Study Objectves:

What is PRP?

- PRP stands for Platelet Rich Plasma. It has been in use for more than 25 years. It contains numerous growth factors that promote healing. It is produced as a liquid by adding anticoagulants before centrifugation.

How is PRF different from PRP?

- PRF stands for Platelet Rich Fibrin. It has been popularized in the last 6-7 years. It is produced from whole blood and no anticoagulants are added. It must be centrifuged within 90 seconds of being drawn or it will begin clotting and poor or no separation will occur. It can be produced as a solid clot or a liquid.

What are the "active" factors in PRP and PRF? What are their primary effects?

- VEGF: Vascular Endothelial Growth Factor; Promotes angiogenesis
- PDGF: Platelet Derived Growth Factor; Recruits other cells to aid healing
- TGF-β1: Transforming Growth Factor: Supports cell proliferation of all cell types
- Leukocytes: Defend against pathogens, modulate immune response to biomaterials, secrete key immune cytokines to aid healing

How is PRF superior to PRP?

- Because PRF is not prepared with added anticoagulants, it will form a fibrin clot. This fibrin mesh structure traps cells and growth factors and releases them slowly over a period of time. The benefits of PRP will last about 2 days where PRF will last 10-14 days. Even though cells and growth factors are released slowly, they are still highly concentrated by the centrifugation process. And cells and factors within the body are being recruited throughout the time the clot is active.
- PRP and PRF will have the same number of cells, PRP growth factors have a half-life of 6 minutes during which they must bind to cell receptor sites. PRF releases growth factors for days. Like watering a flower with a 2 litre bottle all at once versus drip irrigation.

What are the two most common forms of PRF and how are they generated?

- Solid clots are produced using red topped tubes, opened to the air after centrifugation. Exposure to air initiates and accelerates clotting.
- Liquid PRF, is produced in blue topped tubes with the cap left on after centrifugation to avoid contact with air. Liquid can even be withdrawn from the tube with a syringe through the cap to minimize exposure to air and delay clotting.

How do they differ? What are the advantages of one form over another? What are the clinical implications of these differences?

- Solid clots can be pressed into membranes, cut into custom shapes, cut into pieces and mixed with bone fragments, layered under incisions, used to fill periodontal defects and tooth extraction sites or sinuses with or without added bone.

- Liquid PRF can be injected along incisions, injected or microneedled for facial rejuvenation and even hair regrowth, can be formed in custom trays and allowed to clot for membranes, can be injected into joints or other tissues, can also be added to bone graft material and allowed to clot to improve efficacy and handling characteristics of the graft material.

How is horizontal centrifugation different than fixed angle? What are the advantages?

- Fixed angle centrifuges maintain the tube at the same angle relative to the motor axis during the entire centrifugation cycle. The centrifugal force generated by the spinning rotor creates force perpendicular to the motor axis. The force on the cells is outward but the tube bottom is angled downward. The heavier cells do not have a straight path to the bottom of the tube but are forced up against the outer wall of the tube. This collection of heavier red blood cells on the outer wall of the tubes impedes the movement of the lighter cells towards the upper layers. Cells cannot freely pass each other and separation is hindered.
- As the horizontal centrifuge begins to spin, the tube holders rise until the are horizontal, perpendicular to the motor axis, and in line with the centrifugal force vector. Cells are not forced against the tube walls and are free to flow and separate according to their densities. Horizontal separation can result in a 4x greater accumulation of cells in the PRF layer!

What is the difference between RPM's and RCF? Why are they important?

- RPM's measure the speed of the centrifuge motor in revolutions per minute. RCF measures the relative centrifugal force on the test tube. Imagine a ball at the end of a rope. if the rope is one metre in length and you spin it at 1 RPM it would be going very slowly. But if the rope was 5 meters in length and you maintained 1 RPM the ball would be traveling at a much greater speed. The centrifugal force would be greatly increased.
- RPM's is a meaningless measure when it comes to centrifuges unless you also know the distance from the motor axis. Then you can calculate the RCF. Instead of calculating this every time, the Bio-PRF displays settings in RCF and adjusts the RPM's to achieve that degree of force.
- Fixed angle and horizontal centrifuges can both be adjusted to achieve the same RCF but the separation is superior with the horizontal centrifuge.

