Enzyme Corporation
611 Gateway Blvd, Ste 120
South San Francisco, CA 94080

https://www.enzyme.com

MEMORANDUM

29 January 2021

Re: Regulatory Status of SuperShot® PRP

To Whom It May Concern:

Enzyme Corporation ("Enzyme") is a life science regulatory software and services company based in the San Francisco Bay Area. Enzyme's consulting team members each have 10+ years experience in their respective fields (e.g. therapeutics, diagnostics, biologics, medical devices) and collectively the Enzyme team has written or co-written FDA and EMA marketing authorization applications for over 25 products, including products from major life science manufacturers such as Amgen, Inc., Regeneron Pharmaceuticals, Inc., and Sanofi, SA.. Enzyme's regulatory consultants all hold postgraduate degrees in either science or engineering, and are all Regulatory Affairs Certified (RAC) professionals by the Regulatory Affairs Professionals Society (RAPS).

Enzyme was engaged by Forever Labs, Inc. ("Forever Labs") to review the enclosed Position Paper (attached below as "Appendix A") for its SuperShot® Platelet Rich Plasma ("SuperShot® PRP") for accuracy with current regulations and regulatory guidance.

To achieve this end, Enzyme reviewed current and past FDA guidance on the subject of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/P's) as well as current legislation and regulation of this field, particularly 21 CFR Part 1271.

Enzyme's formal opinion is that Forever Labs' position that SuperShot® PRP has the same regulatory status as PRP that has not undergone the SuperShot® process is both reasonable and supported by FDA Guidance on HCT/P's.

Sincerely,

Jared Seehafer, MS RAC
CEO & Cofounder

Enzyme Corporation
611 Gateway Blvd, Ste 120
South San Francisco, CA 94080

https://www.enzyme.com

APPENDIX A

FOREVER LABS POSITION PAPER

January 29, 2021

SUPERSHOT® PRP: INFORMATION AND REGULATORY STATUS

I. Platelet-Rich Plasma

Platelet-rich plasma (PRP) is a preparation for therapeutic purposes that is increasingly accepted for various musculoskeletal disorders, due to its theoretical potential to repair tissues with poor-healing capacity [1]. PRP therapy uses injections of a patient's own concentrated platelets. In a PRP preparation, platelets are concentrated by centrifugation from a peripheral blood draw resulting in three fractions: 1) Red blood cells (RBCs) 2) Platelet-rich plasma (PRP) and 3) Platelet-poor plasma (PPP). According to the FDA, PRP is considered a blood product. Per the FDA guidance "Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use" [2], July 2020, Section V.A, pg. 22:

> for example, platelet rich plasma (PRP, blood taken from an individual and given back to the same individual as platelet rich plasma) is not an HCT/P under 21 CFR Part 1271 because it is a blood product.

1. Radiologia. Nov-Dec 2018;60(6):465-475. https://pubmed.ncbi.nlm.nih.gov/30274850
2. "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" U.S. Department of Health and Human Services Food and Drug Administration, Center for Biologics Evaluation and Research, Center for Devices and Radiological Health, Office of Combination Products; July 2020 https://www.fda.gov/media/109176/download

II. SuperShot® PRP

SuperShot® PRP is a PRP preparation that includes an additional centrifugation step whereby the low-density lipid rich plasma fraction is precipitated out of the Platelet-poor Plasma fraction, following initial centrifugation, and incorporated into the PRP. In a standard PRP procedure, the PRP fraction is injected, leaving many biological molecules such as extracellular vesicles (EVs), discarded with the PPP. EVs mediate a series of cellular functions such as the transport of materials and intercellular communication [1]. EVs are highly specialized messenger molecules, which can deliver biological signals. For these reasons, the potential of extracellular vesicles in therapeutic application in regenerative medicine is drawing increasing interest [2]. The SuperShot® PRP process increases the concentration of EVs in PRP when compared to a standard PRP process.

