

## Study Protocol

### **Analysing the Role of Platelet Rich Plasma As Biological Stimulator for Cartilage Regeneration: An Experimental Study**

#### **ABSTRACT**

**BACKGROUND:** In articular cartilage defects and degenerative cartilage lesions, platelet-rich plasma (PRP) is increasingly used. PRP is an autologous concentration of platelets containing a high number of factors which are responsible for growth. In the healing, recovery and acceleration of biochemical processes, these growth factors play a role in reducing the pain associated with joint injuries. The latest world research shows excellent results in reducing pain by using PRP and it has tremendous scope in reducing the knee pain as well as in regeneration of the articular cartilage. This study aims to analyse the effectiveness of Autologous PRP as biologic stimulator for Cartilage Regeneration in Rabbit Model.

**Method:** This will be a prospective experimental study carried out on 10 rabbits at Animal House in JNMC, Wardha. Osteoarthritis will be induced in the rabbits by destruction of articular cartilage. Then platelet rich plasma therapy. Will be given to induce the regeneration of the cartilage.

**Expected Results:** Evidence of regeneration of articular cartilage in rabbits with osteoarthritis through Platelet Rich Plasma Therapy.

**Keywords:** Platelet Rich Plasma (PRP), Cartilage, Rabbits, Osteoarthritis, Animal, Experimental

#### **INTRODUCTION**

The need for biology and its interaction with inflammatory process is growing with the latest studies and findings in the treatment of knee pain in articular cartilage injuries and degenerative cartilage lesions. The normal function and preservation of articular cartilage is mainly dependent on the balance between aggressive and protective factors [1]. In order to balance these causes, biology and its use are extremely necessary [2,3,4]. In articular cartilage defects and degenerative cartilage lesions, platelet-rich plasma (PRP) is increasingly used. [5,6] PRP is an autologous concentration of platelets containing a high number of factors which are responsible for growth [7,8]. In the healing, recovery and acceleration of biochemical processes, these growth factors play a role in reducing the pain associated with joint injuries [9,10,11]. The latest world research shows excellent results in reducing pain by using PRP [12,13,14].

Among the circulating cells in the blood, platelets are one of the smallest structures; they do not have a nucleus and thus replication cannot occur, varying in diameter from 2 to 4  $\mu\text{m}$  and consisting of cytoplasm and vesicles, lasting in circulation for maximum 10 days[1]. They are most important reservoirs of factors responsible for tissue repair which lies within them. They are necessary for the human regenerative process. Current literature has shown the existence of pre-packaged various growth factors(GFs) which are not in the active form in the platelet microvesicles and exosomes[2]. Platelet growth factor(PDGF), transforming growth factor beta(TGF-beta), fibroblast growth factor(FGF), Insulin like growth factor 1(IGF-1), connective tissue growth factor(CTGF), epidermal growth factor(EGF) and Hepatocyte growth factor(HGF) are the most relevant ones[3,4]. For mesenchymal tissue regeneration numerous microRNAs[5] are involved which[6,7,8] are also present in platelet microvesicles and some of them, such as microRNA-23b, have been thought to be required in differentiating Mesenchymal Stem Cells into chondrocytes[9] or as microRNA210, which have been suggested as a therapeutic alternative to improve healing of ligaments by injections given intra-articularly in animal model which are small[10]. In addition, strong anti-inflammatory effects of platelet concentrates have been looked into to promote tissue healing as a related effect. When coping with articular cartilage lesions, this factor may be mainstay. For tissue repair, an inflammatory response of adequate magnitude and timing is known to be important as most mesenchymal repair occurs from regulated inflammation. In this regard, reducing synovial tissue inflammation can contribute to a decrease in the amount of matrix-metalloproteinases which are enzymes capable enough to destroy the cartilage-matrix[4].

The in vitro and preclinical evidence is the basis for the interesting trophic properties of PRP[3,11] which can be resumed with regard to articular cartilage in

- (i) the involvement of particular chondrogenic factors responsible for growth such as PDGF(which can induce multiplication and synthesis of collagen), Tissue Growth Factor-beta(which can increase synthetic activity of chondrocytes, production of matrix and proliferation of cells and decrease IL-1 catabolic activity) and Fibrinogen Growth Factor(that encourage multiple anabolic pathways);
- (ii) chemotactic resettling of MSC and human subchondral progenitor cells through procedures that can include TGF-beta and FGF synergistic action[12];
- (iii) the stimulation, regardless of the donor age, of the proliferation rate of MSC[13, 14];
- (iv) differentiating the chondrogenic lineage of Mesenchymal Stem Cells and neighbouring cells[12,15]; this results were previously seen in vitro with autologous human peripheral stem cells of the blood;
- (v) PRP's anti-inflammatory effect[4,16,17];
- (vi) a hypothesizable anti-apoptotic effect by inhibition of associated apoptotic factors (i.e. downregulation by IGF-1 of programmed cell death protein 5)[18].

