**Platelets Rich Plasma (PRP) for human body healing**

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**Key word :** platelets rich plasma , PRP , healing , grow factors , injection.

**The aim of the research :** The aim of my research is offering Platelets Rich Plasma PRP therapy technique for tissue healing in order to be used for treating patients .

**Abstract :**

Platelets rich plasma shortly ( PRP ) it’s a new technique used for tissue healing depends on injecting platelets (separated from the patient blood sample) in the damaged tissue .

 Platelets are cytoplasmic fragments of megakaryocytes,(with diameter about 2 µm ) formed in the marrow. They contain more than 30 bioactive proteins, many of which have a fundamental role in homostasis or tissue healing .

A fundamental protein growth factors that are actively secreted by platelet initiate all wound healing process .

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Table 1

platelets growth factors and their specific characteristics .

Activation causes platelets degranulation by fusing platelets granules to its cell membrane where secreted proteins ( such as transforming growth factor beta1 (TGF-β) and other growth factors ) . activated and transformed to the bioactive state by addition side chain of carbohydrate ..

The active proteins ( growth factors ) are binding to the transmembrane receptors of target cells ( target cells include endothelial cells , epidermal cells , mesenchymal stem cells , fibroblasts and osteoblasts ) , this bound activates the intracellular signal protein and causes an expression of gene sequence which directs matrix forming , cellular proliferation , collagen synthesis and osteoid production that’s how tissue regenerated and repaired .

 To give an evidence for this technique therefore I studied the betterment of five various morbidity situations after injection PRP in the damaged tissue .

PRP platelets rich plasma have been prepared by centrifuging blood sample( approximately 20 ml ) that obtained from the patient and have been concentrated , the result is ready platelets rich plasma PRP for injecting in the damaged tissue of the patient .

The first situation is 42 years old male ( Tennis elbow medical epicondylitis ) PRP injection treated the situation completely 100 % step by step through 24 weeks .

The second situation is 55 years old female ( Knee Patellar tendonitis ) PRP injection treated the situation 75 % gradually through 24 weeks .

The third situation is 28 years old female ( shoulder Bicipital tendonitis ) PRP injection treated the situation approximately 65% gradually through about 20 weeks.

The fourth situation is 57 years old female ( foot Plantar fasciitis ) PRP injection treated the situation 70 % gradually through 14 weeks .

The fifth situation is 58 years old male ( Hand Chronic thumb sprain ) PRP injection treated the situation 50 % gradually through 18 weeks .

PRP gave a high betterment through ( 12 – 24 ) weeks for skin tissue healing of 10 situations of various ages and morbidity situations 5 of them are burns , 3 of them are old skin and 2 of them are scars , the range of betterment is( 70 % - 100 % ) depends on the situation kind , depth and scar old .

PRP gave high betterment through ( 10 – 19 ) weeks for hair growth of 4 situations with deferent ages all of them gave a high betterment about ( 50 % – 80 % ) .

**Introduction :**

Platelets rich plasma ( PRP ) it’s a new technique therapy used for medical treatment of many morbidity situations includes separation of blood sample ( from the patient himself ) by centrifuge and concentrating it for obtaining blood plasma rich of blood platelets as a concentrated internal blood platelets source to be ready for injection in where it needs to be treated , because platelets contains a lot of growth factors works to regenerate tissue cells and catalyze healing of tissue and bones .

This technique is completely safe method and absolutely without side effects because it depends on injecting internal substances from the patient himself therefore there is no any fear of body rejection of injected substance or transportation of microbic infections.

According to the classification proposed by Ehrenfest *et al*. (2009), four main families of preparations can be defined, depending on their cell content and fibrin architecture :

 **1** . Pure Platelet-Rich Plasma (P-PRP) or leukocyte-poor PRP products are preparations without leucocytes and with a low-density fibrin network after activation.

**2** . Leukocyte- and PRP (L-PRP) products are preparations with leucocytes and with a low-density fibrin network after activation. It is in this family that the largest number of commercial or experimental systems exist. Particularly, many automated protocols have been developed in the last years, requiring the use of specific kits that allow minimum handling of the blood samples and maximum standardization of the preparations.