SuperShot® Solution: SuperShot is packaged as 2mL solution in a 10x concentrate of: 23.3% Poly(ethylene glycol) 6000 (PEG)/10% Dextran 500 (Dex). It is diluted 10x in PPP to a working concentration of 2.3% PEG/1% Dex.

SuperShot is manufactured in a 503b facility (Compound Preferred, 1125 Hollipark Dr., Idaho Falls, ID 83401). SuperShot® is terminally sterilized during manufacture.

When added to PPP, SuperShot® enables separation of low-density plasma-born molecules upon low-speed centrifugation. PEG and dextran together result in aqueous polymer two phase system, which is required for the purification of biological materials. The PEG/Dex solution results in the precipitation of small low-density molecules, including extracellular vesicles (50-500nm in diameter). PEG precipitation is simple, fast, and scalable; does not deform EVs [3].

The SuperShot® PRP Process: First a PRP centrifugation process is performed. A small portion of the PPP is aseptically removed and combined with SuperShot® 10x solution. The PPP including SuperShot® is then centrifuged at 4200 RPM for 1 min. The result is a pellet of low-density biomolecules including EVs. The supernatant, which now consists of the depleted PPP and PEG/Dex is removed and discarded, leaving behind the EF pellet, which is then resuspended into PRP.

1. Biomater Sci. 2017 Dec 19;6(1):60-78. https://pubmed.ncbi.nlm.nih.gov/29184934
2. Circ Res. 2017 May 12;120(10):1658-1673. https://pubmed.ncbi.nlm.nih.gov/28495996
3. Biomed Res Int. 2018 Jan 30; 2018:8545347. https://pubmed.ncbi.nlm.nih.gov/29662902

III. SuperShot® Regulatory Status

FDA's regulatory authority over Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/P) is defined in 21 CFR 1271. 21 CFR 1271.3(d) defines what is and is not included under the definition of HCT/P:

> (d) Human cells, tissues, or cellular or tissue-based products (HCT/Ps) means articles containing or consisting of human cells or tissues that are intended for implantation, transplantation, infusion, or transfer into a human recipient. Examples of HCT/Ps include, but are not limited to, bone, ligament, skin, dura mater, heart valve, cornea, hematopoietic stem/progenitor cells derived from peripheral and cord blood, manipulated autologous chondrocytes, epithelial cells on a synthetic matrix, and semen or other reproductive tissue. The following articles are not considered HCT/Ps:
>
> (1) Vascularized human organs for transplantation;
>
> (2) Whole blood or blood components or blood derivative products subject to listing under parts 607 and 207 of this chapter, respectively

As stated above, PRP is considered by FDA to be a "blood derivative product" and therefore not within the scope HCT/P, and not subject to the regulatory requirements of HCT/P's.

FDA has clarified its view in the guidance "Same Surgical Procedure Exception under 21 CFR 1271.15(b): Questions and Answers Regarding the Scope of the Exception" [1], November 2017, Section II, pg. 3:

> In sum, FDA's view is that autologous cells or tissues that are removed from an individual and implanted into the same individual without intervening processing steps beyond rinsing, cleansing, sizing, or shaping, raise no additional risks of contamination and communicable disease transmission beyond that typically associated with surgery. FDA considers the same surgical procedure exception to be a narrow exception to regulation under Part 1271.

Based on FDA's analysis, we submit that SuperShot® PRP qualifies for the same exemption in 21 CFR 1271 as PRP does and for the same reasons that FDA defines above. Like PRP: 1) it is a blood derivative product, 2) it is autologous, 3) it is removed from an individual and implanted into the same individual in the same surgical procedure, 4) it does not include intervening processing steps beyond rinsing, cleansing, sizing, or shaping (as in PRP preparation, size exclusion is achieved in SuperShot® PRP by centrifugation), and 5) it raises no additional risks of contamination and communicable disease transmission beyond that typically associated with surgery;

SuperShot® is sterile, PEG/Dex is non-toxic, does not modify the biology, and used only in centrifugation, and is not injected.