This persuasive history of basic science research on PRP has therefore provided the clinician with a promising opportunity over the past 10 years for an innovative approach for the treatment of osteoarthritis and cartilage lesions..

Given its avascularity, cartilage has an exceedingly restricted potential for self-repair[3];thus healing response in cartilage injury is not done by conventional inflammatory

repair because of the avascularity of the articular cartilage which shows no meaning of travelling to the locally damaged tissue. . The reason for the use of Platelet Rich Plasma is that platelet-derived factors are supraphysiologically released at the direct site of cartilage damage or disease and may activate the normal cascade of curing and tissue reconstruction[4].In order to promote synthesis of cartilage matrix and increase phenotype changes, cell growth ,migration and make easier protein transcription within chondrocytes, Activation of platelets leads to the release of a fore mentioned factors which are responsible for growth and plenty of others from its alpha-granules[5,6]. Fibrinogen and Fibrin are proteins which are extracted by chemo attractants which are stored in platelets and the fibrin act as an initial scaffold for stem cells to relocate and evolve. Simple scientific proof has generally showed the capacity of Platelet Rich Plasma to multiply the proliferation of mesenchymal stem cells and chondrocytes, collagen type II deposition and proteoglycan[7,8]. In principle, this may speed-up the development of cartilage repair tissue. The plenty of platelets in Platelet Rich Plasma multiply the concentrations of the material involved regionally, resulting in a prolonged impact on the articular cartilage. The transcription of several degradative cytokines like IL-1 $\beta$ , TNF- $\alpha$ , and IL-6 are subject to upstream nuclear factor KB (NF-KB)regulation, and the alpha-granule contents in platelets which hinder this catabolic pathway on the downstream end and avert the otherwise harmful results of the osteoarthritis process on articular cartilage changes[9,10,11]. Activated PRP raises hepatocyte growth factor levels in vitro, which improves the expression of cellular I $\kappa$ B $\alpha$  and ultimately disrupts the transactivating function of NF-KB. It does so through cytosolic retention and nucleocytoplasmic shunting of the NF-KB-p65 subunit, thereby reducing its downstream proinflammatory impact[12]. In addition, Platelet Rich Plasma has antinociceptive and anti-inflammatory effects arising from PRP's capacity to lessen the expression of synoviocyte matrix metalloproteinase-13 as shown in cartilage explant studies, which would otherwise play a principle part in the deterioration of the cartilage matrix when undergoing osteoarthritic modifications. Some studies showed significantly increased expression of hyaluronan synthase-2 in samples treated with Platelet Rich Plasma, which is an enzyme known to generate large isoforms of hyaluronic acid(HA) & thus contributes to the cartilage construct[11]. Finally, Platelet Rich Plasma reduces the expression of target genes of COX-2 and chemokine-receptor CXCR4 that can control regional inflammation while being utilized in the articular cartilage injury[12].

## **OBJECTIVES:**

The above aim will be met by following objectives-

1. To analyse effect of multiple APRP injections on Cartilage and Histopathological Examination
2. To Test frequency of Therapeutic APRP for Cartilage regeneration.

## **MATERIAL AND METHODS**

- **Type of study** : Experimental study
- **Study design** : Prospective study

- **Study area** : Animal laboratory, Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi (Meghe)
- **Study subjects**: Rabbits / Animal Models
- **Sample size**: The sample size for this study will be 10.
- **Duration of data collections and follow up**: September 2020 to April 2023
- **Data analysis** : June 2023 to October 2023
- **Method**:  
10 rabbits will be prospectively enrolled who will be undergoing destruction of articular cartilage and inducing osteoarthritis then regeneration of the cartilage with use platelet rich plasma therapy.

#### **BLOOD WITHDRAWAL:**

During clinical evaluation or blood sampling, rabbits easily get shocked and begin to scratch the manipulating individual or leap from the examination table. As a result, rabbits were restrained in a wooden cage.

Amounts upto 5ml of blood was drawn from the auricular marginal veins from both ears, using a 22G to 25G needle or a 22G butterfly attached to a syringe.