**3** . Pure platelet-rich fibrin (P-PRF) or leukocyte-poor platelet-rich fibrin preparations are without leucocytes and with a high-density fibrin network. These products only exist in a strongly activated gel form, and cannot be injected or used like traditional fibrin glues.

**4** . Leukocyte- and platelet-rich fibrin (L-PRF) or second-generation PRP products are preparations with leucocytes and with a high-density fibrin network.

This classification system was largely cited, advocated, and validated by a multi-disciplinary consensus conference published in 2012 .

**Materials And Methods :**

**Materials :**

1 . PRP Kit .

2 . blood sample from the patient .

3 . centrifuge .

4 . Needle .

5 . Test tube .

6 . Antiseptic substance .

**Methods :**

**1 .PRP Preparation :**

#### PRP method :

**1** . WB were obtained by venipuncture in acid citrate dextrose ( ACD ) tubes .

**2** . The obtained blood were centrifuged by using soft spin .

**3** . The supernatant plasma containing platelets that results by centrifuging were transferred into another sterile tube ( without anticoagulant ) .

**4** . The last tube were centrifuged at a higher speed using hard spin to obtain a concentrated platelets .

**5** . As a result of hard spin centrifuging 3 layers obtained the first layer 1/3 rd is Platelets Rich Plasma ( PRP ) , the upper layer 2/3rd is Platelet Poor Plasma ( PPP ) and the lowest layer in the bottom of the tube is Platelets pellets .

**6** . Platelets Poor Plasma ( PPP ) were removed and platelet pellets were suspended in a minimum amount of plasma 2-4 ml by gently shaking the tube .

#### Buffy coat method :

**1** . Obtained WB were stored at 20°C to 24°C before centrifugation .

**2** . WB were centrifuged at a high speed .

**3** . Three layers are formed because of its density: The bottom layer consisting of RBCs, the middle layer consisting of platelets and WBCs and the top PPP layer.

**4** . Supernatant plasma were removed from the top of the container.

**5** . the Buffy-coat layer were transported to another sterile tube.

**6** . The last tube were centrifuged at low speed to separate WBCs .

**2 . Injection :**

Prepared PRP ready to be injected in patient body the area depends on the morbidity situation of the patient ..

Injection of PRP must be done by a professional specialist physician

 **Results** **:**

The results of PRP injection for treating various morbidity situations shown in the following diagram

100 Elbow pain ( Tennis elbow)

90 Knee pain ( Patellar Tendonitis)

80 Shoulder pain ( Bicipital tendonitis)

70

60 Foot pain ( Plantar fasciitis )

50 Hand pain ( Chronic thump sprain )

40

30

20

10

**pain**

 2 4 6 8 10 12 14 16 18 20 22 24 26 28

 **Time by weeks**

**Discussion :**

The results shown in the diagram expresses the betterment after PRP injection for various illnesses as the following :

**1** . The blue carve expresses the betterment after injecting PRP in the elbow ( Tennis elbow medical epicondylitis ) of a male 42 years old suffering severe elbow pain fixed completely 100 % step by step through 24 weeks .

**2** . The green carve expresses the betterment after injecting PRP in the knee ( Patellar tendonitis ) of a female 55 years old suffering a severe knee pain decreased 75 % gradually through 24 weeks .

**3** . The brown carve expresses the betterment after injection of PRP in the shoulder ( Bicipital tendonitis ) of a female 28 years old suffering a severe shoulder pain decreased approximately 65% gradually through about 20 weeks .

**4** . The purple carve expresses the betterment after injecting PRP in the foot ( Plantar fasciitis ) of a female 57 years old suffering foot pain decreased 70 % gradually through 14 weeks .

**5** . The gray carve expresses the betterment after injecting PRP in the Hand (Chronic thumb sprain ) of a male 58 years old suffering hand pain decreased 50 % gradually through 18 weeks .

PRP gave a high betterment through ( 12 – 24 ) weeks for skin tissue healing of 10 situations of various ages and morbidity situations 5 of them are burns , 3 of them are old skin and 2 of them are scars , the range of betterment is( 70 % - 100 % ) depends on the situation kind , depth and scar old .