1. "Same Surgical Procedure Exception under 21 CFR 1271.15(b): Questions and Answers Regarding the Scope of the Exception Guidance for Industry" U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research; November 2017 https://www.fda.gov/media/89920/download

IV. Safety Profile of Poly(ethylene glycol) 6000 and Dextran 500

Poly(ethylene glycol), 6000: PEG is a non-ionic hydrophilic polymer available in different molecular weights. PEG and dextran together result in aqueous polymer two phase system, which is required for the purification of biological materials [1]. PEG is soluble in water and has a low intrinsic toxicity that renders the polymer ideally suited for biological applications [1]. PEGs are FDA-approved, generally nonimmunogenic, and are frequently used in many biomedical applications including bioconjugation,[1] as excipients in drug delivery,[2,3] surface functionalization,[4] and tissue engineering [5]. Polyethylene glycol (PEG) is used as a non-penetrating cryoprotectant [6].

Laxatives have PEG listed as an active ingredient: MiraLAX® (17g per dose).

The following medicines have PEG listed as an inactive ingredient in: Acetominophen, Ibuprophen, Oxycontin, Benadryl®, Muscle relaxers, Mavyret® (hepatitis C treatment).

PEG is also found in lubricating eye drops, skin cream, toothpaste, processed foods [7].

Dextran, 500: Dextran is a high molecular weight, inert, water soluble polymer that has been used in a wide variety of bio-medical applications. Dextrans are characterized by their moderate-to-high molecular weight, good water solubility, and low toxicity. They also generally exhibit low immunogenitiсy [1]. Dextrans are biologically inert due to their uncommon poly-(α-D-1,6-glucose) linkages, which render them resistant to cleavage by most endogenous cellular glycosidases. Dextrans are used therapeutically as plasma volume expanders and anticoagulants [8]. Dextran can be part of vaccines as a carrier, a back-bone and/or as a stabilizer of the antigen or other subunits [9,10]. Dextran can be used as a cryo-protectant in combination with DSMO, glycerol etc. [9,10].

Some dextran-containing medicines: Photrexa®, Macrodex®, Rondex®, Hyskon®, Polyglucin®, Promit®, Debrisan® (Dextranomer), Zuidex®, Solesta®, Exudex®

PEG and Dextran are non-toxic commonly used as stabilizers and excipients. SuperShot® employs PEG/Dex during centrifugation for size-exclusion of low-density extracellular vesicles in the plasma.

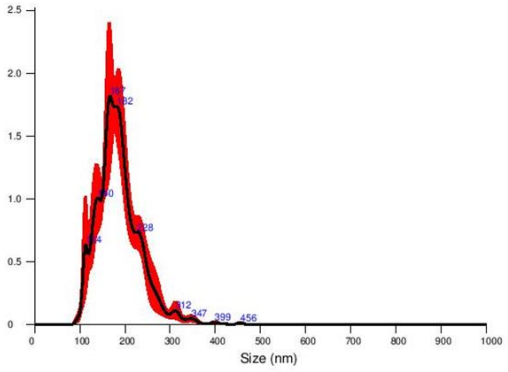
1. Poly(Ethylene Glycol) Chemistry, 2013. https://www.springer.com/gp/book/9780306440786
2. Bioconjugate Techniques; Elsevier Science: Burlington, 2013. https://www.sciencedirect.com/book/9780123822390
3. POLYMERIC DRUG DELIVERY TECHNIQUES: Translating Polymer Science for Drug Delivery; Aldrich Materials Science: Milwaukee, WI, 2015.
4. Eur. J Pharmacol. 2011, 670 (2-3), 372–383. https://pubmed.ncbi.nlm.nih.gov/21951969
5. Gold Bull. 2011, 44 (2), 99–105. https://link.springer.com/article/10.1007/s13404-011-0015-8
6. Biomaterials 2009, 30 (35), 6702–6707. https://pubmed.ncbi.nlm.nih.gov/19783300
7. Polyethylene Glycol: https://www.drugs.com/pro/polyethylene-glycol.html