The hair on the ear was shaved and the skin washed with alcohol before starting the sampling. As the skin on the ear is very sensitive, it was anaesthetized locally with a lidocaine containing cream.

The creamed spot was surrounded by a sheet of plastic and a protective adhesive bandage. The entire thickness of the skin becomes numb after 45 minutes. For the next 60 minutes, the anaesthetic effect will linger. Vessel dilation was obtained by ear massage.

The needle was gently inserted after vein occlusion and blood was removed. Cotton gauze was tightly applied to the venipuncture site for at least 1 min after removal of the needle or until bleeding stopped to avoid hematoma and blood clots.

The rabbit remained under observation for next few hours to ensure that hemostasis was complete.

Around 10ml of blood was drawn through auricular marginal venipuncture from both ears and divided in four test tubes of 6ml capacity containing CPDA anticoagulant solution.

#### **PREPARATION OF PRP:**

The tubes were situated and counterbalanced in the centrifuge machine. At 1200 rpm for 10 minutes (soft spin) or at 220 x g for 20 minutes, the first centrifuge cycle was carried out. The entire blood was divided into red blood cells in lower layer and straw coloured plasma in upper layer. The uppermost area of this plasma contains relatively low platelet

concentration (Platelet poor plasma) and there is higher platelet concentration in the boundary layer, also referred to as "buffy coat".

A spinal needle was attached to 5 ml syringe in order to extract straw coloured plasma from the tubes by pushing the needle above downwards.

When an RBC layer had been reached or in the first 1 to 2 mm of that layer, the draw stopped. The plasma was then represented in another tube without anticoagulant solution. For other tubes, the same process was done.

The plasma tubes were again centrifuged for 10 minutes at 2000 rpm or for 20 minutes at 480 x g. The contents of the tube are now composed of the upper layer of fibrinogen-containing transparent supernatant serum and very low platelet concentration, and the lower layer is mostly red-tinted with heavily condensed platelets.

With the same spinal needle attached to the 5 ml syringe, the upper two-thirds of that substance was removed, leaving one-third of the serum with concentrated platelets in the tube.

To shape red coloured concentrated platelet rich plasma, the remaining one-third material was thoroughly blended.

- **Inclusion criteria :**

Healthy rabbits of weight more than 1 kg without any pathology

- **Exclusion criteria**

Weight less than 1kg and with any existing pathology

- **Research methodology**

After destruction of articular cartilage PRP infiltration will be carried out every 4<sup>th</sup> day or twice in a week. Rabbits will be evaluated at 3 months by MRI/USG/Histopathologically for Cartilage Regeneration.

### **Expected Outcomes/Results:**

Platelet Rich Plasma Therapy will induce the regeneration of articular cartilage in previously affected cartilage with osteoarthritis in rabbits.

### **Discussion:**

In vitro and preclinical studies suggested the ideas of utilization of PRP in cartilage surgery because of trophic effects and the capacity to assist Mesenchymal Stem Cells to evolve toward bone & cartilage in proper conditions.[4]

In 2014, the value of L-PRP injections in a group of 79 patients selectively affected by intermediate grade knee osteoarthritis was again limited by a study of Mangone et al. The WOMAC Scale, VAS at rest and VAS in motion showed progress up to 1 year after the end of

treatment. Three Platelet Rich Plasma administrations per three-week cycle were included in the procedure. Due to the high cost of this procedure relative to conventional HA therapy, they delineated the role of Platelet Rich Plasma as a second solution to the treatment of knee OA.[19]

A group of patients were assessed by Gobbi et al after two years, showed a greater improvement in clinical scores when the Platelet Rich Plasma administrations cycles were repeated after one year and was the first research to support the importance of cyclical Platelet Rich Plasma therapy[20].For Talarosteocondral lesions single stage correlation of bone marrow concentrate and Platelet Rich Plasma may be as productive as other regenerative methods like the autologous chondrocyte implantation.[21]Platelet Rich Plasma is related with microfracture method to enhance the cartilage repair for which L-PRP is used and Platelet Rich Plasma was administered in situ and all over the microfracture holes after extracting arthroscopic fluid from the joint by ensuing the concept of in situ activation[22]. A number of studies on use of Platelet Rich Plasma were reported[23-30].

Platelet Rich Plasma therefore appears to have the capacity to enhance the function of the knee and the quality of life of patients with chondropathy or initial OA by decreasing inflammation and degenerative articular procedures to a lesser degree.

#### **COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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