4):1040-1050. doi: 10.1007/s13577-021-00540-0. Epub 2021 Apr 27. PMID: 33908022.

miRNA Cargo in PPX for Alopecia, Inflammation, Healing

miRNA	Hair Loss	Wound Healing	Inflam-mation	Function
hsa-miR-16	🔴	🟢	🟢	Regulates **cell proliferation and apoptosis**, supporting hair follicle cycling.
hsa-miR-26a	🟡	🟢	🟢	Enhances **dermal papilla cell differentiation and proliferation**, boosting hair follicle regeneration and new hair growth.
hsa-let-7a/f	🟢	🟡	🟢	Supports **stem cell differentiation** and maintains the hair follicle stem cell niche, preventing follicle miniaturization.
hsa-miR-21	🟢	🟢	🟢	Promotes **wound healing and reduces inflammation**, improving scalp health and helping in immune-related hair loss conditions.
hsa-miR-199a/b	🟡	🟢	🟢	Modulates **inflammation and fibrosis**, protecting hair follicles from damage and enhancing regrowth.

🟢 = **Strong evidence**
🟡 = **Moderate evidence**
🔴 = **Limited evidence**

1. Zheng Y, Nace A, Zheng X, Wagle M, Patel A, et al. Glucocorticoid signaling and regulatory T cells collaborate to maintain the hair follicle stem cell niche. Nat Immunol. 2021;22(6):688-699.
2. Liu Z, Hu X, Liang Y, Yu J, Li H, Shokhirev MN, Zheng Y. Glucocorticoid signaling and regulatory T cells cooperate to maintain the hair-follicle stem-cell niche. Nat Immunol. 2022 Jul;23(7):1086-1097. doi: 10.1038/s41590-022-01244-9. Epub 2022 Jun 23. PMID: 35739197; PMCID: PMC9283297.https://pubmed.ncbi.nlm.nih.gov/35739197/
3. Paul S, Bravo Vázquez LA, Pérez Uribe S, Reyes-Pérez PR, Sharma A. Current insight into the functions of microRNAs in common human hair loss disorders: a mini review. Hum Cell. 2020;33(3):711-720. https://pubmed.ncbi.nlm.nih.gov/33908022/
4. Kazi T, Nagata A, Nakagawa T, Matsuzaki T, Inui S. Dermal Papilla Cell-Derived Extracellular Vesicles Increase Hair Inductive Gene Expression in Adipose Stem Cells via β-Catenin Activation. Cells. 2022 Jan 7;11(2):202. doi: 10.3390/cells11020202. PMID: 35053317; PMCID: PMC8773911.
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PPX Safety & Quality

- ✓100% natural autologous source
- ✓Not classified as an HCT/P
- ✓Non-nucleated, acellular
- ✓Minimally manipulated
- ✓Sterile filtered without radiation
- ✓No cryoprotectant
- ✓Endotoxin/contaminant testing on all samples
- ✓Non-Expanded/Non-Cultured
- ✓Manufactured in a designated clean room under cGMP standards

PPX Hair Growth at 6mos

Autologous Plasma Precipitate Fraction
PPX (600 Billion/2ml)

Patient H.L. 66M 6mos s/p 2ml PPX injected to Vertex

HMI 42 → 51 (+21%)
Improvement in Crown/Vertex Coverage
Thickening/Lengthening of Existing Hair

PPX Hair Growth at 6mos

Patient H.L. 66M 6mos s/p 2ml PPX injected to Vertex

HMI 42 → 51 (+21%)
Improvement in Crown/Vertex Coverage
Thickening/Lengthening of Existing Hair

Autologous Plasma Precipitate Fraction
PPX (600 Billion/2ml)

Regulatory & Standardization Considerations

1. PPX, like platelet-rich plasma, (blood taken from an individual and then given back to the same individual) is not an HCT/P* under 21 CFR Part 1271, because **PPX is an autologous blood product**.

2. Because PPX meets the requirements for an exemption, as defined in 21 CFR 1271.15(b), is not subject to FDA's regulations in 21 CFR Part 1271. **NO FDA pre-market approval is required for PPX.**

3. PPX is processed in the United States utilizing rigorous standards of cGMP production in accordance with AATB** and FDA 21 CFR 1271. **PRP has no standardization.**

CFR - US Code of Federal Regulations
* HCT/P - Human cells, tissues, and cellular and tissue-based products
**AATB - American Association of Tissue Banks.

PRP vs PPX

	PRP (Platelet-Rich Plasma)	PPX (Patient Pure X)
Potency	🔴 Lower concentration of bioactive molecules.	🟢 Higher concentrations of exosomes, miRNAs, and bioactive molecules, may have stronger regenerative capacity.
Consistency	🔴 Non-standardized preparation, may lead to inconsistent product/results depending on technique.	🟢 Standardized under cGMP, ensuring reliable, more consistent production/outcomes.
Safety	🔴 Contains cellular material, which may trigger immune reactions or inflammation.	🟢 Acellular product, reducing the risk of immune response and inflammation.
Preparation	🟢 Quick and easy to prepare in-office with centrifugation.	🔴 Requires lab processing (7-10 days), adding complexity.
Storage	🔴 Must be used immediately; no storage options.	🟢 Lyophilized product can be stored for later use, offering flexibility.
Regulatory	🟢 HCT/P exempt. No pre-market approval needed.	🟢 HCT/P exempt. FDA-compliant production, no pre-market approval needed. Injectable.
Cost	🟢 More affordable, widely available.	🔴 Higher cost due to specialized processing but may offer superior therapeutic value.

THANK YOU!

SCAN TO CONNECT!

@DrAlanBauman