8. Dextran, Product Information: https://www.sigmaaldrich.com/technical-documents/protocols/biology/dextran.html
9. Dextran: https://pubchem.ncbi.nlm.nih.gov/compound/dextran
10. Dextran Application Area: https://www.dextran.com/application-areas

V. Analysis of SuperShot® PRP after Processing

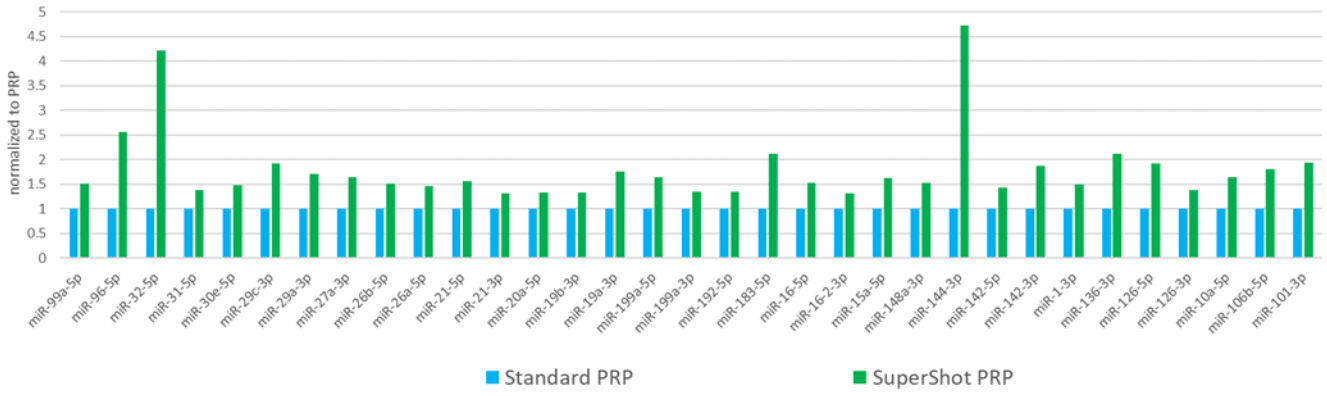
PEG/Dex remaining in EF: Final PEG and Dextran concentrations in the EF were determined by Impact Analytical (Midland, MI). 5mL of PPP from a healthy human donor were processed with SuperShot®. The resulting pellet was suspended in 5mL of Phosphate Buffered Saline, sent for analysis. The sample was analyzed by Agilent 1290 Series liquid chromatograph coupled with a G6530B quadrupole time-of-flight (Q-ToF) mass spectrometer following Impact Analytical standard operating procedure SOP-MOL-020. Concentrations determined were: Dextran: 11 µg/mL, PEG: 812.5 µg/mL. These data demonstrate that PEG/Dex in SuperShot® is used for size-exclusion in centrifugation, and it is not injected.

SuperShot® isolated EVs: The EF isolated from 5mL of platelet-poor plasma by SuperShot®, were analyzed by the NanoSight imaging device.



Results: Mean: 187.2 +/- 0.7 nm, Mode: 181.1 +/- 4.8 nm, Concentration: 1.69e+11 +/- 1.21e+10 particles/mL

SuperShot® PRP miRNA: EVs contain micro RNAs (miRNAs). miRNAs present in PRP processed with SuperShot® or standard PRP determined by via single-end sequencing on an Illumina Hiseq 2500 (LC Sciences, Houston, TX):



The SuperShot® process isolates autologous extracellular vesicles from the plasma and is enriched for plasma-born miRNAs when compared to standard PRP.