So what centrifuge settings should I use?

- There are an infinite number of combinations of RCF and time. At first, researchers only measured the volume of "yellow", the plasma layer and the total number of cells in the volume. Spinning at high speed for an extended time produced total red/yellow separation but all cells were in the red layer. Slower speeds yielded more cells but less volume of PRF.
- Dr. Miron developed a novel way to evaluate RCF/time of centrifugation. People had only measured the cell numbers in the yellow fraction but Dr. Miron asked "where in the yellow fraction and which cells?"
- He divided the tube into 10 1cc increments by pipette and then tested each increment for cell type and concentration. He tested 24 different protocols and settled on three basic settings depending on the need for volume versus cell concentration.

- The nomenclature can be confusing. I will try to explain it as clearly as possible.
- The first protocol is “Solid PRF” spun at 700rcf for 8 minutes. This yields a substantial volume (3cc or more) with a high concentration of cells throughout. When you produce a clot at this setting it will have near uniform distribution of cells end to end. This setting can be used for almost all procedures with good result. A sort of “universal setting”.
- A second protocol is “Liquid PRF” which is spun at 300rcf for 5minutes. The volume of PRF is very low, even 1cc, but the concentration of cells if very high. This can be used for injections or microneedling.
- The third protocol is “C-PRF”, for concentrated. 2000rcf for 8 minutes. Yields a high concentrate of cells at the buffy coat but only about 1cc. The upper yellow layer is acellular and can be used for e-PRF (more about that later). Useful for joint injections.
- Now the confusing part: the only thing that determines whether PRF is in a liquid or solid state (clot) is the test tubes used. Red tubes produce clots. Blue tubes produce liquid, period. The “Solid PRF” protocol has universal applications but the resulting PRF being “solid” or “liquid” depends on which tubes, red or blue, are used. And often both red and blue tubes are used at the same time in the “Solid” protocol.

Now...more about tubes. Why do red tubes clot? Why do blue tubes stay liquid?

- Red tubes are made of glass. Glass is hydrophilic, and platelets are the first attracted to it. Surface contact is a major initiator of clotting and the platelets along the tube walls begin the clotting cascade which spreads throughout the tube. Taking the cap off and exposing PRF to air increases the rate of clot formation.
- Blue tubes are made of PET plastic and are hydrophobic in nature and repel the PRF liquid and discourage clot initiation. With the cap left on, the liquid PRF will resist clotting for up to 4 hours. Clotting is an enzymatic reaction and will be slower at reduced temperatures. The Bio-Cool device keeps the tubes or syringes at 4^0 C to retard the reaction. Conversely, incubating tubes or membrane formers at 37^0 speeds up clot formation.

What determines clot size?

- The clot size is determined by the tubes used and not by the particular centrifugation device. Clot size was shown to vary by as much as 200% from tubes from different manufacturers. (The smallest clots were produced in silica-coated plastic tubes which were claimed to be equivalent to glass tubes.)
- Females tend to produce a larger clot - up to 17% larger due to variation in hematocrit.
- Older people tend to produce larger clots - largest membranes are from 61-80 year olds
- People from elevations of 5000 feet will have higher hematocrit and may have to have 25% more centrifuge time
- Processing of blood from people on anticoagulants is the same as others, but they will take longer to clot. All blood clots eventually.

Time, along with choice of tubes is a major factor in clot formation

- Tubes should be drawn and the centrifugation cycle started within 90 seconds.
- After 90 seconds, clot volume decreases by 13%
- After 120 seconds, volume decreases by 23% and cell concentration decreases by 52%
- If drawing both red and blue tubes, draw the blue tubes first because they are slower to initiate clotting. The red tubes being drawn last, will have the shortest time before centrifugation.

What else should we know about tubes?

- Additionally, silica-coated tubes may shed their coatings into the PRF membranes, embedding silica particles in significant numbers into the clots.
- When these silica microparticles from silica-coated tubes were added to human periosteal cell cultures, they proved to be toxic to cells.
- Tubes can be checked for additives by filling them 1/2 full with water and them vigorously shaking them and observing if foaming occurs.
- Collection kits and tubes are matched by manufacturers. Mixing kits and tubes of different brands may cause a problem with needle depth and penetration of the cap and cause poor or no blood flow.