PRP gave high betterment through ( 10 – 19 ) weeks for hair growth of 4 situations with deferent ages all of them gave a high betterment about ( 50 % – 80 % ) .

The betterment after injecting PRP depends on the prepared PRP and the method and effective factors on the yield of PRP ..

Various factors influence the yield of PRP such as draw of blood; speed, time and temperature of centrifugation and use of anticoagulants.

#### Draw of blood:

The clotting process is influenced from the time of the draw. To avoid unintentional activation of platelets, most protocols use large bore needles (>22) to draw the blood .

In a study by Waters and Roberts , using two cell-salvage devices and two table top devices over the course of 260 clinical cases, they found that there was a downward trend in platelet counts with longer draw time.

#### Centrifugation :

The earth's gravitational force is sufficient to separate many types of particles over time. A tube of anti-coagulated WB left standing on a bench top will eventually separate into plasma, RBC and WBC fractions. However, the length of time required precludes this manner of separation for most applications. In addition, the potential degradation of biological compounds during prolonged storage means faster separation techniques are needed. Hence, to accelerate sedimentation, the effect of gravity is amplified using ‘centrifugal force’ provided by a centrifuge and can be many thousand times the force of gravity.

Separation of cellular constituents within blood can be achieved by a process known as differential centrifugation. In differential centrifugation, acceleration force is adjusted to sediment certain cellular constituents and leave others in suspension. In centrifugation, RCF is the force required to separate two phases, this force also called relative centrifugal field.

It is expressed as multiples of the earth's gravitational field (g). By accelerating the g, speedy sedimentation can be achieved.

‘g’ is the actual force exerted on the contents of the spinning rotor, which separates the aqueous solutions in the centrifuge. Revolutions per minute (rpm) is calculated using the following equation.

#### Formula

g = (1.118 × 10-5) R S2

Where ‘g’ is the RCF, R is the radius of the rotor (from centre of rotor to sample) in centimeters and S is the speed of the centrifuge in revolutions per minute.

It is important to remember that calculation of RCF is dependent on the radius of the centrifuge rotor used. The same centrifuge machine with different rotors can produce different acceleration forces.

#### Temperature :

Temperature during processing is crucial to prevent platelet activation. AABB manual recommends 21°C-24°C for centrifugation of blood for obtaining PRP .

Macey et al , also stated that cooling may retard platelet activation and this may be essential in obtaining PRP with viable platelets.

Many authors have used a temperature level of 12°C-16°C during centrifugation for best platelet recovery.

This is germane to those who use an ordinary centrifuge to develop PRP, which are mainly developed for diagnostic purposes and not for PRP processing and hence may not produce a sufficient platelet yield.

#### Anticoagulants

The importance lies in choosing an anticoagulant capable of preserving the platelets’ best possible functionality, integrity, and morphology.

With regard to the type of anticoagulant for use, most authors agree on not using EDTA because it could damage the platelet membrane. Therefore, anticoagulants with citrate and dextrose of sodium citrate are recommended .

The authors compared the effects of sodium citrate and ACD-A on platelet aggregation, pH and extracellular iCa concentration. The anticoagulant ACD-A is the choice for collection of platelets by apheresis, whereas trisodium citrate (3.2% or 3.8%) is the anticoagulant most commonly used for diagnostic evaluations of platelets. Trisodium citrate and ACD-A solutions differ markedly in pH, with ACD-A having a pH of 4.9 and 3.8% sodium citrate having a pH of 7.8. In addition, the citrate ion concentration in ACD-A is 15.6 mg/mL, whereas 3.8% sodium citrate contains 24.4 mg of citrate ion/mL. It has been reported. In several species that alterations in the pH and extracellular iCa concentration of PRP can affect platelet aggregation in vitro, with aggregation typically impaired at acidic pH and lower extracellular iCa concentrations .

Alternatively citrate phosphate dextrose-adenine) can be used. It is similar to ACD-A but has fewer supportive ingredients and, therefore, is 10% less effective in maintaining platelet viability .

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